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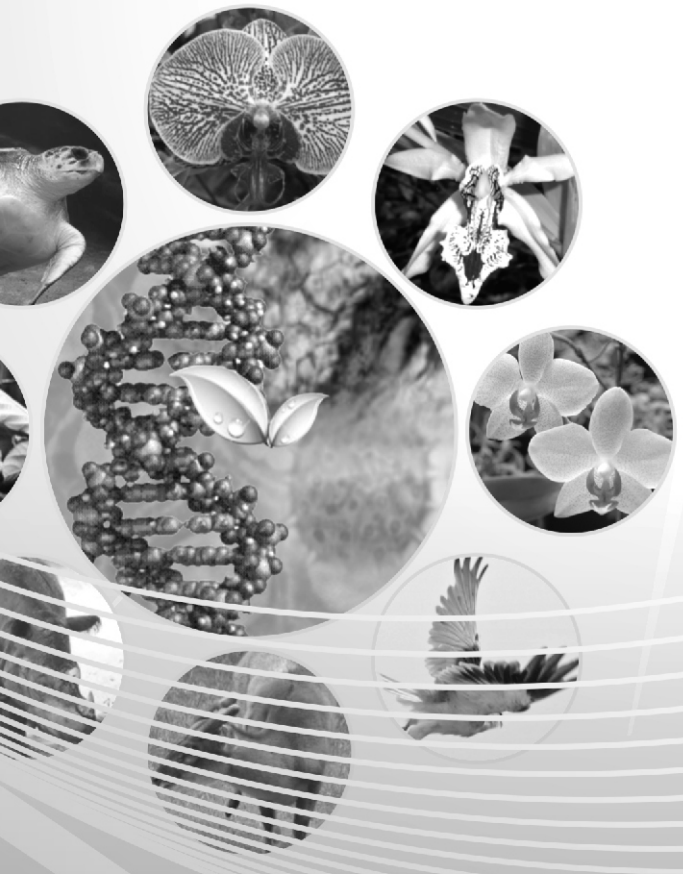
ICBS 2011

Faculty of Biology Universitas Gadjah Mada

ADVANCES IN BIOLOGICAL SCIENCE

Education for Sustainable Development-based Tropical Biodiversity Management and Conservation for Supporting Human Prosperity



September 23rd-24th 2011

Yogyakarta, INDONESIA

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

PROCEEDING ICBS BIO-UGM 2011

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CONTENT

PREFACE

Proceeding of the **International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2011 (ICBS BIO-UGM 2011), Advances in Biological Science: Education for Sustainable Development-based Tropical Biodiversity Management and Conservation for Supporting Human Prosperity,** organized by and held at the Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia on September 23-24, 2011. The conference addressed a range of important research from various fields in biological science likely to play role tropical biodiversity management and conservation for supporting human prosperity. Three kinds of session were held at the conference: plenary session featuring keynote and invited papers, oral presentation session, and poster presentation session. This proceeding features a number of papers presented in these sessions, which represent 5 themes covered in the conference, i.e. genetics and molecular biology, ecology and conservation, systematics and evolution, physiology and developmental biology, and biomedics.

Many people have been involved in the production of these Proceedings, which is started in June 2011 with the launching of a call for abstracts. The abstracts were reviewed by both internal and external reviewers . Those selected abstracts were called for either oral or poster presentations and inveted to submit full papers.

Lastly, on behalf of the organizing commite we would like to all participants for their kindness to be part of this conference. We would like to acknowledge each partnerships and sponsorship that involve during this event. I believe that this proceeding still has some weaknesses, therefore any constructive comments are welcome. We hope that the papers contain in this proceeding will prove helpful toward improving the scientific atmosphere. See you in the next two year ICBS 2013.

Yekti Asih Purwestri

Chair of the Organizing Commitee

WELCOMING SPEECH FROM CHAIR PERSON OF THE ORGANIZING COMMITTEE

Distinguish guests

- Executive Director of Indonesia-Managing Higher Education for Relevane and Efficiency (I-MHERE) Project
- Keynote speaker, invited speakers, participants, sponsorships, ladies and gentlemen

Good morning and May God shower us with His blessing.

On behalf of the Conference Organizing Committee, I extend a warm welcome to all participants to the second **International Conference on Biological Science Faculty of Biology Universitas Gadjah Mada 2011 (ICBS BIO-UGM 2011), Advances on Biological Science : Education for Sustainable Development-based tropical biodiversity management and conservation for supporting human prosperity.** Bio-conservation becomes a critical issue not only in Indonesia but also in global community. A good understanding on Education for Sustainable Development- based tropical biodiversity management is necessary to have the right policy regarding bio-conservation action.

For this year, the organizing committee has put together an interesting Scientific Program to accommodate the areas of Biology. The Program comprises of 6 plenary sessions of keynote and invited speakers. The parallel session of 82 oral presentations and more than 50 poster presentations. I realize that you are fully dedicated to the sessions but I do hope that you all will also take time to enjoy Yogyakarta, the multicultural city and may enjoy the special Merapi scenery, the most active volcano in the world.

I would like to take the opportunity to thank Prof Hubert Gijzen (Director of UNESCO-Jakarta) as a keynote speakers and also to these following invited speakers, Hao Yu, Ph.D (National University of Singapore), Prof. Christ Austin (Charles Darwin University, Australia), Prof. Yasumasa Bessho, Ph.D (Nara Institute of Science and Technology, Japan), Dr. Yam Tim Wing (Senior Researcher Orchid Breeding and Conservation Singapore Botanic Gardens), Drs. Langkah sembiring, M.Sc. Ph.D (Faculty of Biology, Universitas Gadjah Mada) for delivering their valuable scientific information.

To make this program happen, I would like to gratefully acknowledge to Indonesia-Managing Higher Education for Relevane and Efficiency (I-MHERE) which support this conference. We also thank to the valuable contributions from personal and institutional sponsorship and funding including Ms. Sachiko Iida, PT Diastika Biotekindo, PT Roche, Prima Grafika Yogyakarta., and Drs. Agus Suryanto - Indogama Yogyakarta.

I also gratefully thank to the Dean and Vices Dean of Biology Faculty, Universitas Gadjah Mada for giving us opportunity and support to organize this conference. My deep appreciation to the Steering Committee, the Academic Reviewers (internal and external: Dr. Sentot Santoso from Institut fuer Klinische Immunologie und Transfusionsmedizin, Justus Liebig Universitaet Giessen, Germany and Prof. Yasumasa Bessho, Ph.D from Gene Expression Research, Biological Sciences, Nara Institute of Science and Technology, Japan), members of the Organizing Committee for their strong support, active participation, cooperation and hard works in preparing and organizing this event a success.

It is inevitable that there is a lack in organizing this conference and I profoundly apologize to all invited speakers, oral and poster presenters, attendants, donators and committee members.

I wish you a pleasant and rewarding two days of scientific discussion.

Thank you,

Yekti Asih Purwestri
Chair person of the Organizing Committee

OPENING REMARKS FROM THE DEAN of THE FACULTY OF BIOLOGY

Bismillahirrahmaanirrahiim.

Director of UNESCO Office Jakarta, Prof. Dr. Hubert Gijzen,
Executive Direktor of Indonesian-Managing Higher education for relevance and Efficiency (I-MHERE) Project
Honorable speakers and distinguished guest, dear participants,

Assalamu'alaikum wr.wb., may God give us healthy and happier life

Welcome to Yogyakarta, the city of youth, education, and culture. It's been an honour for me to be here in front of you to open the prestigious **International Seminar with the special theme of "Advances in Biological Science: Education for Sustainable Development-based Tropical Biodiversity Management and Conservation for Supporting Human Prosperity",** that invited our honorable speaker from the UNESCO as the keynote, Prof. Hubert Gijzen, Ph.D honorable invited speakers Dr. Yam Tim Wing From Singapore Botanic Garden, Singapore; Prof. Yasumasa Bessho, MD, Ph.D from NAIST, Japan; Prof. Christopher M. Austin, Ph.D from Charles Darwin University, Australia; Dr. Yu Hao from National University of Singapore, and Dr. Langkah Sembiring MSc, from the Faculty of Biology, Universitas Gadjah Mada, Indonesia.

My special gratitute to the speakers who have spent your time travelling to Indonesia in your such busy activity. This international seminar atrracts more than 400 scholars and students mostly come from Indonesia, and some participants come from abroad. This occassion is such a good opportunity for us to share our experiences in research and good practices of ESD based research and community service done, that could inspire students and other researchers, furthermore our keynote speaker today is the Director of UNESCO Jakarta Office, who will talk about Science, Technology and Innovation-an Engine for Sustainable Development.

Honorable and distinguished participants,

The seminar theme taken today is in line with vission of the Faculty of Biology UGM as the center of excellence for higher education that generates biologists who respect to our tropical biodiversity. Since 2010, Faculty of Biology UGM had obtained an ESD based research grant from the World Bank, through I-MHERE (Indonesian Management of Higher Education for Efficiency and Relevance) project. In this project has been conducted 3 activities, these are: improvement of publication and research quality, improvement of integrated collaboration research in tropical diversity with other Institutions, and community based activities that respect to biodiversity conservation. As stated in UNESCO HE information brief, the challenge for higher education in the context of ESD is to innovate the traditional learning environment and learning processes in such a way that they do not only support learning process in the formal education, but also in informal learning.

Our environment is now facing many dilemmas starting from global financial and economic crises highlights the risks of unsustainable economic development models and practices based on short-term goals. These aspects triger economic disparity between the poor and the rich countries, many complex societal contexts, and finally environmental degradation.

Education for Sustainable Development (EfSD) promotes quality education and its inclusive for all people. It is based on values, principles, and practices necessary to respond effectively to current and future challenges. UGM has shown commitment in Education for

Sustainable Development and will continue to conduct ESD in the future. I hope that this Conference will continue to serve as a sustainable forum to provide opportunities for teachers, lecturers, researchers and professionals to share experience and present research activities and action programs. To everyone present here, I wish you have a productive and significant Conference that will benefit humankind, civilization as well as knowledge.

Lastly, I would like to extend my sincere appreciation and profound gratitude to the Director of UNESCO Jakarta and NAIST Japan for their supports. My special thanks should also go to the steering and organizing committee for their hard work in making this event a success. Thank you very much.

Yogyakarta, September 23rd, 2011

Sincerely yours,

Dr. Retno Peni Sancayaningsih, MSc.

WELCOMING SPEECH FROM EXECUTIVE DIRECTOR I-MHERE UGM

Honorable Dean of Faculty of Biology UGM, Dr. Retno Peni Sncayaningsih, M.Sc.
Distinguish Keynote speaker Prof Hubert Gijzen (Director of Unesco in Indonesia)
Distinguish Dr. Yam Tim Wing (Singapore), Prof. Yasumasa Bessho (Japan), Prof Christ Austin (Australia), Dr. Langkah Sembiring (UGM),
Dr. Yu Hao (Singapore)
Distinguish all of participants

Assalamu'alaikum wr.wb.

Welcome to Yogyakarta and participating in International Conference on Biological Science, by Faculty of Biology UGM.

This seminar was supported by IMHERE UGM (Indonesia Managing Higher Education for Relevancy and Efficiency). As we know, UGM get a competitive grant from World Bank trough Directorate General of Higher Education, from 2009 – 2012, and proposed program entitled "Education for Sustainable Development toward World Class Research University" by establishment of Center of Excellence (CoE) on 3 selected academic units, namely (i) "Tropical Biodiversity", in Faculty of Biology (ii) "Medical Herbal and Supplements" in Faculty of Pharmacy and (iii) "Reduction Emission from Deforestation and Degradation (REDD)" in Faculty of Forestry.

Faculty of Biology has attempted for enhancement of the research quality on tropical biodiversity, development of the integrated research on utilizing biodiversity resources to enhance the EfSD and development of network capacity for national and international collaboration on research and community services through Regional Centre of Expertise (RCE) Yogyakarta.

This prestigious international seminar is one of our strategic activities to achieve better key performance indicator, especially in international publication and international research collaboration. As a new paradigm of competitive grant that developed by World Bank, called "Performance Based Contracts", achievement of our key performance indicator in this year was 190% compare to targeted indicator for three years activities. We would like to continuing our "Research based Learning and Services for sustainable reputation as World Class Research University.

Please be enjoy to discuss and active participating in this seminar.

Wassalamu'alaikum wr.wb.

Sincerely yours,

Executive Director I-MHERE UGM

Dr. Cahyono Agus Dwikoranto, M.Agr.Sc.

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ADVANCES IN BIOLOGICAL SCIENCE

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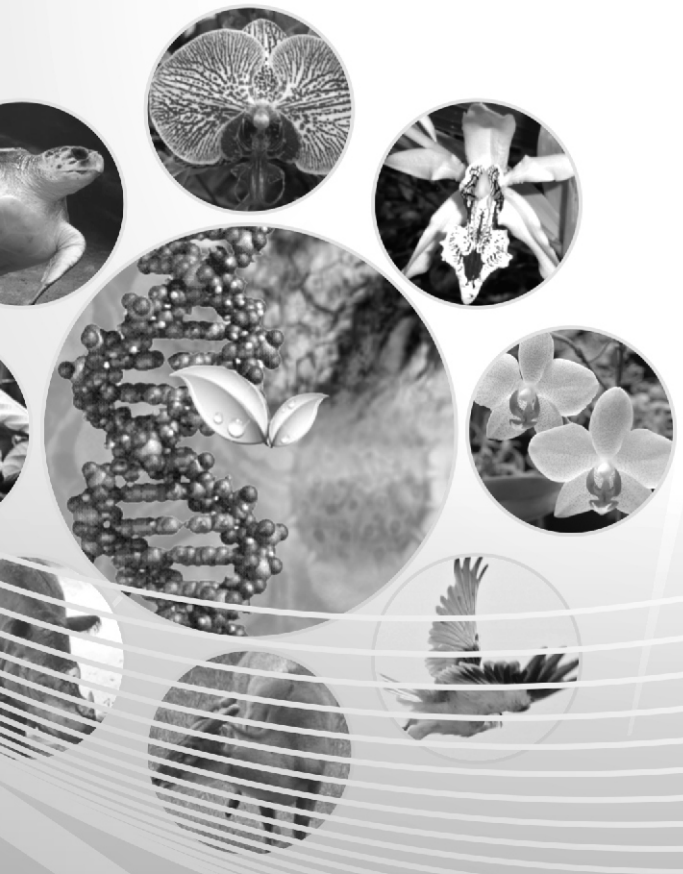

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Conservation and reintroduction of the native epiphytic orchids of Singapore - a physiological and developmental biology perspective

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Singapore Botanic Gardens, 1 Cluny Road, Singapore Botanic Gardens, Singapore 259569

Native orchids of Singapore and their conservation

Singapore is located at about 1º north of the equator, off the southern tip of the Malay Peninsula between the South China Sea and the Indian Ocean. It consists of the main island of Singapore, and 58 nearby islands. The total land area is about 710 sq km. The whole island consists mostly of lowland. The highest point is at Bukit Timah, reaching a height of 165 meters. It has an equatorial climate, with a relatively uniform temperature and high humidity. The average daily temperature fluctuates between 25.2º C to 32º C. Its annual rainfall is about 1,700 mm; the wettest months are November to January.

Although Singapore is a modern city, there are many interesting types of natural habitats. In the heart of the main island there are a primary rainforest and a freshwater swamp forest. In addition, some mangrove also remain. The other habitats consist of secondary forests, shrub, grasslands and urban parks and fields.

According to the Singapore Red Data Book published in 2008 (Davison et al., 2008), Some 226 species of native orchids were recorded in Singapore. Of these, 178 are considered to be extinct, 40 are critically endangered, one is endangered (*Bulbophyllum vaginatum*), two are vulnerable (*Vanilla griffithii, Bulbophyllum trifolium*), and only five are considered to be common (*Arundina gramminifolia, Bromheadia finlaysonianum, Dendrobium crumenatum, Eulophia graminea, Spathoglottis plicata*). An orchid conservation programme was initiated to monitor these species and to attempt to find ways and means to conserve their germplasm and to increase their number for subsequent re-introduction into appropriate habitats in the nature reserves, parks and roadside trees.

Native orchids in Singapore can be divided into two main groups: epiphytes and terrestrials. More than 80% are epiphytes.

Dendrobium is the largest epiphytic genus among the native orchids. The first orchid species that comes to mind always is *Dendrobium crumenatum* (the Pigeon Orchid, Anggrek Merpati). These orchids can be commonly seen growing on the trunks of road side trees. It is a delight to see them in bloom because the flowers are sweetly scented. The species flower 9 to 10 days after a heavy rain storm. Another interesting native is *Dendrobium leonis*, section *Aporum.* Leaves of this interesting species are thick, fleshy and laterally flattened. Each of the yellowish-green flowers is borne near the apex of the stems.

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The flowers measure 1.5 cm across, and have an extremely sweet vanilla fragrance which can be detected from a distance. It is distributed throughout Indochina, China, Peninsular Malaysia, Singapore, Indonesia and Borneo (Seidenfaden and Wood, 1992). The species is extinct in Singapore. Seedlings were raised from seeds collected from nursery grown plants. *Dendrobium aloifolium* has flattened and pendulous stems that are 40–60 cm long. The bottom part of the stem bears flattened leaves that are 25 mm long and 7 mm in width, overlapping each other. The upper portion of the stem is covered only by sheathing leaves, and usually bears the flowers. Flowers are white, about 4 mm long and wide. It can be found in Thailand, Peninsular Malaysia, Borneo, Indonesia and the Philippines, growing under wet and bright areas in lowland forests.

Bulbophyllum is the second largest orchid genus in Singapore. Plants of *Bulbophyllum vaginatum* grow in fairly exposed areas on branches of rain trees and of *Eugenia grandis* in the Singapore Botanic Gardens. They usually cover an extensive area of a branch, and produce a spectacular sight when in full bloom. The flowers are creamy-yellow. About 15 of them are arranged in a fan shape whorl or in a circle at the tip of the scape. There is another Singapore native, *Bulbophyllum medusa*. Flowers of this exotic-looking species are creamy-yellow, some varieties having purple spots on the floral parts. About 15 of them are arranged in a fan shape whorl or in a circle at the tip of the flower stalk. The lateral sepals are 12 cm-long, which is much longer than the upper sepal. When the flowers are seen from afar, the lateral sepals look like some silk threads hanging on the tree. It can be found in Thailand, Peninsular Malaysia, Indonesia, Singapore and the Pacific Islands. The species is extinct in Singapore. *Bulbophyllum membranaceum* is found in Malaysia, Borneo, Java, Sulawesi, Sumatra, Papua and New Guinea, Solomon Islands Fiji, Samoa, Tonga and Vanuatu (Seidenfaden and Wood, 1992). It is a tropical lowland species that can be found from sea level to 450 meters. Among the smallest native orchids, it has very small flowers (ca. 6 mm long) that do not open completely. The upper sepal is pale yellow, whereas lateral sepals are dark red, and the petals, 1.5 mm long, appear translucent. There are only very few naturally occurring populations of *B. membranaceum* left in Singapore. It is an epiphyte but in a particular location in the nature reserve, it grows as a lithophyte creeping on a large piece of granite. *Bulbophyllum blumei* is a very beautiful species that is quite adaptable to a range of habitats as it can be found in both the hot, lowland areas to cooler conditions. It bears one leaf. It flowers quite freely in Singapore. The inflorescence has one to two flowers. Individual flowers are 6cm across. The species distributed in West Malaysia, Singapore, Sumatra, Borneo, to the Philippines, Papua and New Guinea, the Solomon Islands and Australia (Seidenfaden and Wood, 1992). It is extinct in Singapore. Other interesting *Bulbophyllum* species in Singapore are *Bulbophyllum purpurescens* and *B. lepidum.*

Four species of *Cymbidium* have been recorded in Singapore, they are *Cymbidium bicolor* var. *pubescens*, *Cymbidium aloifolium, Cymbidium finlaysonianum* and the most recently discovered *Cymbidium atropurpureum.* All native *Cymbidium* species are epiphytes, the most common being *Cymbidium finlaysonianum*. It can still be found occasionally inside the nature reserve and the catchment areas. It is interesting that it can also be found growing naturally on roadside trees near suburban areas as well. Perhaps seeds were blown there from plants growing at the nearby nature areas. *C. finlaysonianum* grows around tree trunks and its leaves are arranged in such a way that a basket is

formed. As a result, leaf litter falling from the canopy is trapped in the basket. This enables the plant to obtain nutrients from the decaying litter trapped around the roots. Such an adaptive feature is important for the survival of the plant in a nutrient-deficient environment. The species bears 90 cm long inflorescences. Individual flowers are 4 cm across. Sepals and petals are yellow-green with a brown central band. They are accompanied by a purplish lip with some yellow markings. It is distributed in Indochina, Thailand, Peninsular Malaysia, Singapore, Borneo, Indonesia and the Philippines (Seidenfaden and Wood, 1992). *C. finlaysonianum* is critically endangered in Singapore. It has been propagated from seeds collected from native plants.There is only one know naturally occurring plant of *Cymbidium bicolor* spp. *pubescens* in Singapore. Thought to be extinct, it was rediscovered some 10 years ago, growing on a mangrove tree, Buta Buta (*Exoecaria agallocha*), at the Sungei Buloh Wetland Reserve. It was last collected in Sungei Buloh in 1891 by Mr H. N. Ridley. It is amazing that the species has miraculously survived much habitat loss. Leaves of the species are about 45 cm long, 1.5cm wide, wide arching, not drooping in habit, leaf bases persistent, enclosing pseudobulbs within; raceme pendulous to 25cm long; sepals and petals with broad dark purple central band, edges pale green, less than 2cm long, 0.5cm wide; lip yellowish with purple-brown spots; column dark purple, tip pale yellow with purple spots. The species is distributed in Malay peninsula, Singapore, Sumatra, Java and Borneo (Seidenfaden and Wood, 1992). Plants can be found on trees in exposed places near the sea.

Next is the genus *Coelogyne. Coelogyne mayeriana* is characterised by the attractive, clear, apple-green petals and sepals, the lip of the flowers is conspicuously marked with dark red-brown, almost black venations. Because the dark brown-red markings are so intense in some cultivars, the name 'Black Orchid' has been given to them. Unfortunately, the flowers of this beautiful species only last for a few days. It can be found in Thailand, Peninsular Malaysia, Borneo and Indonesia (Seidenfaden & Wood, 1992). The species is extinct in Singapore. *Coelogyne rochussenii* has rather close narrowly conical, ribbed pseudobulbs that bear two ovate leaves, measuring 20–28 cm long and 10–15 cm wide. The pendulous inflorescence can reach up to 70 cm long, bearing some 30 flowers. Each flowers is about 5 cm across, with pale yellow green sepals and petals (Fig. 4). Side-lobes of the labellum are dark- to light-brown with whitish veins on the inside. It can be found in Thailand, Peninsular Malaysia, Sumatra, Java, Borneo, the Philippines and Sulawesi, with large altitudinal amplitude ranging from sea level to 1500 m (Seidenfaden & Wood, 1992). It is extinct in Singapore.

Although Singapore is a small country, it is the home of *Grammatophyllum speciosum*, the largest orchid plant in the world. Although Singapore is a small country, it is the home of the largest orchid plant in the world, *G.* speciosum. Also known as the tiger orchid, because of the markings on the flowers that resemble the skin of a tiger, it is extremely rare if not already extinct in the wild in Singapore. However, it can still be found in Indochina, Peninsular Malaysia, Thailand, Borneo, Indonesia, the Philippines and the Pacific Islands (Seidenfaden and Wood, 1992). *G. speciosum* was last found in the wild in Tuas and Pulau Ubin. Since a mature plant could weigh more than a ton, it is a wonder that such a huge plant could live on the trunk of a tree.

The orchid conservation programme, which started in 1995, aims to monitor existing species, explore ways to conserve their germplasm, and increase their number through subsequent re-introduction into appropriate habitats, including roadside trees, parks and nature areas. Propagation (Arditti et al., 1982). of native species started in the mid 1990s and seedlings were introduced in 1999 . By 2009, we succeeded in propagating and introducing five epiphytic species of native orchids, namely, *Grammatophyllum speciosum*, *Bulbophyllum vaginatum*, *Bulbophyllum membranaceum, Cymbidium finlaysonianum* and *Cymbidium bicolor spp. pubescens* (Yam, 2008). From 2009 to 2012, we expanded our reintroduction effort by planting more than 6000 plants consisting of 15 species in many parts of the island.

One of the challenges of reintroducing epiphytic orchids is that they do not grow in soil. In order to carry out successful planting, we must understand the physiology and developmental biology of these orchids.

Reintroduction of epiphytic orchids - a physiological and developmental biology perspective.

More than 70% of the orchids in the tropics and subtropics are epiphytes. They grow on other plants and other objects such as rocks (these are specifically known as lithophytes) for support. The trees provide wide range of habitats with variable conditions of temperature, exposure and illumination. For example, epiphytes can reach positions where the light is better or where they can avoid competition for light.

How do epiphytic orchids absorb water and nutrients?

Since epiphytes are not in contact with the soil, one of the difficulties they encounter are shortages of water and nutrients.

Some epiphytic orchids shrivel and stop growing when water is in short supply , in fact the plant may appear dead. When water becomes available again, they are able to absorb it rapidly and growth resumes. Epiphytes get their water from dew, moisture in the atmosphere and rainwater running down tree trunks.

Dust in the air and on the tree trunks and leaves contain nutrients, they are absorbed by the epiphytes when rainwater wash them off the bark. Roots of certain species such as *Grammatophyllum speciosum* and *Cymbidium finlaysonianum* form what the ecologists call “trash baskets.” They enable the plant to obtain nutrients from the decaying litter trapped around the roots. Such an adaptive feature is important for the survival of the plant in a nutrient-deficient environment.

Aerial roots of epiphytic orchids are covered with a layer (or layers) of dead cells known as the velamen (terrestrial orchids also have velamen). It works almost like a sponge to absorb water during the wet period and releases it slowly during the dry intervals. The silvery-looking velamen also reflects sunlight so as to protect the roots from excessive heat. When the velamen is filled with water, it becomes transparent allowing light to reach the green tissue in the roots. During dry periods when water is in short supply, the velamen reduces water loss from the roots. It also cools the roots.

The roots, in addition to providing nutrition for the plant, also fix it to the tree or rock on which it grows, either sticking fast to the smoothest surfaces, or anchoring the plant by threading through the interstices of bark and rock. Green roots fix carbon.

How do epiphytic orchids reduce water loss?

Water exits plants through the stomata, pores on the leave surface which open to take up carbon dioxide from the air for photosynthesis. Most plants open their stomata during the day when temperature is high, resulting in increased water loss. To reduce water loss, some epiphytic orchids have succulent leaves which have thick cuticles and cuticular ledges over their stomata . The stomata only open during the night for gas exchange when the air is cool and humid. This reduces transpiration.

Like succulent leaves, the roots of epiphytic orchids fix carbon via Crassulacean acid metabolism (CAM). When the air is cool and humid at night, these plants open their stomata to take in carbon dioxide (CO_2), it is then fixed and stored as malate. During the day, CO_2 is released where it is concentrated around the enzyme Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO), increasing the efficiency of photosynthesis. Since CAM plants close their stomata during the day, water loss due to evapotranspiration is reduced (Arditti, 1992).

Furthermore, many of them have pseudobulbs or swollen stems which store water.

Seed production and dispersal

Most orchids are cross-pollinated, requiring specific pollinators (such as ants, bees, beetles, wasps, moths, butterflies, flies, and birds) to transfer the pollen from one plant to another. They have many extremely complex and ingenious pollination mechanisms to ensure effective and efficient pollination. Many orchids attract their pollinators by having brightly coloured flowers, producing scent and providing food rewards such as nectar, pollen, floral fragrances, resins and oils. Other attraction mechanisms are deceit and insect pheromones.

Orchid seeds are the smallest amongst all the flowering plants. The dustlike seeds consist of tiny elliptical embryos with thin seed coats. They are distributed by the wind, often over considerable distances.

All orchids are myco-heterotrophic at some point in their life cycle, i.e., they rely on fungi for their nutrients. This relationship between orchid roots and a variety of fungi is known as orchid mycorrhiza. The fungi are very important during orchid germination, as the small dustlike seeds have very limited energy reserves (which they have difficulties utilizing) and obtain their carbon from their fungal symbionts. Seeds may or may not germinate in the presence of a suitable mycorrhizal fungus, but they cannot start the germination process until an appropriate fungus has penetrated them. Once mycorrhiza has been established, orchid seedlings grow into a small lump of tissue known as a protocorm. It derives all of its nutrients and energy from its fungal symbiont. Once the protocorm has grown to a sufficient size, it produces shoots and subsequently starts to grow autotrophically. In

orchids which produce green leaves, the first leaves begin to photosynthesize. In order to germinate in nature, the natural habitat must have the correct mycorrhizal fungus.

In light of the specific physiological requirements of epiphytic orchids, we learn that the following points are important to ensure successful reintroduction.

Microclimate of the area such as relative humidity

Seedlings planted in areas with high relative humidity tend to survive better than those in dry areas. For example, among all the planting locations, Kent Ridge Park is located at the highest elevation with the most exposed environment. Seedlings planted there had the lowest rate of survival. Similarly, seedlings of *Cymbidium bicolor* spp. *pubescens* planted at Sungei Buloh planted near the visitor centre (less exposed to direct sun light and strong wind) grew very well and had 100% survival rate On the other hand, of those planted near the exposed mangrove area, only 10% survived. In Pulau Ubin, seedlings established in a damp area inside a secondary forest are healthier and more vigorous than those growing near the sea where the breeze tends to dry the bark faster. Orchids planted at forested area such as MacRitchie Reservoir grow well without any watering even during drought periods.

Host tree species and the presence of other epiphytes

Trees that support more epiphytes tend to be better hosts than those with fewer epiphytes. Seedlings of *Grammatophyllum speciosum* had the highest survival rate on *Pterocarpus indicus* and *Samanea saman*. Only 10% seedlings survived on *Fagraea fragrans*. Both *Pterocarpus indicus* and *Samanea saman* carry more epiphytes than *Fagraea fragrans*. It was observed that if conditions are suitable for other epiphytes, they are also more appropriate for epiphytic orchids too. Young trees do not have epiphytes grow on them because of the fine bark texture. When trees reach 15 to 20 years of age, their bark surfaces become rougher and epiphytes such as mosses and liverworts begin to appear (Tee, 2009). When dead leaves from surrounding trees fall within the cracks and crevices of the old tree trunk, they decay and the resulting humus not only holds water but also provides nutrients for the epiphytes. These pockets of organic matters also create suitable habitats for the germination of orchid seeds dispersed by the wind and subsequent development of seedlings. The best locations for the epiphytes to thrive are the forks of the main branches. When water flows from the top of the tree to the ground, it tends to flow towards the fork area before coming down to the ground. Therefore, the fork areas tend to accumulate more moisture and are more suitable for epiphytes to thrive. Of all roadside trees that are suitable for epiphytes, the rain tree exhibits the most luxuriant growth of epiphytic plants. The most common epiphytes are Bird's Nest Fern (*Asplenium nidus*) and *Dendrobium crumenatum*, also known as the Pigeon orchid.

Size of plants

The size of seedlings is also an important factor in determining survival. For example for *G. speciosum*, larger plants (with at least five shoots, with approximately 25 leaves reaching a length of 45 cm) tend to survive better and become established faster than smaller plants.. Larger plants og *G.*

speciosum, are more mature, the pseudobulbs are longer and fleshier, These characteristics enable the plants to withstand a longer period of drought and dehydration This in turn would allow the plants a longer period to adapt to the conditions of their new home. For small sized species such as *B. vaginatum* and *B. membranaceum*, seedlings were planted on fern bark. They must be grown at the nursery for at least 6 months until new shoots and roots begin to emerge before reintroduction. Such seedlings have high rate of survival when planted under the right environmental conditions. *Time of planting*

The best time for planting is before or during the rainy season. In Singapore, the rainy season starts around October and last until January. March also tends to be rather wet as well. Therefore most of our planting were done from late September to November; planting can also be done in late February to March. Seedlings planted during these periods established themselves quickly by producing new shoots and roots. Once the roots attach themselves to the tree bark, they can absorb water and nutrients directly from the environment.

Growth of reintroduced plant throughout the year

Singapore has a tropical rainforest climate with no distinctive seasons, uniform temperature throughout the year, high relative humidity, and abundant rainfall. Temperatures usually range from 23 to 33 ° C. Relative humidity averages around 80% in the morning and 73% in the afternoon. April and May are the hottest months, with the wetter monsoon season from November to January. Reintroduced plants grow extremely well during the wet months, their pseudobulbs look very turgid and leaves are green. However, during the dry periods, leaves tend to wither, and pseudobulbs shrink. During prolonged drought, some leaves may turn yellow or even drop off. Sometimes half of the plant may look dead. Fortunately most of these plants revive when the rains start. New shoots emerge and the plants regain their former lush appearance.

Maintenance

Our aim is to have as little maintenance as possible. Orchids planted under the right microclimate should be able to thrive by themselves like other epiphytes. Therefore very little maintenance is needed. In case of severe drought, plants can be drenched twice a week. If plants are reintroduced during the dry season, or planted at more exposed locations, it is necessary to water them at least three times a week. Once the roots of the newly planted orchids attach themselves to the tree trunk, watering can be reduced.

When dead leaves from trees fall within the cracks and crevices of the old tree trunk, they decay and the resulting humus not only holds water but also provide nutrients for the epiphytes. The reintroduced orchids should be able to thrive like other epiphytes without the application of fertilisers. In order to help the seedlings to establish faster, a light, balanced foliar fertiliser can be applied one month after planting and for a period of six months after that.

Our hope is that the reintroduced species will act as catalysts in the restoration of at least part of the original ecosystem. For example, pollinators may be attracted to come back to pollinate the flowers.

Orchid seeds that are formed naturally after flowers are pollinated may be blown to the proper environment where appropriate mycorrhizal fungi are present. And we hope that one day, we will be able to see natural populations of native species sprouting up all over the island!

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The mechanism of the biological clock that controls animal development

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ABSTRACT

In mouse development, many biological events sequentially occur in a strict time schedule. However, the mechanism of biological clocks remains to be elucidated. Somite formation is a prominent process under strict temporal regulation in mouse embryogenesis. Somites are formed every 2 hours in mouse by budding off the unsegmented presomitic mesoderm (PSM) in an anterior to posterior direction. This temporal periodicity of the segmentation is translated into the repeated pattern of the even-grained somites, which serve as repeated structures such as vertebrae, ribs and muscles. The periodicity of somite formation is governed by a molecular oscillator termed the segmentation clock. Several genes including *Hes7*, which encodes a negative transcription factor, display oscillatory expression in a 2-hour cycle corresponding to the somite segmentation. In the PSM cells, Hes7 establishes a negative feedback loop that serves as a major mechanism of the cyclical gene expression, which controls periodical somite segmentation as a biological clock. We generated the mutant mice that have Hes7 with a longer half-life, and demonstrated that instability of Hes7 protein is crucial for sustained oscillation of gene expression and periodic somite segmentation. Thus, the segmentation clock generates metameric patterns in mouse development using cyclical gene expression, which depends on the negative feedback loop of Hes7 and rapid degradation of Hes7 protein in the PSM cells.

Introduction

Several medicaments possibly induce malformation of embryo if the mother takes them during pregnant period. For example, Valproic acid (VPA) is a widely used anti-epileptic agent, but its prenatal exposure occasionally induces developmental abnormalities (Ornoy, 2009). The sensitivity to teratogenic reagents, including VPA, often depends on the genetic background or individuals (Tyl *et al.*, 2007). One interpretation of this variability is that the developmental system possesses mechanisms to endure the chemical perturbation and that these mechanisms have variability among genetic background or

individuals. The axial skeleton is one of the most routinely examined developmental patterning that might be influenced by the prenatal perturbation and noise brought by teratogenic reagents. Therefore, we tried to seek the mechanism that gives robustness to developmental processes using formation of axial skeleton of vertebrates as a model system.

The components of axial skeleton, including vertebral bones and ribs, is derived from somites, the metameric unites in the mid-embryogenesis of vertebrates (Pourquie, 2001). Somite is formed as a transient structure that gives rise to vertebrae, ribs, their accompanying muscles and skins. In the embryos of vertebrates, somites are symmetrically arranged on the both side of the midline as even-grained epithelial spheres (Figure 1). Thus, the well-ordered structure of axial skeletons is the result of the metameric structure of somite, and the teratogenic reagents are supposed to disturb the somite formation therefore leading to anomaly of axial skeletons. The precursor tissue of somites, the presomitic mesoderm (PSM), is unsegmented mesenchymal tissue that is located at the most posterior part of embryo. PSM cells vigorously proliferate and form posterior body sequentially toward the direction from anterior to posterior. On the other side, the most anterior part of PSM cells bud off to form a pair of somite every two hours in mouse. In the other words, somites are generated by sequential and cyclic segmentation of PSM, and each species of vertebrae has characteristic periodicity: 30 minutes in zebrafish, 90 minutes in chick, 2 hours in mouse and 8 hours in human. The periodic event, somite segmentation, has been believed to be orchestrated by a biological clock, called the “somite segmentation clock” (Bessho and Kageyama, 2003; Pourquie, 2003). Thus, this periodicity is the origin of the even-grained structure of somite and well-ordered structure of the axial skeletons (Figure 1). Therefore robustness of the somite segmentation clock might be the mechanism that gives robustness to axial skeleton formation.

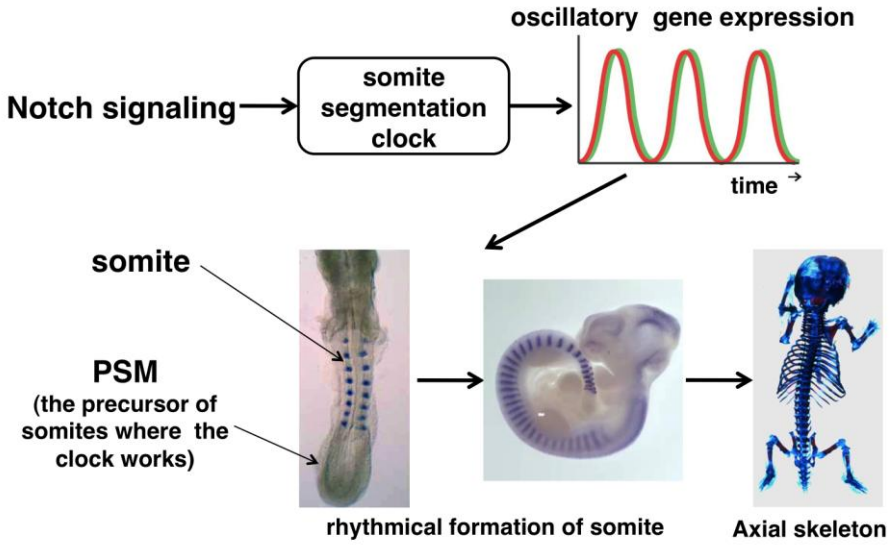

Figure 1

Figure 1. The somite segmentation clock is the underlying mechanism of well-ordered structure of the axial skeleton. Notch signaling activates several gene expression and the mechanism including several feed back loops produces the oscillatory gene expression, which works as the somite segmentation clock. The somite segmentation clock orchestrates the rhythmic somite segmentation and sequentially forms the array of somites, which give rise to the vertebrae and ribs.

In 1997, Olivier Pourquie and his collaborators made a breakthrough with regard to this somite segmentation clock (Palmeirim *et al.*, 1997). They discovered oscillatory expression of mRNA of a transcription factor gene, *hairy1* in chick PSM. Because this oscillatory expression of *hairy1* is associated with the cyclic somite segmentation, this is the first example of the molecular oscillator working in embryos, and it was believed that this molecular oscillator controls the timing of segmentation as the somite segmentation clock. Following this discovery, in addition to *hairy1*, several oscillating genes were identified, and some of them are the components of Notch signaling, which is a cell-cell contact dependent signaling pathway (Dequeant *et al.*, 2006). The transmembrane protein Notch interacts with its ligand Delta on the surface of the adjacent cell, and this binding induces limited proteolysis of Notch and the intracellular domain of Notch (NICD; activated form of Notch) translocates to the nucleus, where NICD activates transcription of several target genes (Lai, 2004). A transcription factor, *Hes7*, which is the counter part of *hairy1* in mouse, is one of a target of Notch signaling, and is specifically expressed in the PSM in a cyclic manner as

same as *hairy1* in chick (Bessho *et al.*, 2001a; Bessho *et al.*, 2001b). *Lunatic fringe* (*Lfng*) and *Notch-regulated ankyrin-repeat protein* (*Nrarp*) are target genes of Notch signaling, and displays oscillatory expression in the mouse PSM (Forsberg *et al.*, 1998; McGrew *et al.*, 1998; Aulehla and Johnson, 1999; Sewell *et al.*, 2009; Wright *et al.*, 2009). Both are also modulators of Notch signaling. Lfng reduces the production of NICD and Nrarp promotes NICD degradation, thus, both control Notch activity or amount of NICD via negative feedback loops.

Our group generated knockout mouse for *Hes7* (Bessho et al., 2001b). In the mutant embryo, both oscillatory gene expression and periodical somite segmentation is completely missing, and that the resultant axial skeleton is severely disorganized. Thus, we concluded that oscillatory gene expression works as the somite segmentation clock that controls the timing of segmentation, in which Hes7 plays an essential role. Hes7 is a basic helix-loop-helix type transcription factor that recruits co-repressors thereby acting as a transcriptional repressor. Hes7 binds to *Hes7* promoter and inhibits transcription of *Hes7*, which is activated by Notch signaling (Bessho *et al.*, 2001a). Thus, Hes7 forms a negative feedback loop by itself. After several genetic and biochemical experiments, we revealed that this negative feedback loop of Hes7 is the major mechanism for generating oscillatory gene expression (Figure 2) (Bessho *et al.*, 2003). In each PSM cell, Notch signaling activates transcription of *Hes7*, which increases Hes7 mRNA. After a while (30-60 minutes later), accumulated Hes7 protein inhibits its own transcription, thereby decreasing Hes7 mRNA. However, the half-life time of Hes7 protein is so short (20 min) that the inhibition by Hes7 ends up soon, and then *Hes7* transcription is activated by Notch signaling again. These repetitive processes could generate oscillatory gene expression. However, our group found that short half-life time of Hes7 (< 20 minutes) is essential requirement for generating oscillatory gene expression using combination of experimental and mathematical analyses; longer half-life time of Hes7 (> 30 minutes) leads to damped oscillation (Hirata *et al.*, 2004).

Transcription of other Notch target genes, *Lfng* and *Nrarp*, is also induced by Notch signaling. It is also inhibited by Hes7 protein (Bessho *et al.*, 2003; Kim *et al.*, 2011). Because *Lfng*, *Nrarp* and *Hes7* are inhibited by Hes7 protein, when it is accumulated, The three genes oscillate synchronously in the PSM. In addition, because Lfng and Nrarp are inhibitor of Notch signaling to reduce the amount of NICD, Notch activity or the amount of NICD is cyclic in the PSM. Importantly this cyclic Notch activity plays an essential role in somite segmentation (Morimoto *et al.*, 2005). Thus, the somite segmentation clock consists of several negative feedback loops, and manifests oscillation of gene expression and activity

of Notch signaling, whose periodicity controls the periodicity of segmentation, and is converted to the special periodicity of somites (Figure 2).

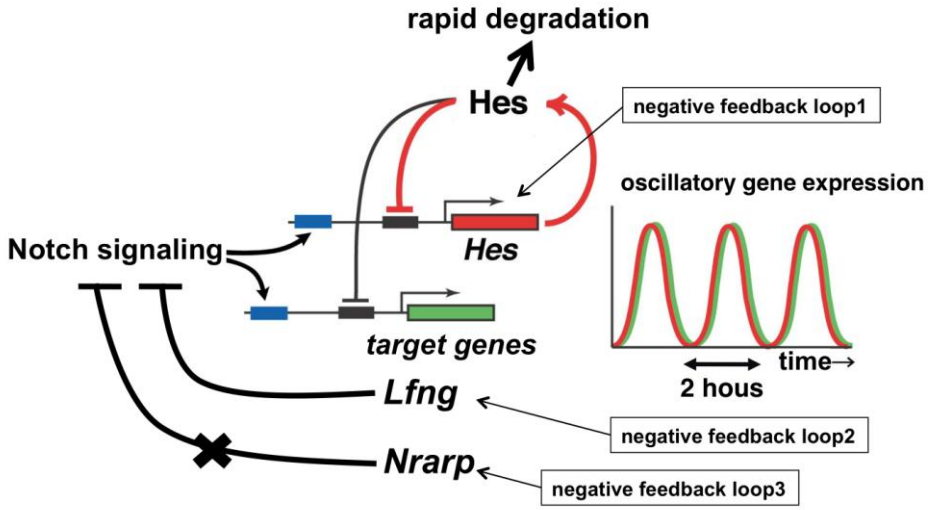

Figure 2. The molecular mechanism of the somite segmentation clock. Notch signaling activates the transcription of several genes. Accumulated Hes7 protein inhibits the transcription of those genes to provide synchronous oscillatory gene expression.

Our group generated a knockout mouse of *Nrarp*, one of the feedback inhibitor genes of Notch signaling (Kim *et al.*, 2011). Nrarp knockout mice lack two vertebrae. In most case, they have five lumber vertebrae whereas wild type mice have six, and they miss one caudal vertebra. We measured the pace of somite segmentation and found that the period of segmentation in mutant embryo is extended by five minutes. We also demonstrated that Notch activity is enhanced in the mutant PSM by two folds. Together with the results of experiment with Notch inhibitor and mathematical analyses, we concluded that the period of the somite segmentation clock is fine-tuned by Notch activity; higher Notch activity leads to a longer clock period and lower Notch activity leads to a shorter clock period. Thus, we propose that the period of the somite segmentation clock is sensitive to Notch activity.

Apart from the missing vertebrae, *Nrarp* mutant mice have slight morphological defects in axial skeleton (Kim *et al.*, 2011). We found anomalies in he shape of some vertebrae or ribs. Because Notch activity is enhanced in *Nrarp* knockout embryo, stronger

Notch activity might lead to anomaly of axial skeleton. However, because Notch inhibitor failed to rescue this phenotype of mutant embryo, it is not likely that stronger Notch activity disturbs proper formation of vertebrae and ribs. These results suggest that Nrarp *per se* is essential for proper axial skeleton morphogenesis.

According to these results, we speculated that Nrarp is involved the mechanism of robustness of the somite segmentation clock. We then established the mathematical model of the somite segmentation clock, which consists of three feedback loops of Notch signaling; one of them is the self-feedback loop of Hes7 as a key of the clock and the others are negative feedback loops of Lfng and Nrarp that modulate Notch activity by reducing the amount of NIDC (Figure 2). Then, we carried out simulation with the mathematical model with three feedback loops, and found that the model produces sustained oscillation with approximately 2-hour period, which mimics the oscillation in the PSM very well. Thus we inferred that the model well describes the somite segmentation clock. As we demonstrated by the experiments, the oscillation period was sensitive to Notch activity in this mathematical model in the wild type situation. However, it was less sensitive in the absence of Nrarp. In the wild type situation, the oscillation period was variable within the range of approximately 10 minutes, whereas it was variable within only a few minutes in the Nrarp null situation. Thus, we proposed that Nrarp provide flexibility to the somite segmentation clock.

We carried out simulation with multiple cells (five cells) and examined the ability of synchronization with each other. In the wild type situation, five cells are synchronized with each other if one of five cells was out of phase. And we speculated that the variable oscillation phase that was provided by Nrarp contributes the synchronization of oscillation. Strikingly, it took more time to recover the synchronization in the absence of Nrarp. Thus, the result of mathematical simulation suggested that the feedback loop of Nrarp is essential for synchronization of gene oscillation between neighboring cells, probably because the flexibility of the oscillation period was missing in the absence of the feedback loop of Nrarp.

Finally, we tried to perturb somite formation of the segmentation clock with teratogenic reagents *in vivo*. VPA perturbs somite segmentation clock thereby affecting the morphology of axial skeleton, if we inject VPA to a pregnant mother. We used a dose of VPA, which induce very small anomaly in the axial skeleton. Administration of VPA in this dose disorganized gene oscillation in the PSM, regular interval pattern of somites and axial skeleton formation in the *Nrarp* knockout embryo. Taken together, we concluded that the negative feedback loop of Nrarp provide a flexibility in the oscillation period to the somite segmentation click, which contributes to the mechanism of robustness of the somite

segmentation clock. This mechanism secures proper morphology of the axial skeletons and it may be one of the mechanisms that endure the chemical perturbation in animal development.

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Figures legends

Figure 1. The somite segmentation clock is the underlying mechanism of well-ordered structure of the axial skeleton. Notch signaling activates several gene expression and the mechanism including several feed back loops produces the oscillatory gene expression, which works as the somite segmentation clock. The somite segmentation clock orchestrates the rhythmic somite segmentation and sequentially forms the array of somites, which give rise to the vertebrae and ribs.

Figure 2. The molecular mechanism of the somite segmentation clock. Notch signaling activates the transcription of several genes. Accumulated Hes7 protein inhibits the transcription of those genes to provide synchronous oscillatory gene expression.

The new genomics era and tropical biodiversity management and conservation

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ABSTRACT

The most extraordinary feature of our planet is life and its diversity and it is in peril. Taxonomy is the foundation science for biology and essential for biodiversity and conservation studies. Traditional approaches to taxonomy are under threat with the "greying" of the taxonomic workforce and the decline in the teaching of taxonomy at universities. The adoption of molecular genetic approaches such as DNA barcoding to solve the "taxonomic impediment" offers potential, but has limitations of its own as it is based on information from a single gene region. Such limitation may soon disappear as we are on the verge of a new revolution of technological advances in DNA sequencing. We will soon have the capacity to sequence complete genomes for thousands of species and many individuals within populations. This genomic information will fundamentally transform current approach to phylogeny, the delineation and identification of species and the study of the amount, distribution and functional significance of genetic variation in natural populations. Much still needs to be done in the short term to fully explore the implications of this genetic data generation revolution for biodiversity and conservation and guidance is required on which genomics tools and approaches will be most appropriate to use for different aspects of biodiversity and conservation studies. There is no doubt that many of the priorities of conservation science that have been intractable through traditional genetic techniques or cost prohibitive will be within the reach of scientist throughout the world and will allow major advances for biodiversity and conservation science.

Keywords: biodiversity, conservation, taxonomy, genomics, DNA barcoding

Introduction

The most extraordinary feature of our planet is life and its prolific variety. Equally extraordinary is its current rate of decline, as rapid as any mass extinction of the planet's history and caused by us. Despite being the intelligent ape we seem powerless to stop our destruction of the natural world and its plants, animals and microorganisms in the face of population growth and the exploitation of natural resources required to support our increasingly technology dependent life styles.

Also astonishing is our capacity to understand the world we inhabit and to develop engineering and technology-based solutions to some of our major challenges. These solutions include advances in food production, transportation, communication and health care, all of which have improved, saved and extended lives through out the world.

One area of rapid technological development, which has seen especially significant growth in recent years, is the field of molecular genetics and genomics and we are now about to enter a new revolution based on new DNA sequencing technologies. This will mean

we will soon have the power to generate individual genome sequences and thereby personalise medicine. An added benefit of this revolution is that we will also be able to produce genetic and genomic data in unprecedented quantities to address fundamental issues in biodiversity and conservation science that have been either prohibitively costly or simply intractable. In this presentation I will discuss the recent advances in genomics and review how this revolution can assist scientists to understand, manage and conserve biodiversity.

Biodiversity

Biological diversity or biodiversity was first used at the National Forum on BioDiversity in 1986 (Wilson 1988) and has become both a central term in environmental discourse and an interdisciplinary science in its own right (Singh, 2002; Khuroo, 2007). The Convention on Biological Diversity (CBD) placed biodiversity, and specifically biodiversity loss on the international agenda, and defined biodiversity to include genetic variability within species and diversity between species and ecosystems (Blackmore, 2002; Khuroo 2007). In actuality it is the species level at which biodiversity is commonly conceived and mostly studied, even though it is genetic variability within species that is essential to support adaptation and evolutionary resilience of species.

A conservative estimate of biodiversity is in the order of 12.5 million species with less than 2 million having been formally recognized and described. Some taxonomists estimate less conservatively that over 100 million species exist, which aligns with molecular genetic studies that suggest that cryptic species and lineages (in the case of microorganisms) may be far more common than previously realized and raises obvious conservation issues (May 1990, Bickford et al 2007).

The foundation of biodiversity and conservation science rests on the science of taxonomy. This discipline, which comprises the discovery, description, naming and classification of living organisms, provides the essential information for the understanding and management of biodiversity on our Earth. Taxonomy, along with related areas of population biology, biogeography and phylogenetics are increasingly utilising or even becoming dependent on molecular genetic data as their primary information source. While the study of genetic variation within and between species has been greatly enhanced with the development of new molecular techniques, it is still costly and not equally available to all biologists especially in tropical countries. The challenges of adequately discovering, documenting and understanding biodiversity, especially in countries with mega-diversity in the tropics are still extremely daunting.

A major constraint to the advancement of biodiversity and conservation science is the uneven spread of taxonomic expertise both geographically and taxonomically and the rapid

decline or “greying” of the taxonomic workforce. The goal of advancing knowledge of the earth’s biodiversity and having sufficient taxonomic expertise to identify species is thus a stupendous and potentially unattainable goal based on the methods of classical taxonomy and current resources (Khuroo, 2007).

Barcoding – a solution to the taxonomic impediment?

As discussed the taxonomic impediment leading to misidentification of species and undetected cryptic species can seriously compromise biodiversity and conservation studies and is also a serious issue for ecologists (Vecchione et al., 2000; Bortolus, 2008). Bortolus (2008) reported that 62.5% of ecology papers by him did not provide any supporting information justifying or guaranteeing the correct identification of the organisms under investigation.

The challenges for biodiversity and conservation scientists and ecologists seeking verification of species identifications in the laboratory, and especially in the field, may not be trivial. Even when adequate taxonomic keys and field guides are available it is often difficult to identify organisms with confidence, as biologists can be dealing with juveniles, undocumented geographic variants, sexual dimorphism etc. Further, accurate identification might require examination of microanatomy or measurements of a complex combination of morphometric attributes. Handling, examining and measuring individuals in the field is often impractical, inappropriate for ethical reasons or simply dangerous, thus exacerbating the problem of securing accurate identifications. Even if experienced taxonomists exist for the organisms under study, it is unlikely that they will be available to assist in the field, especially in remote locations. Voucher specimens can be taken for subsequent lodgement in museums, however, this is often impractical for large species, samples obtained in remote locations and studies involving multiple species. In addition even when voucher specimens can be obtained it does not necessarily guarantee reliable and timely identification.

The DNA barcoding approach to taxonomy (Hebert et al., 2003) potentially offers scientists, who are not expert taxonomists, a powerful tool to support the efficiency and accuracy of field studies involving the challenging identification of diverse taxa. The proponents of this approach mostly advocate the use of a single gene for global bio-identification of animals based on the availability of a data base of sequences linked to voucher specimens, thus making these sequences, in effect, a DNA barcode (Hebert et al., 2003). A 650-base fragment of the cytochrome *c* oxidase I (COI, *cox1)* is proposed as a ‘global’ standard because variation in COI within species is low relative to that among species. While the DNA barcoding approach has its critics when touted as a panacea to the impediments presented by traditional taxonomy (Tautz et al., 2003 Moritz and Cicero, 2004) it does potentially provide a quick and reliable means to confirm the match of individuals to

voucher specimens and to identify groups where there is discordance in the delineation of species boundaries that require further investigation. Nevertheless, the over arching limitation of DNA barcoding is that it is based on the use on a single, relatively short DNA fragment (Moritz and Cicero 2004).

The new genomics era

The world has already seen a revolution in genomics and our capacity to generate molecular genetic data is already extraordinary. The world is now about to have another genomics revolution. To understand the explosive pace of development in genomics and the under pinning technologies development behind the acquisition of molecular genetic information it is useful to start with the Human Genome Project (HGP) which was officially founded in 1990. From inception it took the HGP 10 years to produce the first draft of the human genome at a cost of $3 billion dollars - $1 per nucleotide (down from $10/nucleotide in the 1980s). In addition to making a giant step forward in the genetic knowledge of ourselves it stimulate the technology that has made direct sequencing of gene fragments routine with the costs of conventional sequencing now being close to $0.01 per nucleotide. Today the cost of a whole genome sequence is now in the order of $ 15-20 K with multiple coverage (x25) and it takes as little as 2 days to generate the gene sequences.

A natural extension of the Human Genome Project and the rapid increase in efficiency in sequencing is the study of human geographic and population genetic variation. The 1000 Genomes Project Consortium has already produced map of human genome variation from population-scale sequencing (Durbin, 2010) the project is already moving onto the 2000 human genome project. The next evolutionary step in sequencing will be the introduction of third or “next next gen” sequencing which will go even further by offering the promise of $1000 human genomes generated in a matter of minutes at a cost of $US0.0000005 per nucleotide and thus making the prospect of personalised medicine almost within reach (Schuster, 2008).

Animal and plant genomes can be generally sequenced for a similar cost to humans, however for non-model organism without a reference sequence from a close relative the data generation requirements are higher and the analysis or bioinfomatics component may be 10 x the cost of generating the data. Nevertheless some very ambitious projects have been launched including the Beijing Genomics Institute’s (BGI) One Thousand Plant & Animal Reference Genomes Project announced in January 2010, which called for collaboration from around the world. The goal of the project is to generate reference genomes for 1,000 economically and scientifically important plant and animal species. Together with their collaborators, BGI have so far initiated 505 plant and animal genome projects, completed genome maps for over 100 species and sequenced about 200 species.

The completed projects include a range of species including rice, silkworm, cucumber, panda, camel, oyster, ant, grouper, goose, crested ibis, potato genomes to name but a few (Beijing Genomics Institute, 2011). It is not far-fetched to envisage the initiation of the10,000 and the 100,000 animal and plant genome projects over the next few years with the anticipated further giant leaps forward in nucleotide generation.

While the prospect of inexpensive whole genomes for species and population characterization is tantalizing for biologists, the analysis of 1000s of genes and millions of nucleotides is still prohibitively expensive for most laboratories in both developed countries and emerging economies. Also, it is not necessary for most applications in routine biodiversity studies and conservation genetic application. Nevertheless the new technologies offer opportunities to undertake better and new kinds of studies in biodiversity and conservation science.

Genetics and biodiversity and conservation studies

The measurement of genetic diversity, predominantly at neutral genetic markers, in species, populations and individuals is generally accepted as a fundamental tool in modern biodiversity and conservation and management. Measures of genetic variability, interpreted in the context of population genetic and evolutionary theory enables the identification cryptic taxa, estimation of population genetic subdivision, quantification of inbreeding and genetic relatedness at the population and individual level, provides individual identity, establishes parentage and individual reproductive success. At the landscape scale methods now exist that allow the identification with precision population boundaries (management units) and fundamental aspects of population biology, which are intractable by any other means (mating systems, behaviour, dispersal, effective population size and population trajectories).

Modern genetics also allows the study of evolutionary history in geographic space within species (phylogeography) and at deeper levels among taxa (phylogeny). The study of how evolutionary lineages are distributed in space allows the analysis of areas harbouring endemism and identification of regions with evolutionary significant diversity, which allows conservation priority setting at larger geographic scales.

New genomic methods are allowing the development of new approaches to provide rapid, low cost genetic data acquisition and analysis that makes the process of acquiring genetic diversity data across a variety of hierarchies including, communities, species, populations, and individuals, possible. These new methods will allow the extension of these traditional approaches by increasing our understanding of the functional significance of genetic variation in natural populations by allowing the identification of genes influencing adaptive evolution (fitness-related) and investigation of the impacts of inbreeding depression. This offers the exciting prospect of being able to analyse suites of markers of

functional significance for endangered species that will be relevant to adaption and persistence in the context of environmental change (thermoregulation, water balance, exotic species) and health and fitness more generally (i.e. protection against disease and parasites) (Allendorf et al., 2010).

An understanding of the variety of approaches to genetic data acquisition is essential for applying genomic approaches effectively to biodiversity questions and conservation. There is an ever-growing list of methods of varying complexity for determining DNA sequence differences among individuals and species and these vary in the distribution and concentration of genetic markers across the genome, their ability to target specific loci and the cost of genotyping (Allendorf et al., 2010). Genomic techniques can be placed into three groups: marker-based genotyping, including a diversity of array-based SNP genotyping platforms; partial sequencing, which uses next-generation sequencing technology to target a subset of orthologous regions across the genome; and whole-of-genome sequencing (Allendorf et al., 2010). A major challenge for the application of most genomic techniques will be nucleotide data management or bioinformatics. The methods and software for handling genomic data sets are evolving rapidly in parallel with the technological developments, but still require a substantial investment of time and resources (Allendorf et al., 2010).

An important aspect of the genomic approach is that it does not require existing genetic information and can utilise very small and degraded samples. A small partial genome scan can yield an abundance of data. My laboratory has use 1/16 genome scans costing $1,500 that generates enough data to allow the identification of 100+ microsatellite loci and to construct whole or partial mtDNA genomes for species as diverse as sting rays, ants, crayfish and copepods. Having a large number of microsatellite loci to choose from means that it is possible to optimise the choice of loci for multiplexed PCR and thereby greatly reducing the cost of genotyping. As a single multiplexed reaction consisting of 10-15 loci can cost as little as $2.00 to run on a capillary-based genetic analyser this means a single population sample of 50 individuals can be genotyped routinely for approximately $100.

Despite these new and exciting genomic-based projects, conventional genetic approached are unlikely to be superseded in the short term and in fact can be further enhanced when used in conjunction with the newer techniques. A recurring challenge for biodiversity and conservation studies is that they often target little studied species for which there is limited existing genetic information. This either makes it impractical to gather genetic data or necessitates a long and often tedious and expensive process of marker development (Guichoux, 2011). The selective use of new genomics approaches will relegate these kinds of technical challenges to history.

Conclusions

Much needs to be done to fully explore the implications of this genetic data generation revolution for biodiversity and conservation and guidance is needed on which genomics tools and approaches will be most appropriate to use for different aspects of biodiversity and conservation studies. There is no doubt that many of the priorities of conservation science that have been intractable through traditional genetic techniques or cost prohibitive will increasingly be within the reach of scientist through out the world and will provide major advance for biodiversity and conservation science. A very important implication is that the cost of gathering genomic data is in rapid decline making it more practical for scientist in countries with emerging economies, especially in the tropics, to tackle important biodiversity and conservation questions either within their own country or in partnerships with international colleagues with access to the necessary laboratory infrastructure and instrumentation.

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Systematics and Evolution: A view of Microbial Systematist*)

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ABSTRACT

Study of diversity and their relationship has been very basic discipline in biology since it is a pioneering field in effort to further understand the realm of biology. Such study is called systematics which its main endeavour is to understand the magnitude of diversity of living being by employing its subdisciplines of classification, nomenclature, and identification. The pioneering nature of the systematics means that, the achievement of its study will pave the way for further study by other subdisciplines in biology suchs as genetics, physiology, ecology, and biochemistry of organisms. The object of systematics is organism's diversity while organism's diversity is in turn also viewed as a product of evolution, and consequently, the study of diversity must be significantly influenced by development of evolutionary theory. Classification for instance has been influenced by evolutionary concept since its birth as science within the realm of biology. This paper will described and discuss the essence of systematics as well as its paramount important roles in undertanding the magnitude of biodiversity as a product od evolutionary process. Subdisciplines of systematics is described and discussed by objective exemplification. Biological classification is discussed critically and the school of thoughts in classification (phenetics, cladistics, and evolutionary taxonomy) is described and analysed within the perspective of evolutionary theory. The essence and use of nomenclature and identification within the study of systematics will also be discussed accordingly. Finally, the impact of science and technology development as well as the use of evolutionary theory in systematics will be exemplified throughout the history of living things classification..

A. Introduction

Systematics has been defined in several different ways despite all of definitions still clearly refer to almost similar meaning, namely pertaining to the study of biodiversity and their relationships. For instance, just to quote several examples, in terms of simple and general meaning, **systematics** is defined to be "*the study of the diversity and relationship among organisms*" (Priest & Austin, 1995), "*the study of the diversity of organisms and their relationships*" (Madigan *et al*., 2012), "*the scientific study of the kinds and diversity of organisms and of any and all relationship among them*" (Goodfellow & O'Donnell, 1993), "*the scientific study the organisms with the ultimate object of characterizing and arranging them in an orderly manner*" (Willey *et al*., 2009). However, according to *Merriam Webster's Collegiate Dictionary*10th edition (Anonym, 1993) "*systematics is the classification and study of organisms with regard to their natural relationshps*". Furthermore, in *Dictionary of Biological Terms* 11th edition (Lawrence, 1997), systematics is defined to be *"the study of the identification, taxonomy, and nomenclature of organisms, including the classification of living things with regard to their natural relationships and the study of variation and the evolution of taxa*". Therefore, it seems that as Minelli (1993) stated "*there is no general aggreement as to the definition of systematics*".

Despite the fact that there seem to be no general acceptance on exact definitions of systematics among scientists, according to Myer and Ashlock (1991) "*systematics stems from the latinized Greek word* ***systema*** *as applied to the systems of classification developed by the early naturalist, notably Linnaeus*". Moreover, it can also be traced back to that one of more frequently quoted definition of systematics provided by Simpson (1961) *cit*. Minelli (1993) that is "*systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them*" which has been simplified by Myer and Ashlock (1991) to be only" *systematics is the science of the diversity of organisms*". Hence, based on Simpson's definition it can be described that systematics is the branch of biology which deal with the scientific study of organisms' diversity and their relationships including phenetic, and phylogenetic relationships since it includes any and all relationships.

According to Goodfellow and O'Donnell (1993) "*systematics is a fundamental discipline that embraces classification, nomenclature and identification, and analyses of phylogeny, evolutionary process, and genetic mechanisms*". This is in agreements with with the description provided by Cowan (1978). Therefore, it can be concluded that in order to study diversity and relationships among organisms, systematics applys the three interelated subdisciplines, namely classification, nomenclature, and identification.

One of among products of systematic studys is classification system of living things. The history of development of organismal classification system clearly showed that it strongly influenced by the development of concept in sciene and technology. For instance, the publication of *The Origin of Species* (Darwin, 1859) greatly influneced the view on how to classify organisms based on new evolutionary concept. This is because "*in his book, Darwin developed two main ideas: that descent with modification explains life's unity and diversity and natural selection brings about the match between organisms and their environments* (Campbell *et al*., 2008). Indeed, evolution is inevitably closely related to the work of systematics due to the fact that systematics deals with the diversity and relationships among organisms, including phylogenetic relationships based on the prevail theory of evolutionary biology. At the end, as stated by Stearns and Hoekstra (2000) "*the goal of systematics is to discover the structure of the evolutionary relationships among organisms that result from their having common ancestor*". Therefore, evolutionary biology theory could be seen as a paramount important back ground of any systematic study of biodiversity and even is viewed as unifying concept in realm of biology.

This paper will describe and discuss the important role of systematics in the study of biodiversity and how evolutionary theory has shaped view on how to classify organisms demonstrated by the history of organismal classification system development from the priod of Aristoteles to the nowdays era of molecular biology.

B. Classification

Classification is one of subdisciplines used by Systematics in order to study diversity of organisms. According to Myer and Ashlock (1991) "*the traditional definition of classification is the grouping of objects into classis owing to their shared possession of attributes*". However, some experts, have interchangebly use classification and taxonomy in identical sense. In fact, the original meaning of taxonomy provided by Simpson (1961) *cit* Goodfellow and O'Donnell (1993), "*is the theoretical study of classification, including its bases, principles, procedures, and rules*". Therefore, classification could be viewed as practical aspect of taxonomy. Furthermore, in more elaborate way, classification can be further defined as "*the process of ordering organisms into groups (taxa) on the basis of their relationsips in order to produce orderly arrangement or system of classification designed to express interrelationships of organisms and to serve as an information storage and retrieval system*" (Goodfellow & O'Donnell, 1993). Therefore, classificataion or practical aspect of taxonomy will certainly generate a classification system of organisms. However, the product of classification will much depend on the set purpose of classification as well as.prevail background theory in use

Based on the purpose of classification, it could be grouped into (i) artificial classication and (ii) natural classification. The example of **artificial classification** could be found in a more applicative aspect of biology such as agriculture, medicine, or industry. For instance, plants could be classified into *edible* and *unedible* plants, or pathogenic and non-pathogenic microorganisms, as well as industrially important and industrially un-important plants. **Natural classification** is based on many characters and therefore showing overall resemblance (Goofellow & O'Donnell, 1993), and this classification could be further devided into (i) phenetic and (ii) phylogenetic classication.

On the basis of number of characters used, classification can also be devided into (i) monothetic classification, and (ii) polythetic classification. Monothetic classification "*is based on unique set of features considered to be both sufficient and necessary for the group so defined*" *and in practice, the overreliance placed on small numbers of subjectively chosen morphological and physiological properties led to serious missclassification*" (Goodfellow & O'Donnell, 1993). Therefore, the clasification system resulted from such method will be very subjective and hence has limited use in identification due to lacking of information (data) being used. Whereas polythetic classification is based on as many character as possible in order to show the overall resemblance *e.g*. numerical-phenetic classification.

B.1. Classification and evolutionary theory

Although evolutionary theory is not only restricted to Darwinian one because before him, there had been several experts who also tried to explain the existence of life diversity

such as Aristotle (384 – 322 B.C.) with his natural affinities among organisms, Leonardo da Vinci (1452 – 1519) who explained that fossil is remain of extinct organisms, and Jean Baptiste de Lamarck (1744 – 1829) with his *Philosophie Zoologique* (Solomon *et al*., 2008). However, it must be akcknowledged that Evolutionary theory proposed by Darwin through the publication of his book titled *The Origin of Species by Means of Natural Selection* (1859), has been so influential and powerfull to change dramatically the view on how to explain the diversity of life on the planet Earth could become as we witness today.

The essence of Darwinian evolutionary theory is that the process of evolution of organisms happens *via* a mechanism of natural selection that he termed with descent with modification. Subsequently, the ideas of natural selection as mechanism of evolution could be summerized (Campbell *et al*., 2008) *as " (i) natural selection is a process in which individuals that have certain heritable characteristics survive and reproduce at a higher rate than other individuals (ii) Over time, natural selection can increase the match between organisms and their environment (iii) If an environment cganges, or if individuals move to a new environmen, natural selection may result in adaptation to this new conditions, sometimes giving rise to ne spcies in the process".*

The publication of *The Origin of Species* (Darwin, 1859) did not directly followed by the practice of classification with a specific method in order to determine common descent. Only after Simpson (1945) *cit*. Myer and Ashlock (1993) developed " *a well-thought-out statements of the principles of traditional evolutionary taxonomy and he followed this up in 1961 with a comprehensive treatment*" that the concept of Darwinian evolutionary theory was really put into practice of classification. From there on, classification has much been further supported by molecular biology by using DNA sequence analysis along with molecular evolution method with several different evolutionary algorithms. The revolution of molecular biology together with molecular (population) genetics, and molecular evolution has historically helped the birth of molecular systematics (Hilis *et al*.,1996) which concerns with molecular phylogenetic analysis of life diversity. The development in concept and method of analysis has provoked the revolution of classification of living things. The impact of evolutionary view on classification could be clearly followed along the history of the development of living things classification.

B.2. Three school of thoughts in classification

The essence of practicing Darwinian theory in classification of organisms is how to generate classification system which reflect relationships in terms of similarity and descent because in practice, both of the two criteria of relationships are frequently in conflict (Myer & Ashlock (1993). Furthermore, with regard to evolutionary theory, three schools of thought of classification had been developed based on the priority given to each of the criteria,

namely (i) "***Phenetics,*** *which gives primacy to similarity (ii)* ***Cladistics****, which gives primacy to the branching points of descent, and (iii)* ***Evolutionary taxonomy****, which consider the two sets of criteria equally but sequentially, that is taxa privisionally delimited by similarity and subsequently tested by monophyly*" (Myer & Ashlock (1993). The healthy debate among those three different school of thoughts in classification is continuing although there has also been moves to adopt a polyphasic approach since it was declared by Rita Colwell (1970).

In microbiology, the polyphasic systematic approach has been practiced so far by combining the independent approach of numerical-phenetic, chemosystematics, as well as molecular systematics in order to achieve sounding classification. Congruence among the three independent approaches is viewed as a good basis to generate the rigorous and robust classification system as an excellent basis for devising a useful identification schemes.

C. Nomenclature

This is another subdiscipline of systematics which also responsible to support the study of diversity and relationship of organisms. As Goodfellow and O'Donnell (1993) stated that "*Nomenclature deals with the terms use to denote taxonomic categories, e.g. species, genus, and family, the relaive rank of such catagories, and the process of allocating correct, internationally recognized names to organisms*" . The scientific names of organisms are regulated internationally. Bacterial and Archeal scientific names are regulated by the *International Code of Bacterial Nomenclature* (Sneath, 1992), Plants and Fungal nomenclature are regulated by the *International Code of Botanical Nomenclature* (Greuter *et al*., 1994), and Animal and Protozoa nomenclature are regulated by the *International Code of Zoological Nomenclature* (Ride *et al*., 1985).There is also effort made to unify the three nomenclatual codes as Goddfellow (2000) stated that "*there are moves to produce a Universal code of Nomenclature*" although it seems that there is still time to wait for unification of the Code.

The importance of universal code of nomenclature is undiniable due to its use for internationall communication purpose. Therefore, it has been agreed by each code that the scientific names for organisms are given in Latin or latinized words and written in *binomial* fashion such as *Bacillus anthracis.* The first representing the genus name, *e.g. Mangifera,* and the second consisting the species identity (specific epithet), *e.g. indica.* In this example, the binomial name is *Mangifera indica.* The choice of Latin words as MacDoo (1993) stated "*provides very great importance for two reasons (i) the use of Latin facilitated international uniformity in nomenclature without offence to any one's national pride (ii) it*

guaranteed continuing precision of application unaffected by popular inaccuracy, since no country's population use latin as the language of every day discourse".

The universal aspect of scientific names clearly benefits all scientists who intend to communicate any orgnism's name across the world and prevent misscommunication among parties. International communication among parties would have been impossible if such regulation had not been carried out. Instead of using *banana* (English) equivalent to *pisang* (Indonsian) the scientific name of *Musa paradisiaca* could be used to facilitate universal communication. The same case will also follows for naming a *grass carp* fish (English) equivalent to *ikan mas* (Indonesian) and this could be solved by using scientific name of *Cyprinus carpio.*

However, as Goodfellow (2000) stated that *it is important to recognize that the nomenclature of a group of organisms does not depend on the correct latinization of words, but on the the thoroughness of the preceding taxonomic work becouse when (micro)organisms have been rigorously characterised and classified it is relatively simple matter to apply the rules of nomenclature. Once the name for a taxon has been chosen, the next step is to publish it. The paper submitted for publication should give the derivation of the new name and the name itself should carry the relevant indiction of novelty (e.g. sp. nov., gen nov.*" For example, there were six novel species of Indonesian streptomycetes validly published in the *International Journal of Systematics and Evolutionary Microbiology* (Sembiring *et al*., 2000), namely *Streptomyces asiaticus* sp. nov.DSM 41761^T Sembiring *et al*., 2000, *S cangkringensis* sp. nov. DSM 41769^T Sembiring *et al*., 2000, *S. indonesiesnsis* sp. nov. DSM 41759^T Sembiring *et al*., 2000, *S. javensis* sp. nov DSM 41764^T Sembiring *et al*., 2000. *S. rhizosphaerius* sp. nov. DSM 41760^T Sembiring *et al*., 2000, and *S. yogyakartensis* sp. nov. DSM 41766^T Sembiring *et al*., 2000. The application International nomenclature could in fact be achieved once a thorough characterization has been carried out properly to generate a reasonable basis for proposing of the new species names through international publication.

D. Identification

The last sub-discipline used by systematics to study diversity and relationships of organisms is identification. Goodfellow and Priest (2000) provide that "*identification covers both the act and result of determining whether an unknown organism belongs to a particular group in a previously made classification. It involves determining the key characters of the unknown organism and mathing of these against databases containing information on validly described taxa*". Therefore, "*it is crucially to understand that organism could only be identified if classification has been a preceeding step and the resultant taxa given names or*

codes, but if the organism respresent an undescribed taxon, then it cannot be identified" (Priest & Austin, 1995).

In terms of plant or animal specimens, the identification can be carried out by matching the key characters of the specimens against databases containing information in form of identification keys. However, for microbial strains identification must be performed by matching the key characters of the strain in question with key characters of live reference strain obtained from culture collection or from somewhere else.

Thus, identification is the product of systematics which is mostly useful for anyone who intend to guarantee the identity of organism s(he) work with. Irresspectively of his/her scientific background, s(he) needs to achieve the definite identity of organism in question let alone if it is being communicated scientifically. Therefore, the basic understanding of identification strategy is required for any biologist to be able to solve his/her own problem of identification. Although, they may sometime be helped by institutional identification services such as, *Herbarium* for plant speciemens, *Zoological Museum* for animal specimens, and *Culture Collection* for microbial strains.

Based on the description above, classification and identification is much more dependent on database and therefore they are continuously developed along with the application of new taxonomic concept and methods (Goodfellow & Priest, 2000). It means that the identification scheme will always be based on the preceeding resulted classification system. It also follows that the robustness of any identification scheme entirely dependent on classification system to which its development has been based.

E. Chronological development of Living Things Classification

Classification system developed by Linaeus (1753) grouped all organism only into two Kingdoms, namely Kingdom *Plantae* (Plants, Algae, Fungi, Bacteria, and of course including Archaea) and Kindom *Animalia* (Animal dan Protozoa). Subsequently, the development of reseach instruments, especially microscope, a microorganism (*Euglena*) was found. This organism was not fit to be classified into either Kingdoms, and therefore Haeckel (1866) proposed the three-kingdom system, those were Kingdom *Plantae,* Kingdom *Animalia*, and Kingdom *Protista*. The last kingdom was created to accommodate organisms which were not fit for another two kingdoms.

Furthermore, as a result of electron microscope development, it was known that in fact internal structure of cell showed that cells of plants, animal, algae, fungi, and protozoa possess membrane-bound organell but bacteria and archaea were found to be lacking of it. Therefore, Chatton (1937) termed the first group of cells to be *eukaryotic*, and the second group of cell to be *prokaryotic*. Subsequently, such dichotomous classification system was accepted and used by most of biologists, and eventually dogmatically treated as

fundamentally and evolutionarily correct. Let alone that electron microscopical and biochemical studies also strongly supported the existense of eukaryotic and prokaryotic cells, and therefore inspired the experts to devise a new classification system based on those state of the art.

One of new classification system devised on the basis of the dichotomous paradigm of prokaryotic-eukaryotic was poposed by R.H. Whittaker (1969) who groupped organisms into five Kingdoms of life. In this system of classification, Fungi (including mushroom, molds, and yeasts) were excluded from Kingdom *Plantae* to be independet Kingdom as Kingdom *Fungi*. The exclusion of Fungi from Kingdom *Plantae* was based on the facts that fungi are non photosynthetic, heterotrophic, cell wall consists of chitin, their body structure, as well as their nature of reproduction. Furthermore, Kingdom *Monera* was created to accommodate bacteria and archaea which were regarded as fundamentally different due to their nature of prokaryotic. Therefore, Whittaker (1969) proposed a five kingdom system consisting of ***Monera, Protista, Fungi, Palntae***, and ***Animalia*** which was based only on three main characteristics, namely (i) internal structure organization of cells (ii) cellular organization, and (iii) nutritional type.

Further development continued since the finding of double-helix structure of DNA molecule by Watson and Crick (1953) enhanced the bitrh of molecular biology which in turns strongly infulenced view of biologists on life itself. During 1970s, Carl Woese of University of Illinois (USA) pioneered the application of molecular biological techniques, that was sequence analysis of small sub unit rRNA (SSU-rRNA) to study phylogenetic relationship of living organisms. He used variational analysis of the universal molecules (rRNA) to challenge the old dogma that hold all prokaryotes are closely related and very similar one among theother. However, based on the result of rRNA molecule variations analysis, Woese (1987) proposed that indeed prokaryotic organisms (Kingdom *Monera*) consisting of two groups of fundamentally different organisms, namely *Archaea* (formerly called Archaebacteria) and *Bacteria* (formerly called *Eubacteria*). Therefore, based on rRNA sequence analysis, it was proposed that prokaryote covers two among three of evolutionary lines, those are Archaea, Bacteria, and Eukarya (Woese *et al*., 1990).

The proposal of three domain of life by Woese *et al*. (1990) was subsequently getting support from Carol J. Bult and collegues (1996) who reported that genome sequence similarity between an archean (*Methanococcus janashii*) and bacteria (Domain *Bactria*) was only less than 50%. Based on this molecular biological data and further studies, eventually most of nowdays biologists come to agreement that prokaryote is diveded into domain Archaea and. Bacteria.

Based on fundamental molecular difference between archaea, bacteria, and eukarya, most of nowdays systematists, especially microbial systematists use a taxon hierarchi above

Kingdom, that is *Domain*. Thus, living things is now classified into three Domain, namely Domain *Archaea, Bacteria*, and *Eukarya*. Results of rRNA sequence analysis showed that member of Archaea possess genes which are combination between genes which are very similar to bacterial genes and eukaryotic genes. From further molecular biological studies it showed that Domain *Archaea* even more closely related with Domain *Eukarya* compared to Domain *Bacteria*.

Consequently, on the basis of molecular biology data, within the prespective of application of evolutionary concept, most biologists accepted the classification of Three Domain and 6-Kingdom System of life. The universal acceptance of such system has been clearly demonstrated by the fact that many biology text books, *e.g*. *Biology* by Solomon *et al*. (1995-2008), *Biology* by Campbell *et al*. (2008), and Biology by Hoefnagels (2008), and most of all microbiology text books, *e.g*. *Microbiology* by Prescott *et al*. (1997-2009), *Brock Biology of Microorganisms* (2000 -2012), *Microbiology* by Tortora *et al*.(2007) have totally adopted the Three Domain-Six Kingdom System, those are Kingdom *Archaea, Bacteria, Protista, Fungi, Plantae,* and *Animalia*. All of domains and 4 Kingdoms among which containing microorganisms, namely Kingdom *Archaea, Bacteria, Protista,* and *Fungi*

According to further analysis of molecular phylogenetic systematics, Kingdom *Archaea* has been classified further into 4 Phyla, namely Phylum *Crenarcheota, Euryarcheota, Korarcheota,* and *Nanoarcheota*. And based on phylogenetic classification, Kingdom *Bacteria* consisting of 23 Phyla (Prescot *et al*., 2009). Finally, it can also be added that the structural and molecular (*molecular signature*) differences amongest the three Domains strongly supported the existence of Domain Archaea, Bacteria, and Eukarya (Atlas, 1997).

F. Concluding remarks

Systematics is a basic subdiscipline in biology which deal with the scientific study of organisms as well as relationship among them, including phenetic as well as phylogenetic relationship. Since the object of systematics is living organisms which based on modern concept of biology is a product of evolutionary process, the study of diversity must also be equipped with evolutionary concept in order to understand the nature of diversity itself. In practicing the diversity study, systematics make use of its three interelated sub-disciplis, namely classification, nomenclature, and identification. Classification is also much influenced by kind of data as well as method of data analysis. Adopting the evolutionary theory in classification means that the best classification to be achieved will be one that could reflect relationships in terms of similarity and descent. Based on this perspective, there have been developing three different school of thoughts within the realm of classification, namely (i) phenetics, which gives primacy to similarity (ii) cladistics, which gives primacy to the branching points of descent, and (iii) evolutionary taxonomy, which consider the two sets of

criteria equally but sequentially, that is taxa privisionally delimited by similarity and subsequently tested by monophyly. The debate among the three schools has been continuing in order to sort out better approach in classification. The impact of evolutionary theory on systematics is very significant since the study of diversity is strongly influenced by the development of concept and method of data analysis. Along the history of living things classification it has been clearly demonstrated the impact of science and technology development as well as development of concept and method of data analysis including molecular evolutionary concept and molecular data anlytical methods.

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A genetic framework for flower initiation

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The transition from vegetative to reproductive growth is the most dramatic phase change in the life of flowering plants. This developmental switch responds to various environmental and endogenous signals and results in the generation of flowers, which bear reproductive organs for seed production. In the last two decades, intensive investigations have progressively unraveled the underlying mechanisms of flower initiation in the model plant Arabidopsis. Our recent studies suggest that several MADS-box transcription factors play key roles in mediating the successive changes of flower initiation, including flowering time control, floral meristem specification and floral organ patterning. This talk will focus on the regulation of flower initiation by these MADS-box transcription factors.

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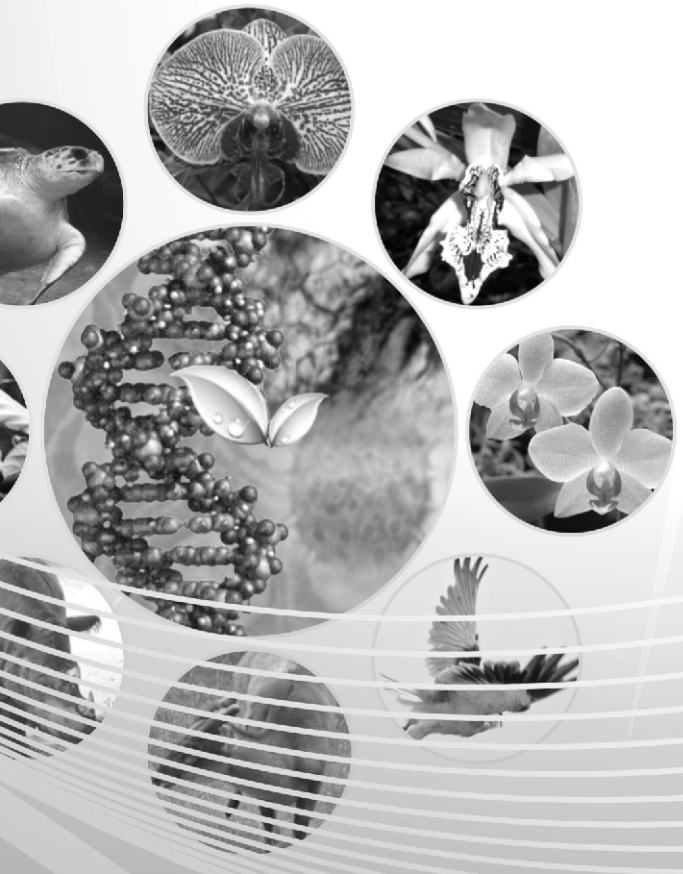

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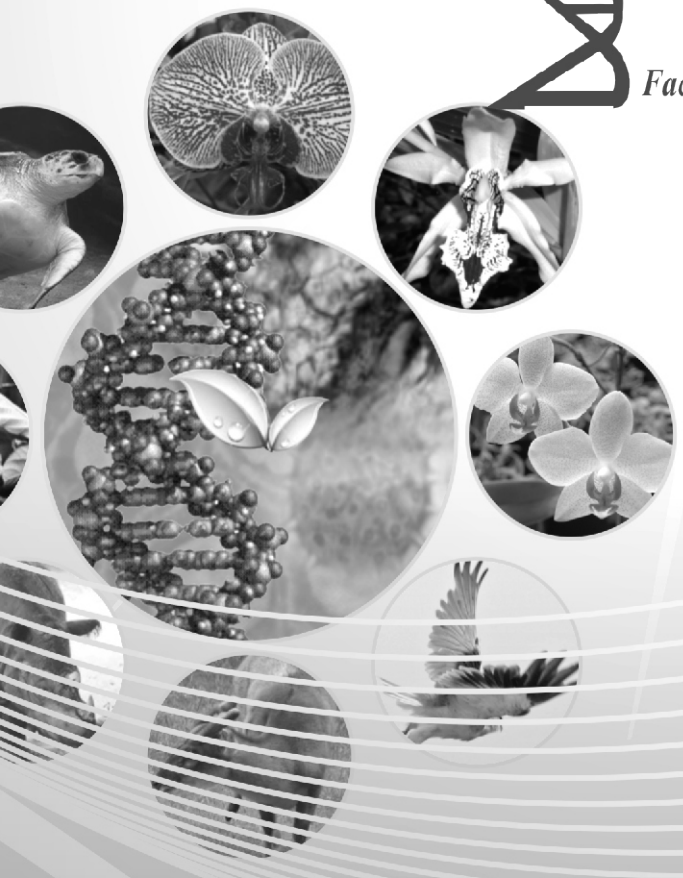

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O-MB01

The Continous Function of *KNAT1* gene on Secondary Shoot Growth in Micropropagation of Indonesian Black Orchid *Coelogyne pandurata* Lindley Transgenic

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Abstract

Agrobacterium-mediated genetic transformation has become increasingly important tools for improving cultivars and studying gene function in plants. This is particularly true in orchids, which are highly valued ornamental plants that are continually being genetically altered. To improve the quality of Indonesian black orchids, we developed a convenient method for the genetic modification of this orchid using *Agrobacterium tumefaciens*. The T-DNA of a disarmed Ti plasmid containing the coding region of a neomycin phosphotransferase II gene as a selectable marker was successfully introduced into intact protocorms of the Black Orchid (*Coelogyne pandurata* L. Form East Kalimantan). The *BREVIPEDICELLUS (BP)/KNAT1* gene was under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter, and is a member of the family of class 1 *KNOTTED*-like homeobox (*KNOX)* genes in *Arabidopsis thaliana* that is required for the maintenance of indeterminate state of cells. the T-DNA containing *BP/KNAT1 was* transformed into the black orchid. The protocorms that were transformed with *BP/KNAT1* produced multiple shoots, indicating that the *BP/KNAT1* gene can be used to improve shoot formation for mass propagation of these orchids. *In vitro* culture using leaf discs of the 35S::KNAT1 transgenic Black orchid on hormon-free medium also resulted in multishoots production. These data indicate that the *KNAT1* gene maintained its function in secondary shoot growth of transgenic black orchid. The method can be applied to the commercial production of orchids in Indonesia for both domestic and international trade.

Keywords: Black orchids, secondary shoot induction, genetic transformation, *Agrobacterium tumefaciens.*

Introduction

Techniques on plant tissue culture for orchid micropropagation are useful for mass production. Since the needs of orchids are always increase by the time for commercial trades, the conservation efforts should also be elaborated. Mass propagation through *in vitro* culture will become a good tool for these efforts. But, in orchids, there are many obstacles to do tissue culture, due to the slow growth rate and the long life cycle of orchid. Recently, we developed an efficient technique for orchid micropropagation through Agrobacterium–mediated genetic transformation of *Knotted1-like Arabidopsis thaliana* (*KNAT1*) gene into genomes of three genera of Indonesian orchids, i.e *Phalaenopsis amabilis* (L.) Blume, *Vanda tricolor* Lindley and *Coelogyne pandurata* Lindley (1, 2).. The insersion of *KNAT1*

gene into orchid genome resulted in multishoot formation in *P. amabilis* and *C. pandurata*, but not in *V. tricolor.* In *P. amabilis*, there was 31-90 shoots emerged from one protocorm (developing orchid embryo), and in *C. pandurata* there was 4-7 shoots emerged from one transformant's protocorm. There is still a question to be addressed: is there any stable orchid transformant that maintained the function of interest gene in their secondary growth?

In this report, we analyze the continous function of *KNAT1* transgene on secondary growth of black orchid transformant's shoots in tissue culture condition, to understand the stability of *KNAT1* as a foreign gene in orchid genome. It is worth to elaborate for this orchid due to the rareness of the black orchid *C. pandurata* as an Indonesian endemic orchid. The method could be implemented for other Indonesian natural orchids.

Materials and Methods

Plant materials and culture condition

Four developing independent shoots of 35S::KNAT1-black orchid transformants that are growing up on 100 mg. l^{-1} Kanamycin-containing New Phalaenopsis (NP) medium were used as plant materials in this experiments. The shoots as source of explant were cut into two leaf discs and a stem to induce new shoot formation on regeneration medium *in vitro*. Explants were cultivated on half strength of *New Phalaenopsis* (NP) medium (3), with addition of 150 $ml.l^{-1}$ coconut water, and combination of plant growth regulators *2-isopenthenyladenine* (2iP) and *Naphtalene acetic acid* (NAA) with ratio of 1: 1 (0.15 and 3 µM). The cultures were incubated at 25°C with 1000 lux continuous light. The growth of protocorm like bodies (PLBs), and shoots from the explants were examined every week. For control experiment, a similar set of experiment was also done using non-transformant black orchid plant.

T-DNA Construct for Genetic Transformation and Detection of Transgene by PCR

Genetic transformation of plasmid 35S::KNAT1 and pGreen vector into orchid was carried out according to the method of Semiarti *et al.* (1). The structure of 35S::KNAT1 containing T-DNA construct that inserted into orchid genome is shown in Fig. 1.

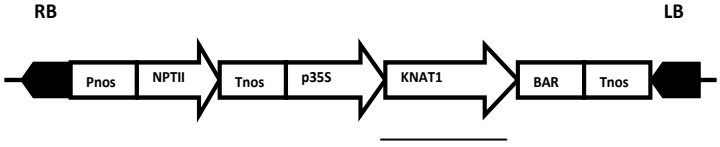

Figure 1. Schematic Structure of 35S::KNAT1 containing T-DNA. LB, Left border; RB, Right Border, 35S: CaMV promoter; KNAT1 gene; HPT: Hygromycin phosphotransferase; Tnos: Nos terminal. Bar: 1.2 kb.

Genomic DNA of transformant plants and non transformant were isolated and extracted using QIAGEN gDNA extraction kit (GmBH, Germany) according to the manual instruction from the manufacture. Pured gDNA from the emerged shoots from selected transformant explants were detected by polymerase chain reaction (PCR) method for the existance of *KNAT1* gene using *KNAT1* gene specific primer KNAT1F1 (5'-CTTCCTAAAGAAGC-ACGGCAG-3') and KNAT1R1 (5'- CCAGTGACGCTTTCTTTGGT-T-3'), that amplified 1.2 kb DNA fragment.

Results and Discussion

Phenotypic analyses

Morphology of the shoot and leaves of transformant are normal as the same as non transformant plant. The growth rate of shoot(s) from transformant and non-transformant stem explant on half strength NP medium with various concentration of growth regulators treatment showed that generally, induction of shoot formation in transformant was faster than that of non-transformant (Table 1).This data indicates that the growth of shoots from transformant explant may be induced by *KNAT1* gene activity that integrated in the orchid genome, than that of induction by growth regulators endogenously or exogenously.

Table 1. The Growth of Shoots from transformant and non-transformant stem explants on half strength NP Medium and Various Concentration of Growth Regulators Auxin and Cytokinin.

Growth regulators NAA: 2-IP	The first time shoot emerging from explant (week)							
	1	2	3	4	5	6	7	8
(0.00 : 0.00)		**T**		NT				
(0.15 : 0.15)		**T**		NT				
(3.00 : 3.00)						**T**		NT

T= transformant; NT = Non-transformant

The fastest emerged shoot(s) from transformant stem was two weeks after explant inoculation on ½ NP medium without additional growth regulators and ½ NP+ 0.15 µM NAA and 0.15 µM 2iP, though in non transformant explant the shoots emerged at 4 weeks after inoculation. Interestingly, when the higher concentration of growth regulators was added into medium, the shoot formation delayed up to 6 weeks in transformant explant and 8 weeks for

non transformant explant. It is inline with our previous data in *P.amabilis*, that shoots formed on leaf discs of 35S::KNAT1 transformant grown on hormon-free NP medium (1).

Multishoots production from 35S::KNAT1 Transformant Explants

In the case of number of shoot production, the transformant stem produced multishoots from one stem explant. The higher number of shoots were produced in transformant explants,than that in non transformant stem (Fig.2, Table 2). Multishoot production were also reported by Yu *et al.* (4), when *Dendrobium Orchid Homeobox1* (*DOH1*) introduced into orchid hybrid *Dendrobium* "Madame Thong In". Introduction of KNAT 1 gene into some Dicot was also induced multishoot formation, i.e Chuck *et al*. (5) observed multishoot production in *Arabidopsis*, and Nishimura *et al*. (6) obtained multishoots in tobacco transgenic explants. Semiarti *et al.* (7) reported that the expression of *KNAT1* gene was improved in a leaf mutant of *Arabidopsis, assymetric leaves2* mutant, that produced multishoots on mutant leaf disc in that were cultured on hormone free medium.

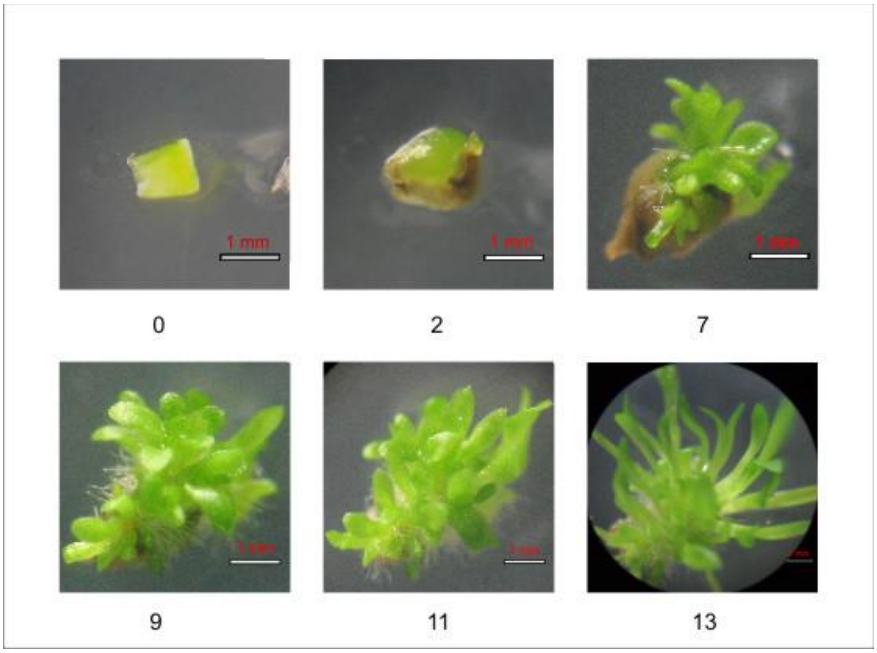

Figure 2. Multishoot formation from stem explant of 35S::*KNAT1* on ½ NP + 0.15 µM NAA+ 0.15 µM). 0 ; week 0, starting inoculated explant, 2; Shoot initiation (emerging leaf primordia) come out from explant, week-2; 7; Week-7, 9 ; Week-9, 11; Week-11, and 13; multishoots emerged at week-13 (Bar: 1mm)

Multishoots production might also be related to the arrangement of endogeneous phytohormone biosynthesis pathway, such as cytokinin and gibberelic acid (GA) that involved in cell division, cell elongation and shoot formation. As described by George *et al.*

(8), that in plant tissue culture, during adventif shoot formation the concentration of cytokinin in cell increase, but the concentration of GA will be decreased. In tobacco transgenic plants, overproduction of KNAT1 protein suppressed the activity of *GA20ox* (*Ntc12*) that bound to some sequences in the first intron of the GA20ox gene. This complex reduced the synthesis of GA, in turn it will activate cytokinin synthase gene,that caused multishoot production.. Overexpression of *KNAT1* gene will also eliminate apical domination in the shoot tip, so that the determinated cells will switch into undeterminated cells (9, 10). It is reasonable that multishoot production in this experiment has also proved the activities of overexpressed *KNAT1* gene in the black orchid stem.

Table 2. Number of Shoot Production from 35S::KNAT1 transformant stem explant after 13 weeks cultivation on ½ NP Medium supplemented with various growth regulators.

No.	Growth regulators (NAA: 2-IP) (µM)	Number of shoots	
		Transformant	Non transformant
1	(0.00 : 0.00)	11	5
2	(0.15 : 0.15)	36	4
3	(3.00 : 3.00)	13	20

Detection of *KNAT1* gene in 35S::KNAT1 transformant plant's genome

Seven shoots of transformants and three non-transformant plants were analyzed to prove the prescence of 35S::KNAT1 into its genomes. The genomic DNA of each plants were amplified using specific oligonucleotide primers for *KNAT1* genes (KNAT1F1 and KNAT1R1), that resulted in about 1.2 kb amplified DNA fragment. Four out of seven shoots showed positive results, but the other three were negative (Fig. 3). These results indicate that *KNAT1* gene still integrated into the genomes of four orchid transformant lines and maintained its activity for shoot production in these orchids. Using these four lines, micropropagation of black orchid (*C. pandurata*) can be improved, as well as the use of the transgenic technology for other character improvement of this orchid. The use of Green Flourescent Protein (GFP) as a reporter gene as described previously (11), combine with *KNAT1* gene will improve the orchid quality in both shoot multiplication and flourescence plant. Hopefully, it will give benefit to support both conservation and commercial trade of Indonesian natural orchids.

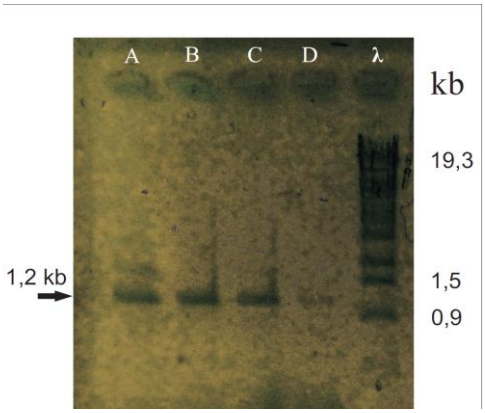

Figure 3. Detection of *KNAT1* gene in 35S::KNAT1 Black Orchid Transformants. Lanes (A-D) show that 1.2 kb DNA fragment could be amplified from four transformants. λ indicates λ DNA digested by Sty I enzyme that used as DNA size marker.

Conclusion

The Arabidopsis *KNAT1* gene can be used for improvement of shoot formation in micropropagation of Black Orchid (*C. pandurata*). The *KNAT1* gene has stably maintained its function in secondary shoot growth of black orchid transformant. The method can be applied to the commercial production of orchids in Indonesia for both domestic and international trade.

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O-MB02

Regulation and signaling of phosphate starvation genes in *Arabidopsis thaliana*

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Abstract

One of the most important nutrients for plants is phosphorous. Phosphorus is critical to bioenergetics, photosynthesis, and regulation of a number of enzymes. Plants acquire phosphate as inorganic phosphate ion (Pi). When Pi is limited, plants respond by changing their root architecture to aid the uptake of Pi. Plants reduced primary root length and increased length and density of lateral root and root hair to enhanced uptake of soil phosphate. This study evaluated several genes involved in Pi regulation under Pi starvation in a model plant *Arabidopsis thaliana* using RT-PCR. Results showed that some of the genes are up regulated under Pi limited condition, and some are down regulated. These results suggest that plants feature complex regulatory networks to maintain P homeostasis and optimise their phosphate (Pi) uptake and storage capacities to meet metabolic and developmental demand.

Keywords: phosphate starvation, gene regulation, RT-PCR, *Arabidopsis thaliana*

Introduction

One of the most important nutrients for plants is phosphorous. It is involved in the regulation of many biochemical and physiological processes and is a structural component of nucleic acids, phospholipids and numerous other biological molecules. Phosphorus is also critical to bioenergetics, photosynthesis, and the regulation of a number of enzymes [1].

Plants acquire phosphate in the form of inorganic phosphate (Pi). The concentration of soluble Pi of many soils ranges from about 1 to 10µM, while for optimal growth, plants require intracellular Pi concentrations range from 5 to 20mM [2]. When Pi supply is limited, plants respond through morphological, physiological and biochemical adaptations [3]. Morphologically, to cope with Pi deficiency, plants change their root architecture to aid the uptake of Pi. Plants reduced primary root length and increased length and density of lateral root and root hair to enhanced uptake of soil phosphate [4]. Pi deficient plants usually accumulate excessive anthocyanin. Absorption of ultraviolet light by anthocyanin can protect nucleic acids and the chloroplasts from damage caused by Pi starvation [1].

Plants are reprogramming their genetic pathway in respond to Pi starvation. This study studied several genes involved in Pi regulation under Pi starvation in a model plant

Arabidopsis thaliana using RT-PCR. Morphological responses of plant growth to Pi limitation were also evaluated.

Materials and Methods

Plant materials and growth conditions

Arabidopsis thaliana Col-o were grown in vertical plates in ½ strength of Hoagland medium supplemented with 0.25 mM phosphate in the form of KH2PO4. Seedlings were maintained in a growth chamber under controlled condition (10h light, 25^{o}C). After five days, the seedlings were transferred to fresh medium without phosphate and continue to grow for another 4 days to induce Pi starved condition. Seedling were then again transferred to medium with phosphate and harvested after 1, 2, 3 and 7 days.

Root morphological and fresh weight analyses

Images of the root system were recorded from plants growing in petridishes using a desktop scanner (resolution: 300 dpi). Fresh weight of shoot and root were measure at day 1, 2, 3 and 7 in minus phosphate medium and after phosphate resupplied.

RT PCR

Isolation and generation of cDNA was done using magnetic beads [5]. Quantification of transcripts was performed using SYBR Green RT-PCR from Applied Biosystems. Several genes studied were shown in Table 1.

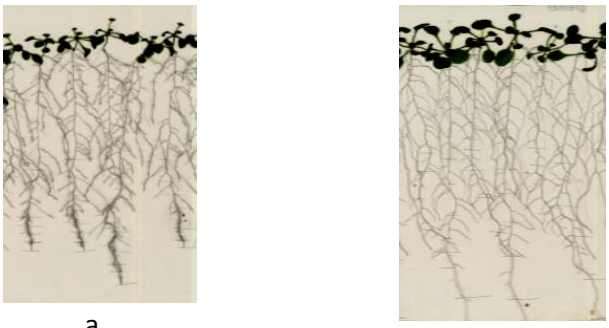
Table 1. Primers used for quantitative RT-PCR

Genes	Primer
Pht1;4	5'TGTGCCGGCCGAAATCT3' 5'TTGCTCCTAATTTTCCTGATGCT3'
Pht1;5	5'GACCTAATGCGACGACGTTTG3' 5'CGCCGATATCCCATGACAAG3'
Pht1;7	5'CGCGGCTTCTGGAAAATTAG3' 5'TGGAGGATATCCATGCTCTGTCT3'
PHF1	5'AGTGGAAAGAGTGGCAGATATATGC3' 5'CTCGAAGAATACGTATGCAGCTATCA3'
PHR1	5'ATATCGGCCAGAACCATCAGAAAC3' 5'TGTAATACCTATCCCACCTTTCAAATC3'
NMT3	5'GCACCAAGAAGGAGTATCCTGATAA3' 5'TGCTGGCTTGTCTTGGATATGT3'

Results and Discussion

Root morphology of phosphate starved seedlings and normal seedling was shown in Fiure 1. As can be seen in Figure 1, Arabidopsis seedlings growth in phosphate depleted

medium showed a decrease in elongation rate of primary root. In addition, Pi-starved seedlings increased root hair growth and density. The primary root suppression in Pi-deprived seedlings was induced by inhibition of root meristematic activity. It was reported this condition was caused by the inability of inactive meristematic roots to respond to auxin, which lead to the decrease of mitotic activity [6].



a b

Figure 1. Root architecture of phosphate starved plant (a) and normal plant (b). Plants were grown in 250mM of phosphate for 5 days, were then transferred to Pi-lacking medium for 7 days (a). Plants after 5 days in 250mM of Pi-medium were transferred to fresh medium with 250mM phosphate and grow for 7 days (b).

Root hair density increased in low-phosphorous root (Figure 1). This adaptation is an important strategy to facilitate uptake of phosphate by increasing surface area of absorption. A piece of evidence has been shown that root hair participated in phosphorous uptake. A study proved that 63% of total P uptake was contributed by root hair when 70 percent of the root hairs grew into the labeled soil [7].

Fresh weight of root to shoot ratio after 1 day, 2, 3 and 7 days in minus phosphate medium as well as after resupplied with phosphate is demonstrated in Figure 2.

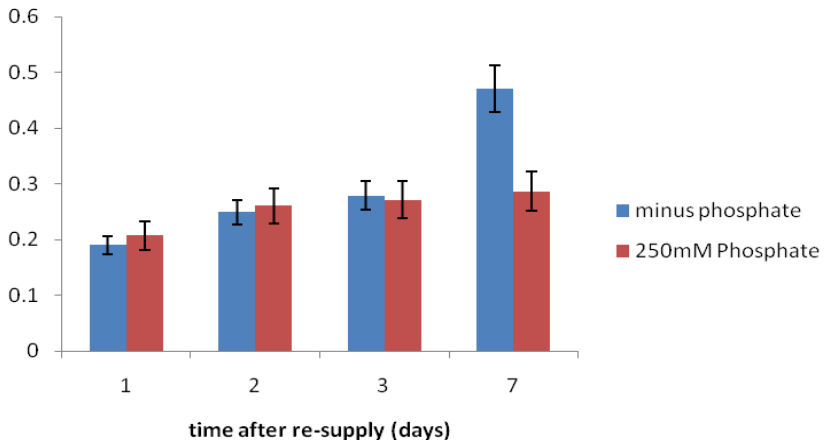


Figure 2. Fresh weight of root to shoot ratio of seedlings grown in minus phosphate and plus phosphate medium after 1, 2, 3 and 7 days.

Figure 2 demonstrates the increase of root to shoot ratio of seedling fresh weight under phosphate starvation. This alteration correlates with the promotion of lateral root and root hair growth in deprived-phosphate seedlings

Phosphate starvation responses in plants are coordinated by expression of huge number of genes [9]. Among them, three transporter genes were evaluated in this study (Figure 3).

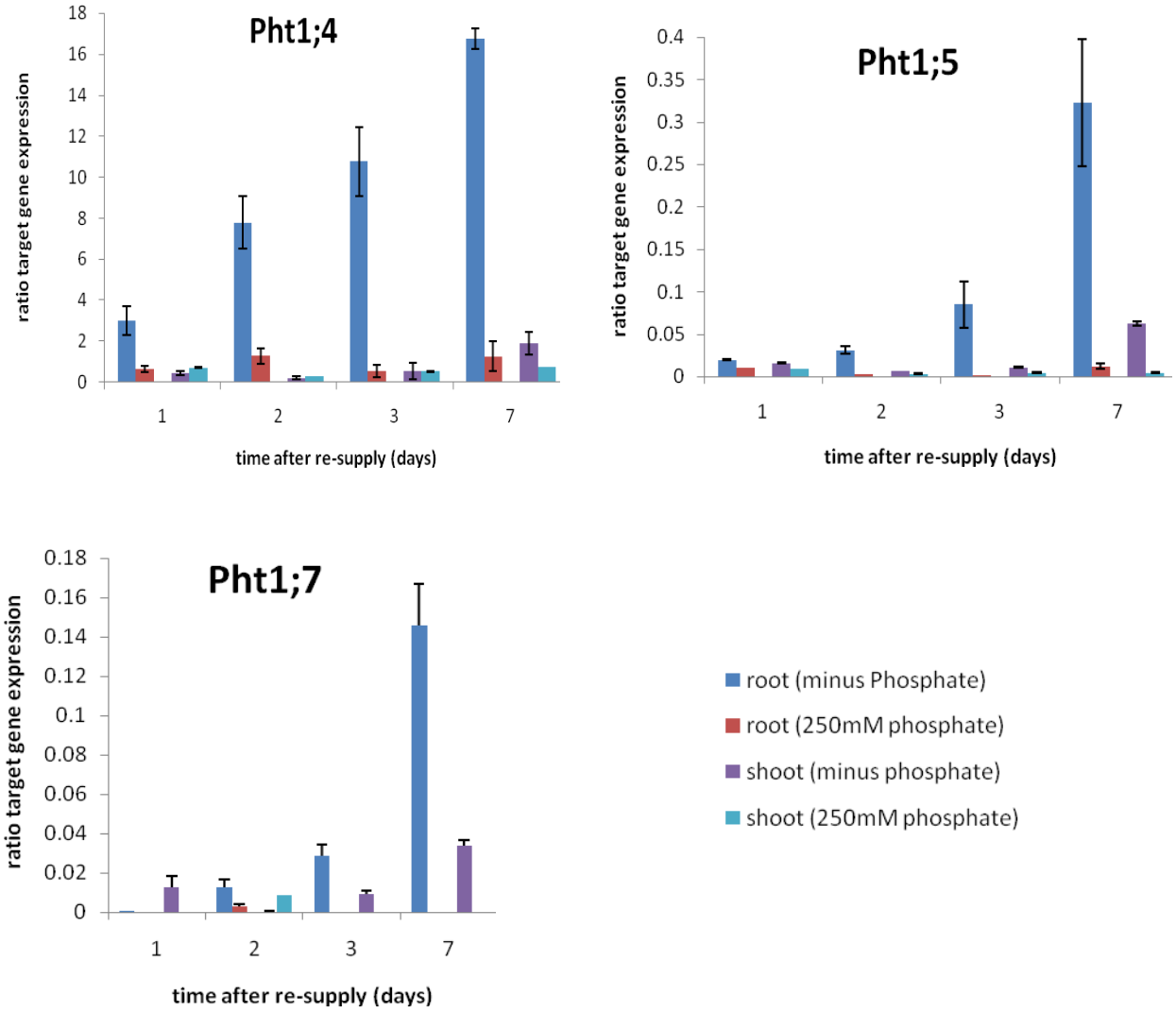


Figure 3. Expression of *Pht1;4*, *Pht1;5* and *Pht1;7* in Arabidopsis seedling during Pi-starved and normal conditions

As indicated in Figure 3, the expression of phosphate transporter genes both in root and shoot were higher in seedling grown in lacking phosphate medium. This up regulation of transporter genes allows transporter to actively assimilate Pi. It was reported that knockout of *Pht1;4* leads to the decrease of Pi acquisition during Pi deficiency [8]. Beside

phosphate transporter genes, other genes which were studied included *PHF1*, *PHR1* and *NMT3* (Figure 4).

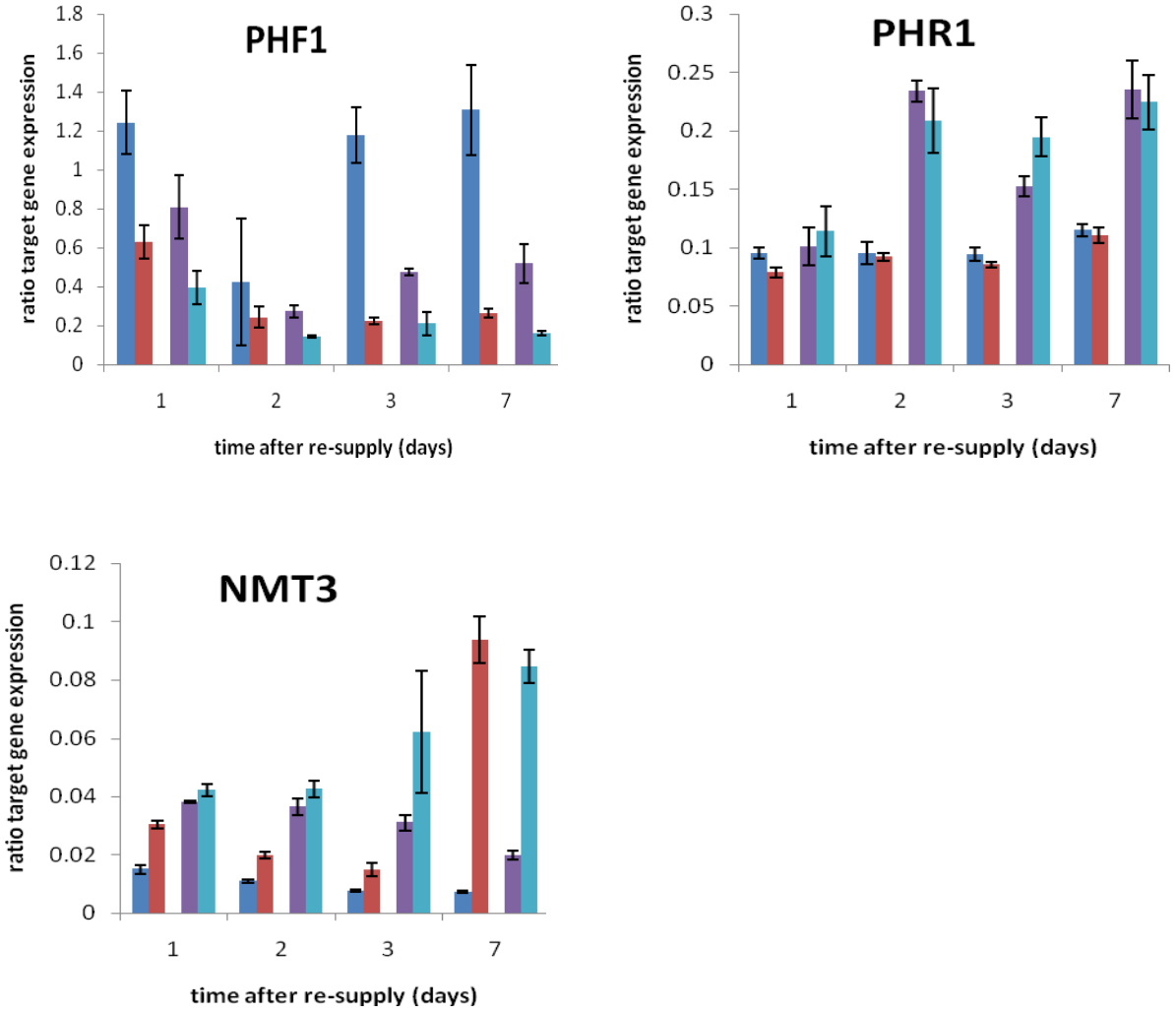


Figure 4. Expression of *PHF1, PHR1* and *MT3* in Pi starved-seedlings compared to those in normal seedling

In Arabidopsis starved seedling, *PHF1* was upregulated. *PHF1* acts as facilitator of *PHT1* to exit from endoplasmic reticulum [10]. The *phf* mutan undergoes retention of Pi transporter in endoplasmic reticulum and shows reduce Pi content [9]. At low phosphate, the expression of *PHR1* remained unchanged (Figure 4). *PHR1* involves in activation of Pi starvation-induced genes by binding a P1BS (*PHR1* specific binding sequence) cis-element (GNATATNC). The phr1 mutant shows a reduced concentration of Pi under both Pi-sufficient and Pi-limited conditions [10].

The expression of *NMT3* was also evaluated. As can be seen in Figure 4, there was a down regulation of *NMT3* gene. *NMT3* encode enzymes phosphoethanolamine N-

methyltransferases which involved in phospholipid biosynthesis [11]. Plants under Pi-lacking conditions have a lower concentration of phospholipids [11], therefore the phospholipid biosynthesis is repressed by down regulation of *NMT3* gene.

From this study, it can be concluded that highly coordinated mechanisms involved in respond of plant to Pi-limited condition. Those include alteration of root architecture, increase of root to shoot ratio and upregulated as well as down regulated of Pi-starved responsive genes.

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O-MB03

Cellular dynamics of nucleolar small G protein AtNOG1-1 in plant cells

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O-MB04

The Identification of Species and Relationship of Javan Langur (*Trachypithecus auratus*) in Javan Langur Rehabilitation Center (JLC) Based on Cytochrome b Gene Sequence

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ABSTRACT

Ebony leaf monkey or Javan Langur, locally named Lutung Jawa, (*Trachypithecus auratus*) is one of the endemic species of Indonesia which is suspected as a vurnerable primate by the *International Union for Conservation of Nature* and *Natural Resources* (IUCN). There are several species of ebony leaf monkeys in the Javan Langur Rehabilitation Center (JLC), Dau subdistrict, Malang Regency. Relationship identification of Javan Langur in JLC has always been done based on morphological characteristics which gives limited information. DNA analysis is considered to give more accurate information. In this research, two types of Lutung Jawa, which are suspected to be *Trachypithecus auratus auratus* (named Rus) and *Trachypithecus auratus mauritius* (named Ijem) were studied. To amplify *Cytochrome-b* gene (*cyt-b*) forward primer used was 5'-CTTCCATGAGGACAAATATC-3' (modification of L15162) and reverse primer was RMuch 5'-GTGGAGTATAGGTATGATTGC-3'. This *Polymerase Chain Reaction* (PCR) process resulted on 500 bp *cyt-b* fragment for Rus and 501 bp for Ijem. Phylogenetic analysis using *Maximum-likelihood* (ML) method, and *pairwise distance* using MEGA5 *software* show that Rus and Ijem have a close relationship and is in a same species with *T. a. auratus*. *Pairwise distance* value both sample is 0.4% ± 0.0027. Phylogenetic tree using ML shows both of samples are close with *gi|148970501|gb|EF465117.1|T. a. auratus haplotype aaJ*, gi|148970497|gb|EF465115.1| haplotype aaG, and gi|148970487|gb|EF465110.1| haplotype aaB, and also placed in one group with others *T. auratus*.

Keywords: Relationship, Javan Langur, Lutung Jawa, *Trachypithecus auratus*, Cyt-b.

Introduction

From 200 species of primate in the world, 25% or 50 kinds lives in Indonesia. 70% among them are endangered, where 20 of them are endemik to Indonesia (1). One of these endemik animal is Javan Langur (2), localy named Lutung Jawa. Javan Langur, Ebony Leaf Monkey, or Lutung Jawa is synonymus with *Trachypithecus maurus* Horsfield (1823), *Trachypithecus pyrrhus* Horsfield (1823), *Trachypithecus sondaicus* Robinson & Kloss (1919), *Trachypithecus kohlbruggei* Sody (1931), and *Trachypithecus stresemanni* Pocock (1934) (3). Indonesian government considers Javan Langur as a protected animal since 1999. *International Union for Conservation of Nature* and *Natural Resources* (IUCN) consider Javan Langur as a primate that is vulnerable against habitat disturbance and poaching for trading.

Taxonomic position of the Javan Langur species has not been able to be clearly determined (4). Lee (5) and Roos et al., (6) categorize Javan Langur into two different species those are *T. auratus* and *T. mauritius* as each has different morphology and clear biogeographical source. Biogeographically, Javan Langur can be divided into two which are Javan Langur that comes from western java (*T. a. mauritius)* and those that come from Eastern Java (*T. a. auratus*) (Fig. 1) (3). *International Union For Conservation of Nature and Natural Resources*/ IUCN (2006) divide Javan Langur into two subspecies those are *T. auratus auratus* and *T. auratus mauritius*. This ambiguity may cause problem in conservational efforts of this animal (7).

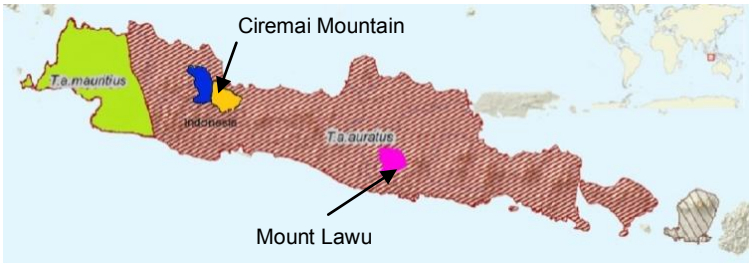

Fig. 1: Distribution of Javan Langur (*T. auratus*) (3).

According to its morphological characters, Lee (5) has described the difference between *T. auratus* and *T. mauritius*. *T. auratus* have Facial skin with yellow eye rings and bluish face (black morph) or eye rings-in some forms, the whole face ‘‘depigmented’’ and freckled face (red morph); pelage glossy black except for the red morph and not ‘‘frosted’’ (i.e., no silver tips). Circumfacial hair (cheek hairs and hair on crown) is erect and forward-curled, forming a ‘‘*mussel-shaped”* semicircle around the orbits. There is no pointed crest. In red morph, tips of hairs on head, arms above wrists, near elbow, and legs above ankles are whitish; there may be a dorsal tinge of black. Females differ from males in having a pale, usually yellowish white pubic area . *T. mauritius* has Black pelage hairs without white tips; face black or gray with no eye rings; pointed crest; long, outward-pointing cheek hairs. *T. a. mauritius* is smaller in size than *T. a. auratus* (8).

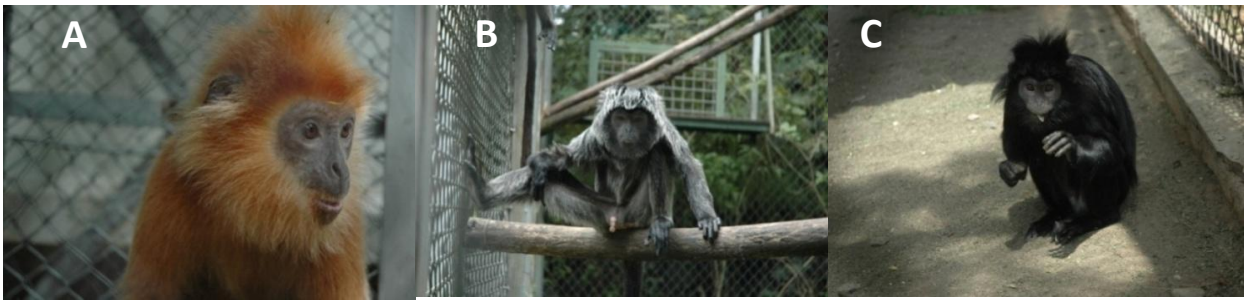

Fig. 2: Javan Langur (*Trachyphitecus auratus*) . A. *Red Morf* and B. *Black Morf* come from East Java, C. from West Java (C). (Photo: Kurniawan, 2008)

The Javan Langur Rehabilitation Center (JLC) is a conservation center that was previously own as Javan Langur center (JLC). This organization is a rehabilitation facility for

Javan Langur before being released into the wild. Javan Langur in this conservation are langurs saved by the Indonesian government or were given by citizens voluntary. Accommodation of Javan Langur is done before being released to its natural habitat. JLC contains langur with black fur with white tip and pure black langurs. Based on these morphological characteristics, the first langur is thought to be *T. a. auratus*, while the second langur is thought to be *T. a. mauritius*.

Species and relationship identification in JLC has been always done using morphological characteristic and original source. Identification using these methods often causes ambiguity (9,10). DNA variation analysis, especially mitochondrial DNA (*mtDNA*) is often used to reconstruct phylogenetic relationship between species or populations in the same species. This is because *mtDNA* has higher rate of mutation (5-10 times) compared to nucleic DNA (11,12). *mtDNA* also has a high copy number, which is 10^3 - 10^4 (12). *mtDNA* is in a circular double helix shape consisting of several regions: 12S rRNA, 16S rRNA, ND1, ND2, CO I, CO II, ATP, CO III, ND3, ND4, ND5, ND6, *cyt-b* and *D-loop* (*displacement loop*) evolve in replication process (13).

Cytochrome b gene (cyt-b) has been used as a reference in determining relationship (14). Cyt-b is relatively conserved making it ideal to identify at species level (11,15) or to clarify phylogenetic relationship into deeper levels (16). Cyt-b gene is directly involved with electron transport in the respiration process of the mitochondria, consisting of eight transmembrane with helical shape and conecting between the outer and inner membrane (17). Cyt-b gene can be used as a gnetic marker that has a high rate of mutation, making it able to give information about haplotype in spesies (18).

On the previos research, cyt-b sequence has been used to understand genetic diversity for conservation management, one of them being the tibetan moose (*Procapra picticaudata*), an endagered species in the high lands of Qinghai-Tibet, China (15). Cyt-b gene has also been used to identify relationship of *Trachypithecus barbei* (19) and was also able to diffrentiate between subspecies of Javan Langur (6).

Materials and Methods

Sample collection

Samples were taken from Javan Langur Rehabilitation Center (JLC), Dau sub district, Malang Regency. The samples were taken from two langurs, named Rus and Ijem. The first langur named Rus is thought to have come from Mount Lawu, Sragen, East Java, while Ijem is thought to be from Ciremai Mountain, Kuningan, West Java. Sample taken was the langur's blood (Fig. 1). Blood sample were stored in EDTA 3 ml tube. In the laboratory, the blood samples were stored at 20ºC until use.

DNA extraction and PCR amplification

Whole genome of mithochondrial DNA isolation follows protocol from *Roche isolation DNA Mini Kit*. Polymerase chain reaction (PCR) used two primers; Forward Primer that was modified from Primer L15162 (20), 5'- CTTCCATGAGGACAAATATC -3' and Reverse Primer Rmuc1 5'-GTGGAGTATAGGTATGATTGC-3' which self-created. The cycling parameters used were as follows: Initial denaturation for 5 minutes at 95ºC, denaturation for 1 minute at 94ºC, 40 siklus, annealing for 2 minutes at 47ºC, extention for 2 minutes at 72ºC, and final extention for 5 minutes at 72ºC. The results of the PCR amplifications were checked by running an aliquot on a 1.5% agarose gel. Sequensing of the PCR Products were performed using both the forward and reverse primers of PCR amplificaton with the Big Dye terminator AB1 3130 and 3130 xl Genetic Analyzer.

Phylogenetic Analysis of the Cyt-b Sequence Gene

Optimalize of chromatogram reading use Peak trace program, while consensus sequence were carried out with DNA baser. Multiple alignment were carried out with the Clustal W at BioEdit. Sequece gene validity were hold in software *Basic Local Alignment Search Tool* (BLAST) from www.ncbi.mln.nih.com. Phylogenetic tree reconstructions were carried out with the Maximum likelihood (ML) algorithms as implemented in MEGA5. ML trees were constructed with the Kimura-Nei model of sequence evolution with selected as best fitting model according to a hierarchical-likelihood with *T. obscurus* (gi|62161253:14192-15332) was used as outgroup. internal nodes was performed by bootstrap analyses with 1000 replications. To identify species we compare topology analisys and pairwise distance (MEGA5) value.

Results and Discussion

Results

Analysis using BLAST showed a degree of similarity with query reaching 92%. A query of 43% shows that sequences generated by two samples are partial of cyt-b. Cyt-b gene position of Rus and Ijem were located around the 400th until 900th base from the

haplotype T. a. auratus	9	42	62	69	72	78	141	159	177	255	303	310	319	369	384	148	441	468
Rus (T._a._a._*haplotype*_mcA)	C	T	T	T	T	G	C	T	T	C	C	A	T	C	A	C	C	C
Ijem (T._a._a._*haplotype*_mcB)	G	T	T	T	C	G	C	T	T	C	C	A	T	C	A	C	C	C
T._a._a.	C	T	C	T	C	G	C	T	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaB	C	T	T	T	C	G	C	T	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaC	C	T	C	T	C	G	C	T	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaD	C	C	C	T	C	A	C	A	C	C	A	G	T	C	A	C	C	C
T._a._a._*haplotype*_aaE	C	C	C	T	C	A	C	A	C	C	A	G	T	C	A	C	C	C
T._a._a._*haplotype*_aaF	C	T	C	T	C	G	C	T	T	C	C	A	T	T	A	C	C	C
T._a._a._*haplotype*_aaG	C	T	T	T	C	G	C	T	C	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaH	C	T	C	C	C	A	C	A	T	C	C	A	T	C	A	T	C	T
T._a._a._*haplotype*_aaJ	C	T	T	T	C	G	C	T	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaK	C	T	C	T	C	G	C	T	T	C	C	A	T	C	G	C	C	C
T._a._a._*haplotype*_aaL	C	T	C	C	C	A	C	A	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaM	C	T	C	C	C	A	T	A	T	T	C	A	T	C	A	C	T	C
T._a._a._*haplotype*_aaN	C	T	C	C	C	A	C	A	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaO	C	T	C	T	C	A	C	A	T	C	C	A	C	C	A	T	C	C
T._a._a._*haplotype*_aaP	C	T	C	C	C	A	C	A	T	C	C	A	T	C	A	C	C	C
T._a._a._*haplotype*_aaQ	C	T	C	C	C	A	T	A	T	T	C	A	T	C	A	C	T	C

Table 1: Character Diagnostics of Individual *T. auratus* Haplotype

cyt-b query. Based on the above analysis, it can be concluded that the sequence formed is a part of the cyt-b. gene

Cyt-b gene sequence gained from both samples are very conserved compared with *T. a. auratus*, *T. a. mauritius*, *T. cristatus* and *T. germaini*. This difference was only found on a few bases, and not more than two consecutive bases. Substitution took place on several bases, such as on base 42, base 69, base 72, base 141, base 159, base 177, base 255, base 303, and base 310 (Table 1).

Sequences obtained were then analyzed using MEGA5 software to determine the relationship between Rus and Ijem. Phylogenetic tree reconstruction using ML method showed that Rus and Ijem are located in one cluster with *T. a. auratus* haplotype aaJ, *T. a. auratus* haplotype aaB and *T. a. auratus* haplotype aaG and are in one group with other *T. auratus* (Fig. 3). Pairwise distance analysis (± = standard error) using the Kimura-2 parameter model showed that Rus and Ijem (using 2% standard) has a really low value which is 0.4% ± 0.0027. This indicates that both of them are from one species. Based on topology and phylogenetic tree, both samples are closely with related *T. a. auratus* haplotype aaJ, *T. a. auratus* haplotype aaB and *T. a. auratus* haplotype aaG and has bootstrap value of 62.

Species determination analysis was done by comparing pairwise distance value between Rus and Ijem with *T. a. auratus* and *T. a. mauritius*. The result of the comparison of Rus with *T. auratus* showed that the lowest pairwise distance is 0.2% ± 0.0019 and the highest pairwise distance is 1.62% ± 0.0054, while for *T. a. mauritius* the lowest pairwise distance is 3.08% ± 0.0077 and the highest pairwise distance reached 3.29% ± 0.008. The result of comparison for Ijem with *T. auratus* has a lowest pairwise distance of 0.2% ± 0.002 and highest value of 1.62% ± 0.0055, while when compared with *T. mauritius* showed a lowest pairwise distance of 3.3% ± 0.008 and highest of 3.51% ± 0.0083. Based on these analyses, it can be concluded that Rus and Ijem belongs to *T. a. auratus.*

Discussion

The result of multiple alignment showed that samples from Rus and Ijem us very similar and was only diffrentiated by two bases; the first diffrence was on the 9th basem while the second was on the 72nd (Table 1). The diffrence in bases is the result of diversity between individuals, as basically no two individuals has the same base sequence as others. Diversity in gene sequenceinside a species can show the relation between one individual and another (21).

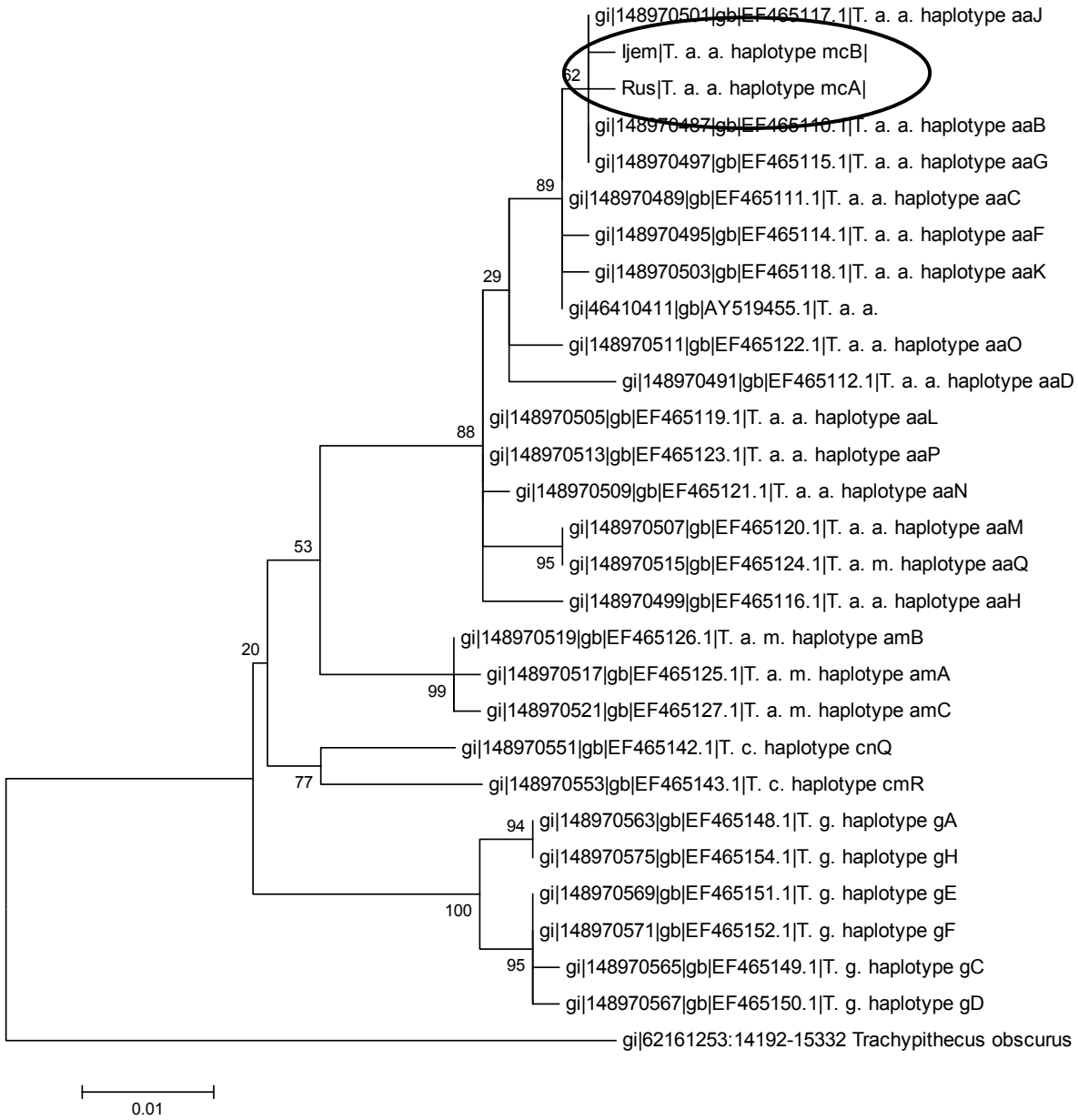

Fig. 3: Analysis of Gen Cyt-b Between Rus, Ijem, and Others Trachypithecus Using *Maximum likehood.* T. c. = *Trachypithecus cristatus*, T. a. a. = *Trachypithecus auratus auratus*. T. a. m. = *Trachypithecus auratus mauritius*. T. g. = *Trachypithecus germaini.*

The formation of phylogenetic tree was done to find the relationship between Rus and Ijem compared to sequence reference from the NCBI. The result of phylogenetic tree topology analysis using the maximum likelihood metho (ML) showed that Rus and Ijem are in the same cluster and is grouped with *T. a. auratus* haplotype aaJ, *T. a. auratus* haplotype aaG, and *T. a. auratus* haplotype aaB, and is in one big group with other T. auratus (Fig. 3). This topological result showed that Ijem Rus are closely related with the *T. a. auratus* group.

Pairwise distance anylises was done to find the genetic distance between one individual with another that was done using the MEGA5 software. The genetic distance ,between invertebrate species using cyt-b is larger than 2% (22,23). Previous research (6) showed that pairwise distance value between *T. a. auratus* is around 0.92% ± 0.001. Pairwise distance analysis between Rus and Ijem showed value between 0.4% ± 0.0027. This value indicates that Rus and Ijem are of one species.

Ijem's charachtersitics of having pitch black fur, which morphologically represent *T. a. mauritus*, has actually different result if examined genetically. Based on pairwise distance anylisis, comparison between Ijem and *T. a. mauritus* has a higher value (more than 3.3% ± 0.008) when compared with *T. a. auratus* (less than1.62% ± 0.0055). Species determination analysis on Rus showed the same result with Ijem. Rus's pairwise distance when compared with T. a. mauratus showed a higher value (more than 3.08% ± 0.0077) when compared with *T. a. auratus* (less than 1.62% ± 0.0054). Based on phylogenetic tree tophology Rus and Ijem are located in one cluster with *T. a. auratus*. Based on the pairwise distance analyses and philogenetic tree topology, its can be concluded that Rus and Ijembelong to *T. a . auratus*.

Acknowledgments

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O-MB05

Genetic Characterisation of Indonesian Swamp Eel Populations Inferred From Mitochondrial Gene Sequences

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ABSTRACT

Indonesian swamp eel are an economically important freshwater fish due to their reputation as delicious food, their ability to survive and grow in poorly oxygenated waters, and to be transported live. However, the taxonomy and phylogeny of this fish species are poorly understood. Although it is commonly accepted that the Indonesian swamp eel belongs to the species described as Monopterus albus, much of debate has centered on the number, distribution and taxonomic status of Monopterus species in southeast Asia. This is due to these fish being phenotypically plastic, which has lead to an extensive and confusing taxonomic nomenclature.

In this study, Indonesian swamp eels were collected from twenty-two sites through out Indonesia. Partial sequences of 16S rDNA and Cytochrome c Oxydase Subunit I (CO1) mitochondrial genes were used to examine genetic variation between populations and reconstruct evolutionary relationships. The sequence analysis of the two mitochondrial genes revealed two distinct group, which are genetically distinct from each other and distinct from other forms from southeast Asia.. Further studies are required to clarify species boundaries, identify diagnostic morphological traits and establish phylogenetic relationships of swamp eels in Indonesia and more widely in southeast Asia.

Keywords : swamp eel-mitochondrial genes-taxonomy-phylogenetics

O-MB06

Identification of *Green Fluorescent Protein (GFP)* Gene on Three Species of Jellyfish in Semarang's Waters

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ABSTRACT

Green Fluorescent Protein (GFP) is a gene encodes GFP protein which emits green fluorescent light when excited by blue light. *GFP* is widely used in cell and molecular biology researches as a reporter gene. *GFP* is obtained only in some genera of jellyfish, i.e *Aequorea, Mitrocoma, Obellia,* and *Phialidium*. Research on *GFP* from the local jellyfish of Indonesia has not been reported, although Indonesia has many different types of jellyfish. This research was conducted to determine whether Indonesian local jellyfish has the GFP gene and to determine the differences from *Aequorea victoria*'s GFP. The jellyfish were sampled on the Marina Beach Semarang. Amplification was done by using four sets of GFP-specific primers designed from specific parts of *A.victoria*'s GFP gene as the sequence reference. Morphological observations showed that the local jellyfish from Semarang is *Rhizostoma* sp., species x, and y (not yet identified). Using GFP's primer, only *Rhizostoma* sp. shows positive result. The amplified genomic DNA from the local jellyfish using GFP primers showed differences in the DNA sequence to local jellyfish and *A.victoria* as the positive control (plasmid pCambia). There are 64 nitrogen base and 11 amino acid in *Rhizostoma's GFP* differences. The amino acid 163 *valine* become *alanine* and *serine* 175 become *glycine* on *Rhizostoma's GFP*, make this *GFP* can give better fluorescent at 35-37^{o}C. *Rhizostoma's GFP* have chromophore structure as *A.victoria's GFP*. *Isoleucine* 167 become *threonine* revert the sensitifity of GFP from 395nm became 475nm. From this research we conclude that Semarang's *Rhizostoma* sp. has *GFP* gene which differ to *A.victoria's GFP.*

Keywords: GFP, DNA markers, local jellyfish Semarang, *Rhizostoma* sp.

INTRODUCTION

Green Fluorescent Protein (GFP) is a non-destructive reporter gene that can report gene expression directly, so that it widely used in cell and molecular biology research [1]. GFP is capable to emit green fluorescent light when highlighted by ultraviolet light. By utilizing Green Fluorescent Protein, it is expected to increase research on molecular detection moreover the going on process in the living organism. For example, the development of nerve cells in the brain or how cancer cells spread in the human body. Tens of thousands of different proteins obtain in the bodies of living things. These proteins control important chemical processes in comprehensive manner. So the Green Fluorescent Protein is also expected to map out a variety of proteins that exist in the human body for medical purposes.

GFP gene was first isolated by Osamu Shimomura (1962) from *Aequorea victoria* jellyfish, that usually live in the northern Pacific region. This species has bioluminescent

proteins called Aequorin that can emit blue light fluorescence. GFP reporter gene has the advantage over other reporter gene. This gene may function as a screenable marker which is non-invasive, non-destructive, and does not require exogenous substrates and cofactors to fluorescent [2]..

It has been reported that GFP is obtained only in some genera of jellyfish, i.e Aequorea, Mitrocoma, Obelia, and Phialidium [3]. Research on GFP from the local jellyfish of Indonesia has not been reported, although Indonesia has many different types of jellyfish. The research on the detection and isolation of GFP gene in the local jellyfish is needed. Because there are no data base on Indonesian GFP, tropical GFP and The isolated GFP gene can be used for advanced research on gene cloning using either GFP cloning vector or expression vector. This research was conducted to determine whether Indonesian local jellyfish has the GFP gene and to determine the differences from *Aequorea victoria*'s GFP.

MATERIAL AND METHOD

A. Sampling and Identification

The research was conducted from January 2010. Samples of the jellyfish were found in the north coast sea of Java, in the Marina beach Semarang. Jellyfish were photographed and then placed in ice boxes to be brought to the laboratory as research material. Jellyfish identification was done by morphological observation based on morphological characters of jellyfish in "the Marine Conservation Society jellyfish Survey"www.mcsuk.org as reference.

B. Genomic DNA Isolation of Jellyfish

Jellyfish body placed into a bowl and it molten by itself. Then 200 mg of the jellyfish liquid use as sample. The genomic DNA was isolated from the powder according to protocol Genomic Qiagen Midi Kit (QIAGEN GmBH, Germany). The isolated DNA is checked by 0.8% agarose gel electrophoresis, 50 mA, for 45 minutes. DNA quantification was done by spectrophotometer λ260/280 nm.

C. Isolation of Green fluorescents protein (GFP) gene(s) by polymerase chain reaction (PCR)

Jellyfish genomic DNA was amplified using four kinds of oligonucleotide primers specific for GFP gene using PCR. The primer sequences designed specifically based on *Aequoria victoria* GFP gene [5] (Table 1). The primers are stick to four specific locations on the GFP gene (Figure 1). Frozen Jellyfish DNA were thawed by hand and then brief vortexed and spindown. Jellyfish DNA was used as a DNA template for PCR reaction. The PCR reaction mixture consists of genomic DNA, PCR kit (Roche ®), and specific primers for the GFP gene. PCR performed with Thermalcycler using programs pre-denaturation 94ºC for 2

minutes and 30 second as one cycle of denaturation 94 º C, annealing temperature varies based on the Tm of each primer, and elongation at 72ºC for 1 minute and 30 second with 30 cycles and final phase of elongation of 72ºC for 5 minute as one cycle. Plasmid pCambia that carrying the GFP gene was used as positive control. Visualization use agarose electrophoresis, agarose 1%, 50mA for 45 minute.

Table.1 Specific *GFP* gen Primer

Set Primer	Nukleotide Sequens	Tm (°C)	anneali ng (°C)	Amplikon Size
GFP A	F1 5' ATGAGTAAAGAAGAAGAAC 3'	49,4º	50,8°	714 bp
	R1 5' GTATAGTTCATCCATGCC 3'	52,3º		
GFP B	F1 5' CTTTTCACTGGAGTGGTCCC 3'	63,5º	61,6°	700 bp
	R1 5' GCCATGTGTAATCCTAGCAG 3'	59,7º		
GFP C-B	F1 5' GGGAACTACAAGACACGTGC 3'	62,2º	60,9°	400 bp
	R1 5' GCCATGTGTAATCCTAGCAG 3'	59,7º		
GFP B-D	F1 5' CTTTTCACTGGAGTGGTCCC 3'	63,5º	60,7°	400 bp
	R1 5'ACAAAGATGACGGGAACTAC 3'	58,0º		

Design primer

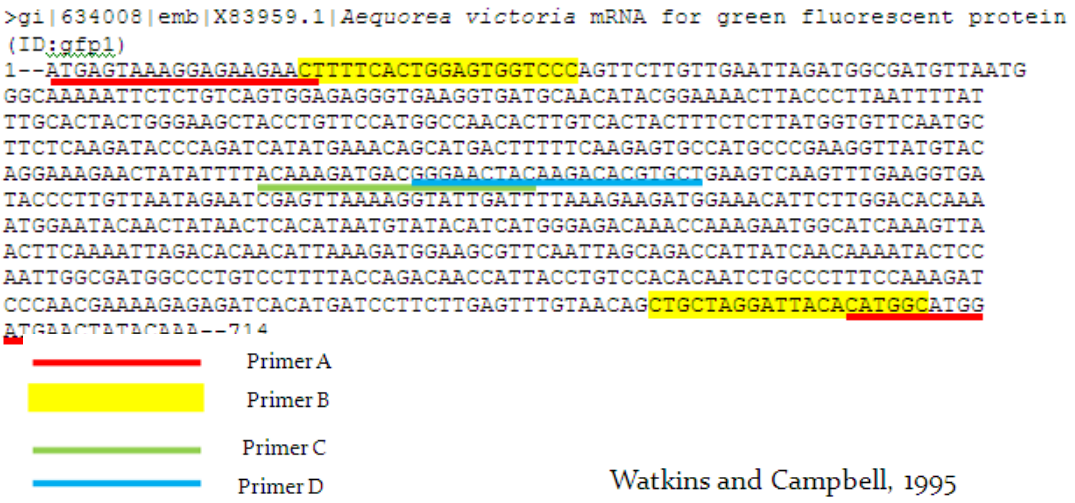

Figure 1. Primer construction design from cDNA of *Aequorea victoria*'s GFP.

D. Sequencing and Bioinformatics Study

Forty micro liters of PCR product that shows positive result send to 1st Base Singapore to sequence. The data compare with *Aequorea victoria's* GFP to analysis the amino acid component use BioEdit software.

RESULT AND DISCUSSION

From the sampling on Marina beach Semarang there are 3 species of jellyfish. The first Jellyfish has white color transparent, cone-shaped tentacles and umbrella speckled with size 25 cm. Based on morphological characters described in the literature of "Jellyfish Conservation Society Marine Survey" (accessed on www.mcsuk.org, April 15, 2010), indicates that the jellyfish from Semarang was a member of the genus Rhizostoma (Figure 2). The second jellyfish also has white color transparent, non marking umbrella with size 15 cm and tabulate-shaped tentacles (Figure 3). The last jellyfish has brown color, umbrella speckled with size 35 cm, and dark brown branched tentacles (Figure 4). The second and the third jellyfish not yet identified and called with x and y jellyfish.

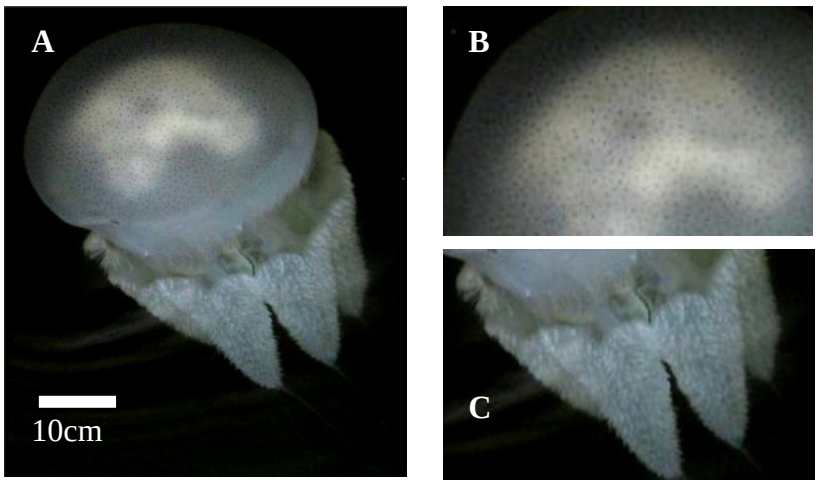

Figure 2. (A) Habitus of jellyfish Semarang (Rhizostoma sp.), (B) Umbrella with black spots, (C) Conical tentacle.

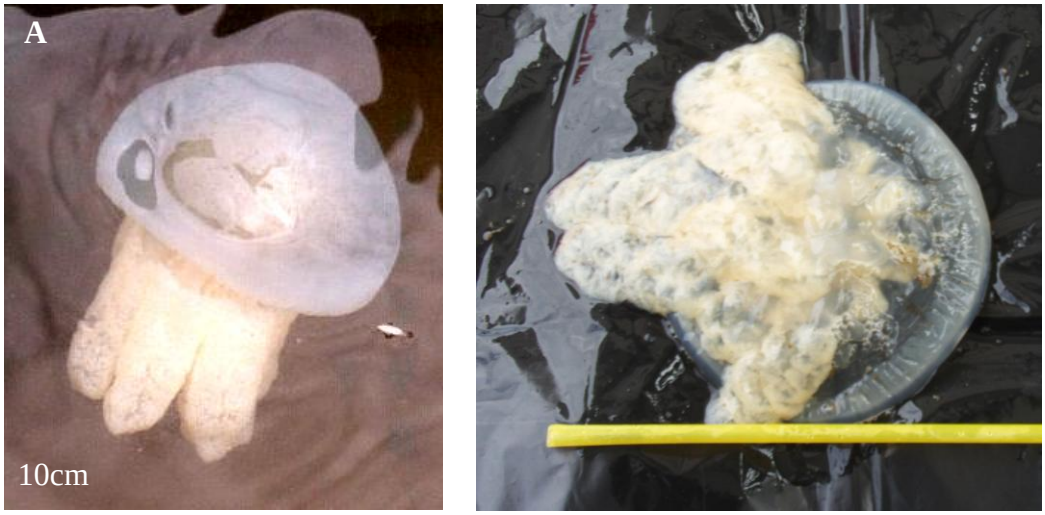

Figure 3. (A) Habitus of jellyfish Semarang (X) with white color transparent (B) non marking umbrella and tabulate-shaped tentacles

Figure 4. Habitus of jellyfish Semarang (Y) has brown color, umbrella speckled with size 35 cm, and dark brown branched tentacles

From the amplification using specific primer only *Rhizostoma* sp. shows positive result (Figure5). The negative result on the other sample, indicate that they not have GFP. On *Rhizostoma* sp. only primer B shows positive result. This is can be caused the sequence on *Rhizostoma* sp.'s GFP different on the edgeand in the middle, proofed with negative result using primer A, C and D.

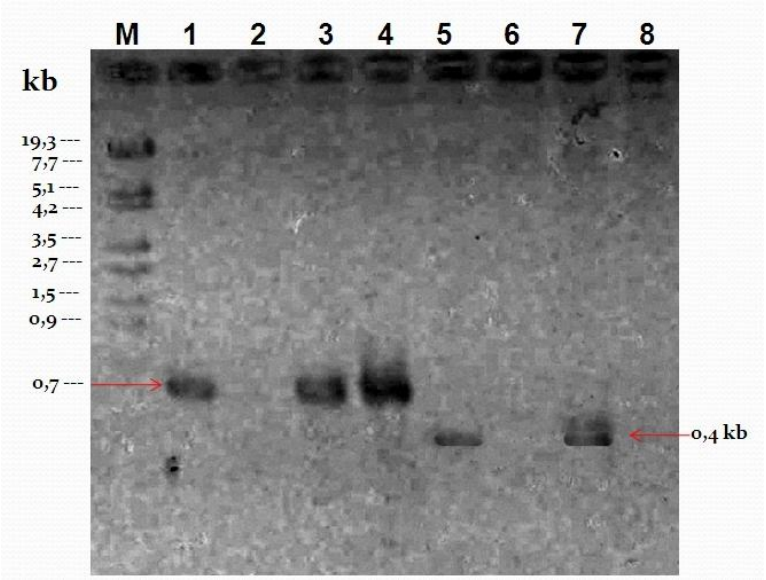

Figure 5. The amplified DNA fragments with specific primers GFP in A. Victoria (pCambia) and Rhizostoma sp. M, marker λ / styI; lane 1, pCambia with primer A; lane 2, Rhizostoma sp. with primer A; lane 3, pCambia by primer B; lane 4, Rhizostoma sp. with primer B; lane 5, pCambia with primer B (fw) and D (rev); lane 6, Rhizostoma sp. with primer B (fw) and D (rev); lane 7, pCambia with primer B (rev) and C (fw); lane 8, Rhizostoma sp. with primer B (rev) and C (fw).

Sequencing result shows there are 64 nitrogen base and 11 amino acid different between *Rizostoma*'s GFP and *Aequorea victoria*'s GFP (Figure 6). This different makes primer C and D not amplified. From the literature study there are 3 differences hat can make different on *Rhizostoma*'s GFP properties. The amino acid 163 *valine* become *alanine* and *serine* 175 become *glycine* on *Rhizostoma's GFP*, make this *GFP* can give better fluorescent at 35-37^{o}C. this GFP became more sensitive and more lighter at this temperature. *Rhizostoma's GFP* have chromophore structure same as *A.victoria's GFP*. *Chromophore* consist of *phenilalanin*, *serine*, *tyrosin*, *glycine*, *valine* and *glutamine* (amino acid number 64 until 69) [3]. This is indicate that *Rhizostoma's GFP give* same color as *A.victoria's GFP.* Amino acid *Isoleucine* 167 become *threonine* revert the sensitifity of GFP from 395nm became 475nm. *A.victoria's GFP* is more sensitive at 395nm than 475nm [6] [7]. So the characteristic of *Rhizostoma*'s GFP is very unique. From this research we conclude that Semarang's *Rhizostoma* sp. has *GFP* gene which differ to *A.victoria's GFP.* To prove this theory need to do further research about the expression of *Rhizostoma*'s GFP.

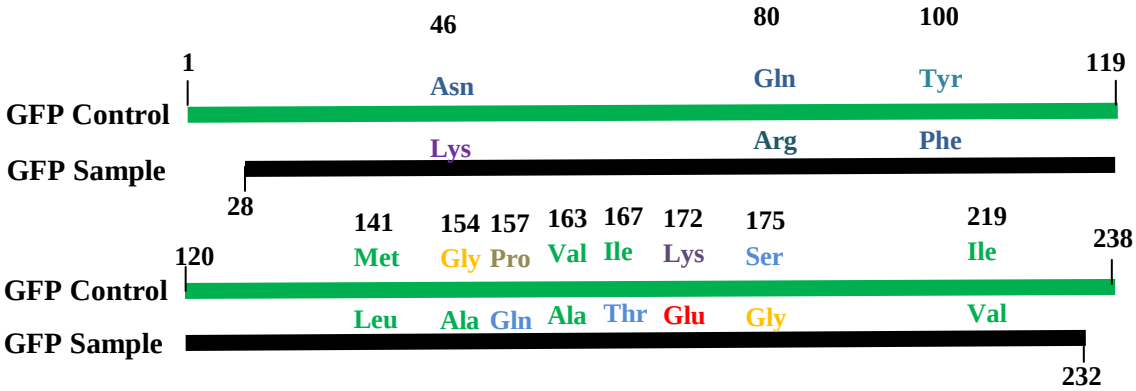

Figure 6. Comparison of Amino acid sequens between *Aequoria vctoria* (control) and *Rhizostoma* sp.(sample).

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O-MB07

Epidermal metabolomic comparison of *Senecio jacobaea*, *Senecio aquaticus* and their hybrids

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ABSTRACT

The epidermis protects the inner-cell leaf from the external environment. Therefore, metabolome in the epidermis is important as the first barrier against abiotic and biotic factors. In this study we investigated the epidermis metabolome of *Jacobaea vulgaris*, *Jacobaea aquatica* and their hybrids. For isolation of epidermis extracts, carborundum abrasion (CA) technique was applied. Subsequently, ^{1}H nuclear magnetic resonance (NMR) spectroscopy and multivariate data analyses were applied to compare the metabolome of the epidermal extracts with the abraded (mesophyll) extracts. Orthogonal partial least-squares-discriminant analysis (OPLS-DA) of the processed ^{1}H NMR showed a clear separation among the two different tissue extracts. The epidermal extracts contained significantly higher amounts of phenylpropanoids which were four times as much 5-*O*-caffeoyl quinic acid (CQA) and one and a half times as much 3-*O*-CQA and feruloyl quinic acid (FQA) compared to the mesophyll extracts. Both CQA and FQA are known for their inhibitory effect on herbivores and pathogens [1)]. Also the defence compounds jacobine-type pyrrolizidine alkaloids (PAs) were identified. They were slightly increased in the mesophyll. Comparison of the epidermal extracts revealed a clear discrimination between parental species and hybrids. *J. vulgaris* contained higher amounts of proline, succinic acid and jacobine-like PAs while *J. aquatica* contained higher amount of sucrose, fructose, and the PA senecionine. The hybrids metabolome contained compounds of both parents in intermediate amounts and no new compounds were detected. Our results emphasize the importance of studying the appropriate leaf tissue for chemical defences of herbivores and pathogens.

Keywords: *Senecio* sp, defence compounds, tissue distribution, metabolomics, NMR

O-MB08

STUDY ON GENETIC DIVERSITY AND CONSERVATION OF ORCHIDS IN WONOSADI FOREST, GUNUNGKIDUL BASED ON MOLECULAR ANALYSIS

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ABSTRACT

Wonosadi forest is located between Dusun Duren and Dusun Sidorejo, Beji village, Ngawen, Gunungkidul. The biodiversity inside Wonosadi is protected by people around Wonosadi using local wisdom. One of the endemic biological diversity in Wonosadi is natural orchid. Conservation which is related to biodiversity were needed to maintain the existence of natural orchid in Wonosadi sustainly. Study on natural orchid in Wonosadi can be used as the database for conservation programs. In this study, genetic variation was analysed using random amplified polymorphic DNA (RAPD), while viruses were detected using reverse transcript-polymerase chain reaction (RT-PCR).

The results show that there were genetic diversity in the populations of natural orchid in Wonosadi. It can be concluded that the population of natural orchid in Wonosadi can be adaptive to environmental change. Genetic diversity is required for populations to evolve to cope with environmental change. It can be used as a database to develop the potential of natural orchid in Wonosadi forest. The viruses found in population of natural orchid in Wonosadi were Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV). Viruses existance might be recognised from the physical symptom on plant and analysis of coat protein (CP) gene using polymerase chain reaction (PCR).

The research of diversity and conservation of natural orchid in Wonosadi forest may be used to support education for sustainable development (EfSD) concept in conservation of biological diversity. Exploration activity can be focused at area which is protected by local wisdom and involves local people. By doing this, local people may be actively included on protecting and developing biological diversity at their own comunity. Thus, it may result a sustain condition of natural resources. This research can be used in supporting and developing programs of natural orchid in Wonosadi forest in order to develop the conservation programs.

Keywords : genetic diversity, natural orchid, CyMV, ORSV, conservation

INTRODUCTION

Wonosadi forest is located between Dusun Duren and Dusun Sidorejo, Beji village, Ngawen, Gunungkidul. The biodiversity inside Wonosadi is protected by people around Wonosadi using local wisdom. The entire area of Wonosadi forest is about 25 Ha. About 15 Ha of the entire area is located in Dusun Duren, and the rest (10 Ha) is located in Dusun Sidorejo. Wonosadi forest consists of core zone and buffer zones. In the core zone, there was customary rules that forbid on taking anything in the forest. Whereas, the buffer zones were utilized by local people for plantation, especially woody plants.

Wonosadi forest is managed by local wisdom. It makes the biodiversity inside Wonosadi is totally protected by people around this forest. One of the endemic biological diversity in Wonosadi is natural orchid. Orchid in tropical forest can grow naturally and diverse, from epiphyte to terrestrial [2 & 3]. Orchids that natively grow in Wonosadi are terrestrial orchids that can be found in open area with high intensity of light. Conservation which is related to biodiversity were needed to maintain the existence of natural orchids in Wonosadi sustainly. It can be done through the study on diversity of natural orchid in Wonosadi [11 &14].

The maintenance of the natural orchid existence in Wonosadi forest can also be done by detection of disease that harm the orchid. Orchids in Wonosadi grow in the area which are not maintained by local people. There was great possibility that the orchid was attacked by viruses. Two viruses that commonly found in orchid are Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) [9]. The study on genetic diversity and virus detection of natural orchid in Wonosadi forest has not been done. This research can be used as the database in supporting and developing programs of natural orchid in Wonosadi forest in order to develop the conservation programs. The people around Wonosadi can be involved to support education for sustainable development (EfSD) concept in conservation of biological diversity.

MATERIALS AND METHODS

Plant Materials

Young leaves approximately 50-100 mg of specimen will be used as the source of DNA and RNA extraction for PCR-RAPD analysis and viruses detection. Before used, the specimen will be kept in vinyl zipper bags with silica-gel until they were stored at -20° C in the laboratory [4]. Those samples were collected in May 2010 on the wet season, when terrestrial orchid in Wonosadi usually grown.

Genetic Diversity

Total DNA was extracted from young leaves collected using *Nucleon Phytopure* kit and adapted to Orchid as follows: 0,1 g young leaves extracted with Phytopure I reagent. Then, the Phytopure II reagent was added. The mixture was incubated at 65°C for 10 minute, followed by incubation at 4°C for 20 minute. Then Phytopure ressin was added, followed by extractions with isopropanol. Isopropanol was used to precipitate nucleic acids, and the pellet obtained was dissolved in Tris-EDTA (TE) buffer (10 mM Tris-HCl, pH = 8.0

and 1 mM EDTA, pH = 8.0). The total DNA was quantified by spectrophotometry [13]. DNA samples were stored at 4°C.

Six decamer oligonucleotides (Table 1) were used for polymerase chain reaction (PCR) amplification [10] following the procedures of Lim *et al.* (1998) with some modifications. Experiments were carried out with SuperHot *Master Mix* PCR *kit* which consists of 0,4 mM dNTPs of each, $MgCl_2$, Taq DNA Polymerase, 32 mM $(NH_4)_2SO_4$, and 130 mM TrisHCl, pH 8,8. The thermal cycler was programmed to have a cycling profile of 1 min denaturation at 95 °C, 2 min annealing at 35 °C and 2 min extension at 72 °C for a total of 45 cycles, using the fastest possible transitions between each temperature. A final extension at 72 °C for 10 min was included after the last cycle. The DNA fragments produced were visualized in a 1,5% agarose gel and stained with ethidium bromide. Replication of the RAPD reaction for every combination of template DNA and primer was carried out to ensure reproducibility. Only reproducible RAPD markers were included in the analysis.

The molecular sizes of the amplification products were estimated using 100 bp DNA ladder plus (Microzone, Ltd, UK). Bands on the photos were then scored. The RAPD bands were represented as '1' (present) and '0' (absent). The PCR was repeated at least twice in order to check reproducibility. The dendrogram following the NTSYS, UPGMA algorithm was generated with the Jaccard coefficient based on all the markers generated [12].

Table 1. Primers used in RAPD analysis.

Code	Sequence 5' to 3'
OPU3	CTATGCCGAC
OPU8	GGCGAAGGTT
OPU10	ACCTCGGCAC
OPU12	TCACCAGCCA
OPU13	GGCTGGTTCC
OPU16	CTGCGCTGGA

Virus detection

RNA virus was isolated from orchid's leaf samples by grinding 0,1 gram of leaf in 1 ml Redzol reagent, followed by chloroform extraction and ethanol treatment. RNA was separated from other contaminant by centrifugation of homogenate in SiMax™ *membrane spin column*. The yield of RNA was diluted in 50 µl DEPC water. cDNA synthesis was carried out using gene specific primer (reverse primer) with reverse trancription (RT) kit from Two step RT-PCR kit (SBS Genetech). PCR step was done directly after cDNA synthesis by

using the same kit. Specific primer which were used in RT-PCR were specific for amplifying the gene coat protein of CymMV and ORSV.

Table 2. Sequences of specific primer used in RT-PCR step [7]

Primer	Nucleotide sequence 5' – 3'
CymMV CP-F1	ATGGGAGAGYCCACTCCARCYCCAGC
CymMV CP-R1	TTCAGTAGGGGGTGCAGGCA
ORSV CP-F1	ATGTCTTACACTATTACAGACCCG
ORSV CP-R1	GGAAGAGGTCCAAGTAAGTCC

Amplification of cDNA was done by Thermocycler (Eppendorf) using time-design : Pre-denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 1 minute, annealing 50^0C for 1 minute, elongation 72^0C for 2 minutes, and post-elongation 72^0C for 7 minutes. Cycle was programmed for 34 cycles. For further analysis, PCR products were analysed by electrophoresis in 2% agarose gel in TBE buffer. gel was stained with ethidium bromide (1 µg/10 ml aquades. The DNA bands on gel were examined under UV-transilluminator. DNA marker 100 bp was used to estimate the size of PCR products.

RESULTS AND DISCUSSION

The diversity of orchid in Wonosadi forest were analysed using RAPD method. The samples were taken from 3 populations : Pelataran Ngenuman (population 1), east buffer zone (population 2), and west buffer zone (population 3). From the observation, there were 3 species of natural orchid in Wonosadi forest, *Pecteilis sussanae*, *Liparis sp.*, dan *Spathoglottis sp.* RAPD results were shown in DNA fragments :

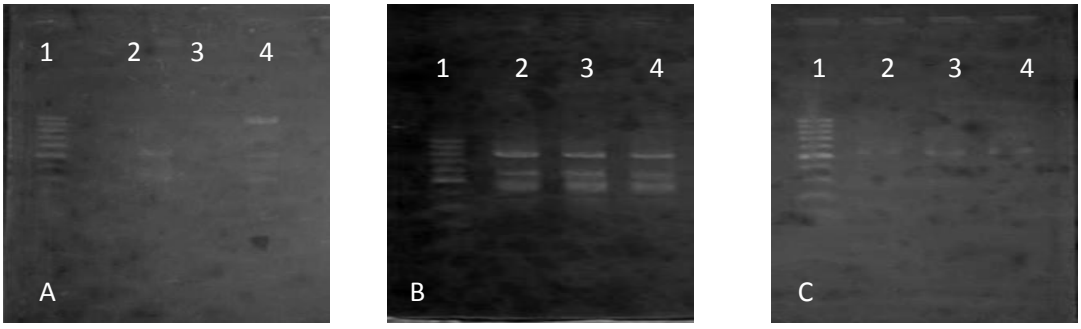

Figure 1. RAPD profiles of *Pecteilis susannae* using primer (A.) OPU 8, (B.) OPU 3, (C.) OPU 10, (1.) DNA ladder, (2.) Population 1 (3.) Population 2, and (3.) Population 3.

DNA profiles than analysed using NTSYS program to construct the dendrograms for each species :

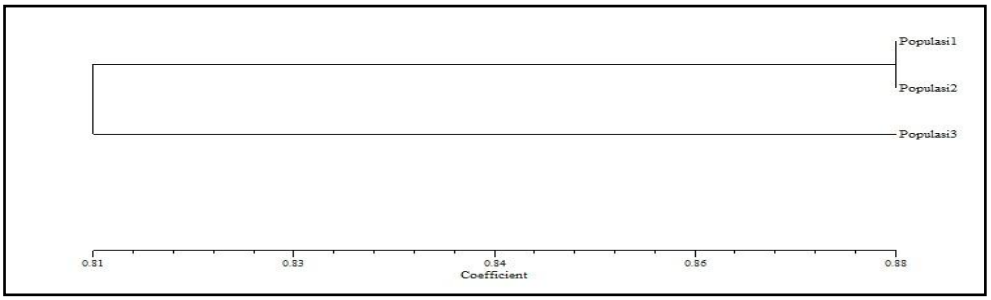

Figure 2. *Pecteilis susannae* dendrogram on 3 populations in Wonosadi forest.

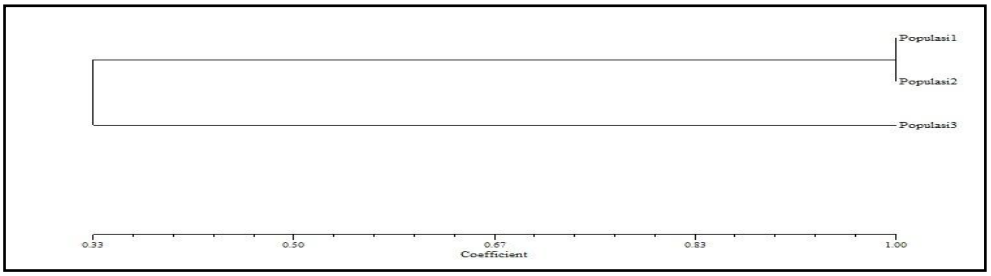

Figure 3. *Liparis sp.* dendrogram on 3 populations in Wonosadi forest.

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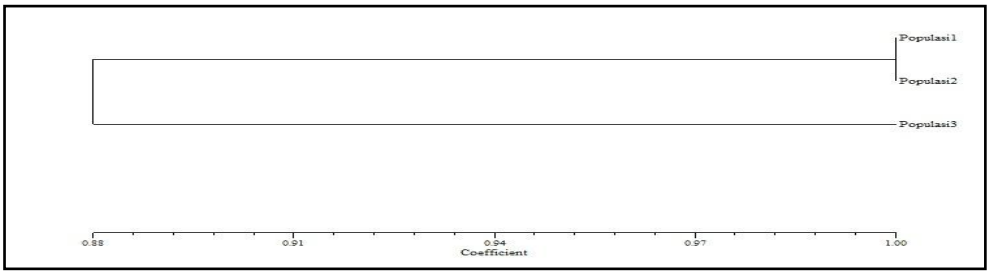

Figure 4. *Spathoglottis plicata* dendrogram on 3 populations in Wonosadi forest.

The results showed that 3 populations of orchid in Wonosadi were separated in 3 different branches. It revealed that there were genetic diversity in those orchid populations caused by adaptation in different habitat. Population 1 and 2 were located inside Wonosadi forest. Both were natural populations with minimal influence of human. Whereas, population 3 were located outside Wonosadi forest which is bordering with villages. Genetic diversity describes the evolutionary potential of population. Since evolution, at its most basic level, is a change in the genetic composition of a population, it only occurs when there is genetic diversity [6]. Genetic diversity allows populations to tolerate a wide range of environmental extremes. Loss of genetic diversity is often associated with inbreeding and reduction in

reproductive fittness and survival [1]. Genetic diversity in populations were required to respond the environmental change and avoid extinction [5].

Virus Detection

Based on electrophoresis result, it was known that Wonosadi orchid was infected by ORSV. DNA band at ±474 bp was appeared *Liparis* sp. sample (L2) and was the only positive sample for ORSV. Liparis was also assumed being infected by CymMV since there was faint band at ±669 bp. Compared to other Wonosadi orchids, Liparis has a thinner and smoother leaf which may lead to it's higher sensitivity to virus than other.

Other orchid that were not infected by virus (according to electrophresis result) may show similar symptom as virus-infected orchid since virus' symptoms is varied among orchid. The similar symptom may appear due to other pathogen attacks or extreme environmental factors.

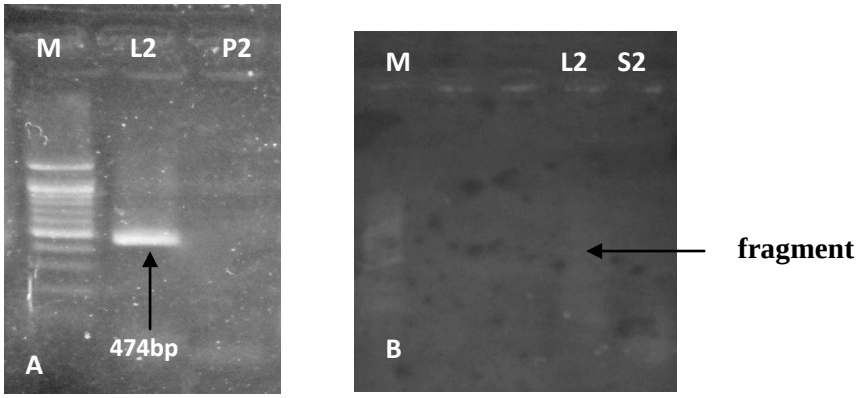

Figure 5. Electrophoresis result of PCR product for ORSV at 474 bp (left) and CymMV at 669 bp (right). M = marker; L2 = *Liparis* sp.

Activities Based on EfSD implementation

Research on genetic diversity and virus detection of natural orchid in Wonosadi forest has become potential effort to support Education for Sustainable Development (EfSD) concept. The activities may be foccused on biodiversity exploration around Wonosadi. Exploration activity can be focused at area which is protected by local wisdom and involves local people. By doing this, local people may be actively included on protecting and developing biological diversity at their own comunity. Thus, it may result a sustain condition of natural resources.

The implementations of EfSD concept were based on the results of the research. By understanding the genetic diversity of natural orchid, local people can be more familiar to orchid species inside Wonosadi, its potential, and how to maintain in an appropriate way.

Whereas, the research of virus detection in natural orchid can assists local people in understanding the symptoms and how to cope with the illness caused by viruses. The results of this research can be used to optimize the natural orchid conservation. The implementation of EfSD in this research was manifested by building of “Rumah Anggrek”. “Rumah Anggrek” was expected to be the center of ex-situ conservation of natural orchid which located near Wonosadi. Natural orchids from Wonosadi were taken and grown in ”Rumah Anggrek”. It also facilitates people who want to see the natural orchids in Wonosadi, without entering the forest. The making of “Rumah Anggrek” was conducted by cooperation with local people and students of KKN-PPM program from Gadjah Mada University. The development of natural orchid’s potential in Wonosadi Forest can also be used in ecotourism activities. It could support the economy condition of local people around Wonosadi.

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O-MB09

Embryonic Calli Induction ,Proliferation and Regeneration of Rodent Tuber Plant (*Thyphonium flagelliforme* Lodd.) by single node culture

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Abstract

The rodent tuber plant (*Thyphonium flagelliforme* Lodd.) is a medicinal plant which shows detoxificying, antineoplastic or anti-cancer agent, antibacterial and antiviral activities. Contains bioactive compounds such as alkaloid, flavonoid, saponin, steroid and glycoside. However, the genetic variation in this plant is relatively low. The purpose of this study is to found optimal media for calli induction, proliferation of calli and shoots induction from embryogenic calli. Using tissue culture technique and to obtain optimal composition of plant regulators having the ability to regenerate plantlets from embryogenic calli. Single node of rodent tuber was sterilized and cultured on MS basal medium. Embryogenic calli were induced on MS basal medium and treated 1 mg/l NAA and 0.5 mg/l BAP. Proliferated calli were treated within various concentration of 2.4-D : 0.5 mg/l, 1 mg/l and Kinetin : 0.1 mg/l , 0.2 mg/l , 0.3 mg/l. The best embryogenic calli were produced on medium using 2.4 D 0.5 mg/l and Kinetin 0.1 mg/l. The embryogenic calli have genereted up to 14.38 plantlet per explant on MS basal medium and treated with 1 mg/l NAA dan 0.5 mg/l BAP. This study suggests the plantlet can be regenerated in significant amounts through the induction of embryogenic calli from single node.
Keywords : *Thyphonium flagelliforme*, single node, embryogenic calli, 2.4 D, NAA, Kinetin

Introduction

Rodent tuber (*T. flagelliforme* Lodd.) is a medicinal plant belonging the family Araceae, native to Indonesia which are found in Java and grow well at an altitude of 100-300 m above sea level (Essai, 1986). It is an herbal plant that has a detoxifying agent. This plant is found to have the potential to cure cancer. All parts of plant, namely roots, stems, leaves and flowers contain bioactive compounds that function as anticancer agent. It can grow up 30 cm tall. This plant is also found in India and Sri Lanka (Nicolson and Sivadasan, 1981).

Rodent tuber is known to be useful in treating some diseases including cancers of breast, colon, prostate gland, liver, leukemia and cervical cancer (Hoesen, 2007; Heyne, 1987). It contains antineoplastic or anticancer and antiviral cpds as well as (Teo and Ch'ng, 1996). Compounds which are efficacious in this plant are alkaloids, saponins, steroids and

glycosides (Syahid, 2007). Medicinal plants contain bioactive compounds that can inhibit pathogenic microorganisms such as bacteria, fungi and viruses (Lai et al., 2003). Choon *et al*. (2008) has stated that the rodent tuber as an anti-cancer activity and induces apoptosis.

Rodent tuber plant is generally propagated vegetatively by tillers separation / hump (Essai, 1986). Micropropagation of shoots can be induced by provision of optimal plant growth regulators. The effectiveness of plant growth regulators auxin and exogenous cytokinin depends on endogenous hormones in the plant tissues. Furthermore, cytokinins (Benzyl Adenine) commonly used in the regeneration of *in vitro* culture of plant growth regulators for this function in cell division and differentiation of adventitious buds (Bhojwani and Razdan, 1981). In this study micropropagation in vitro methods through a single node or shoot meristem can be induced shoot multiplication. The addition of plant growth regulators BA and NAA on the media is expected to produce an optimal shoot multiplication.

The purpose of this study is to found optimal media for calli induction, proliferation of calli and shoots induction from embryogenic calli.

Materials and Methods

Plant Material

Rodent tubers were obtained from Balai Tanaman Obat Bogor. The material used was single-node culture of node rodent tuber from Bogor. Node rodent tuber with buds were aseptically used as explants.

Sterilization of explants.

The rhizomes of *T. flagelliforme* were washed thoroughly with detergent and rinsed in running tap water to remove any soil particles. The single node tuber of *T. flagelliforme* were used as explants resources. The buds were excised from the rhizomes and soaked in a a solution fungicide and bactericide each for 2 hour. Thus, The explants of node tuber were sterilized using 2.5 % and 1.5 % Clorox bleach with three drops of Tween-20 for 10 minutes respectively. Explants node tuber were sterilized again using Clorox bleach 1% for 5 minute and HgCl2 0.1 % for 5 minute respectively. Explants were rinsed again using sterile water three times. Explant node tuber were grown in MS medium.

Experiment 1. Calli induction in basic culture medium for optimum in vitro culture growth of *T. flagelliforme.*

Callus was induced on MS basal medium treated with growth regulators 2,4 D : 0 mg/l, 0.1mg/l , 0.5 mg/l, 1 mg/l with BA 0.3 mg/l and NAA 1mg/l with BA 0.5 mg/l. The sterilized explants cultured in five kinds of MS medium with addition of plant hormones. Four explants of node tuber were used for each culture medium. The best medium was induced embryogenic calli with supplemented NAA 1 mg/l and BA 0.5 mg/l. Parameter is observed when the callus induction begins to form.

Experiment 2. Effect of 2,4 D and Kinetin on Proliferation embryogenic calli

The material used were the calli induced from single-node culture. Calli were obtained from previous treatment media and then sub-cultured to a new media. Embryogenic calli were proliferated on MS basal medium treated with growth regulators 2,4D and NAA. The design used was completely randomized design in factorial pattern with 3 replications per treatment. The first factor is 2.4 D :0.5 mg / l and 1 mg / l and the second factor is the third level of kinetin: 0.1 mg / l, 0.2 mg / l and 0.3 mg / l. The observed parameters is diameter of the calli, colour of calli and texture of calli.

Experiment 3. Effect of NAA and BA on Shoots Induction from calli embryogenic

Calli embryogenic were have proliferated and then its subcultured to media of shoot induction. Calli embryogenic were regenerated to be shoots on MS media with treatment NAA : 0.5 mg/l ; 1 mg/l dan 1,5 mg/l and BAP 0.5 mg/l. The parameters observed were the number of shoots formed. These experiments the number of shoots formed from each calli embryogenic after 8 weeks of culturing was recorded. The data were analyzed using ANOVA and the means compared using Tukey's pairwise comparisons at P= 0.05.

The pH of the culture media for all the above experiments was adjusted to 5.6-5.8 before autoclaving at 121^{o}C for 20 minute. The cultures were placed in a culture room with the temperature regulated 22^{o}C and 16 h fluorescent lighting with a light intensity 1000 lux.

Result and Discussion

1. Percentage of calli Induction

Callus induction in basic culture medium for optimum in vitro culture growth of *T. flagelliforme* by single node culture. MS Media were supplemented with 2,4 D 0, 0.1 ,

0.5 or1 mg/l, or 0.3 mg/l BA couldn't induce calli from single node culture of rodent tuber. This could be due to the balance of the addition of auxin 2,4 D and cytokinin BA were not optimal to calli induction.

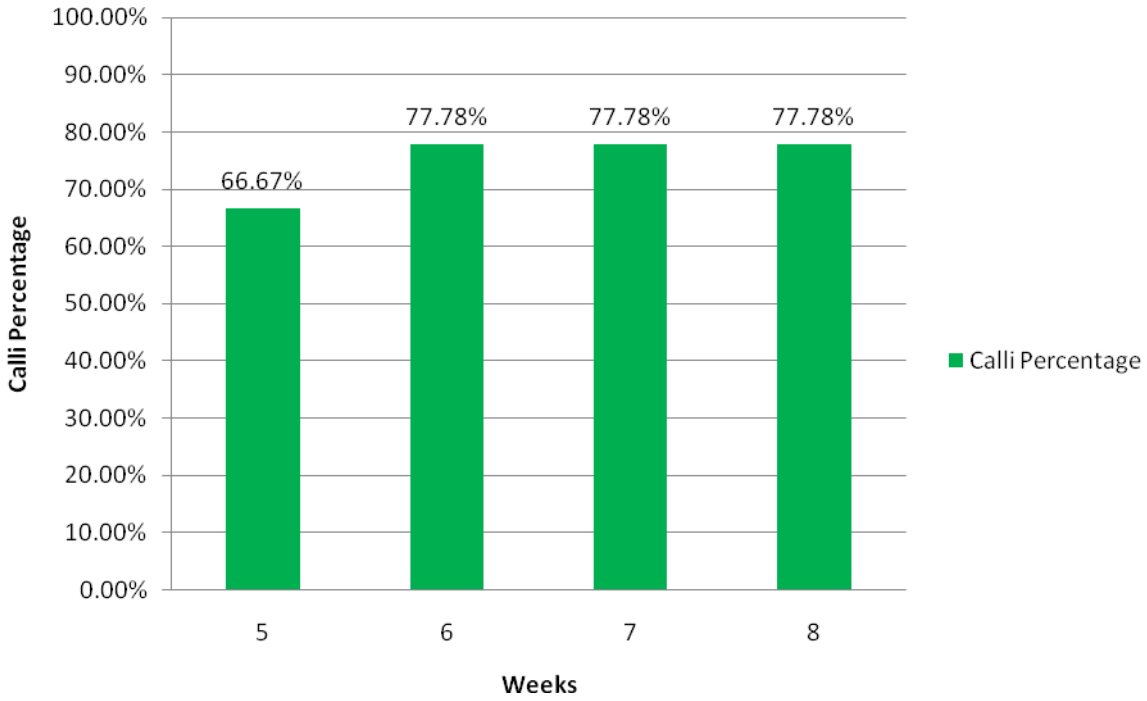

Figure 1. Percentage of induced Calli in the media MS supplemented with 1 mg/ NAA with 0.5 mg/l BA

The MS media added with 1 mg/l NAA or 0.5 mg BA induced calli from single node culture of rodent tuber up to 77.78 % (Fig.1.) The result showed that auxin NAA and cytokinin BA can be induced calli of rodent tuber after 5 weeks. According to Bhojwani and Radzan (1996), 2,4 D was a powerful normally used for callus induction. The same study was done with the induction of callus from embryo using auxin 2.4 D in Wheat (Rahman *et al.*, 2008; Kamil, 2002).

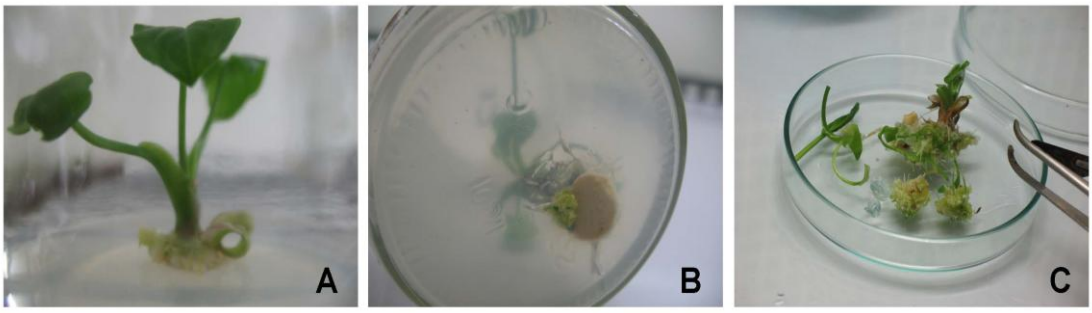

Fig.2. Calli Induction from single node culture of rodent tuber from Bogor: (A) Explants within three weeks of culture; (B) Calli induction within 5 weeks of culture; (C) Embryogenic calli winthin eight weeks of culture.

2. Proliferation Embryogenic Calli

The embryogenic calli were induced from single node culture could be proliferated in optimal medium. The best medium to proliferated embryogenic calli was MS media supplemented with 1 mg/l 2,4 D and 0.3 mg/l kinetin or 0.5 mg/l 2,4 D and 0.3 mg/l kinetin. The resulted data obtained from various treatments showed that every treatment was different (Table 1). The induced calli were friable, compact and globular structure. Produced Calli were embryogenic indicated with a light green and yellowish green color (Table 1.)

Plant growth regulators of 2,4-D is a strong auxin often used to induce callus formation from various plant tissues (Bhojowani and Razdan, 1996). Plant growth regulators of 2,4 –D is effective to initiate callus (Nagasawa and Finer, 1988). The use of auxin (2,4 D) and cytokinins (Benzyl Adenine) will enhance the process of callus induction (Litz *et al*., 1995). Cytokinins BA are commonly used in the process of regeneration in vitro culture because this plant growth regulators are function in cell division and differentiation of adventitious buds from callus (Bhojwani and Razdan, 1996).

Table 1. Effect 2.4 D and kinetin to calli proliferation of colour of calli and texture of calli after 10 weeks

Treatment	Colour of calli	Texture of calli
1 mg/l 2.4-D + 0.3 mg/l Kinetin	Light green	friable, compact, globular
1 mg/l 2.4 -D + 0.2 mg/l Kinetin	Greenish Yellow	friable, compact
1 mg/l 2.4 -D + 0.1 mg/l Kinetin	Light Yellow	Translucent, slimy
0.5 mg/l 2.4-D + 0.1 mg/l kinetin	Brownish yellow	Translucent, slimy
0.5 mg/l 2.4-D+ 0.2 mg/l Kinetin	Brownish yellow	friable, compact
0.5 mg/l 2.4-D+ 0.3 mg/l Kinetin	Yellowish green	friable, compact, globular

The effect in addition of plant growth regulator 2.4-D and kinetin showed that each treatment produced a significant different texture and colour of the embryogenic calli. The combination of auxin and cytokinin concentrations determined optimal embryogenic callus formation (George and Sherrington, 1984). Oluk and Kaskar (2005) stated that the addition of kinetin and NAA can be induced embryogenic callus on *Papaver somniferum* plant.

Table 2. Effect of 2,4 D and Kinetin to calli proliferation of Rodent Tuber Diameter after 10 weeks

Source	DF	Seq SS	Adj SS	Seq MS (mm)	F	P
24D	1	30,6	30,6	30,6	0,26	$0,614^{ns}$
Kin	2	5,6	5,6	2,8	0,02	$0,976^{ns}$
24D*Kin	2	79,3	79,3	39,6	0,34	$0,716^{ns}$
Error	18	2092,5	2092,5	116,3		
Total	23	2208,0				

Note : analyzed using ANOVA

The results showed that supplementation of 2.4 D and kinetin on MS medium could propagated calli but not significant for calli diameter. Diameter of calli can be achieved 39.6 mm per clump (Table 2). The development and proliferation of calli can be produced a embryogenic calli (Fig. 3 and 4). Among the six treatment of MS media, addition of 0.3 mg /l kinetin and 1mg/l 2,4-D or 0.5 mg/l 2.4 D tended to make the texture of calli was friable, compact and globular stucture.

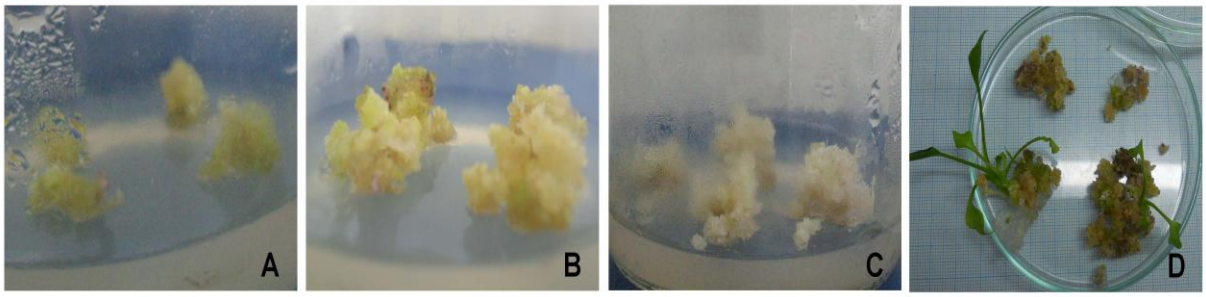

Fig 3. Development and proliferation of embryogenic Calli with supplemented 1 mg/l 2,4 D and 0.3 mg/ l Kinetin (A) Embryogenic calli within one week (B) Embryogenic Calli within four weeks (C) Embryogenic Calli within six weeks (D) Embryogenic Calli within ten weeks

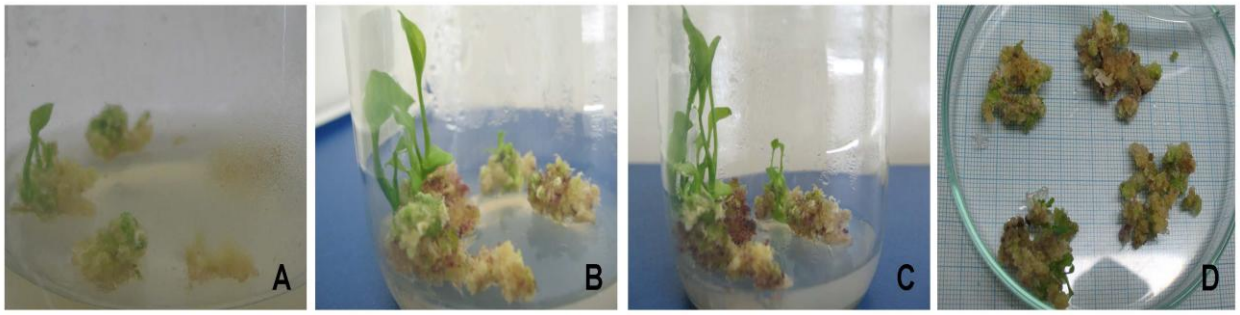

Fig 4. Development and proliferation with supplemented 0.5 mg/l 2,4 D + 0.3 mg/ l Kinetin (A) Embryogenic calli within one week (B) Embryogenic Calli within four weeks (C) Embryogenic Calli within six weeks (D) Embryogenic Calli within ten weeks

3. Shoots induction from calli embryogenic

Embriogenic calli could be regenerated on various medium. Medium used for shoots induction from embryogenic calli were MS medium with NAA and BA. Embriogenic calli were regenerated to plantlet. All shoots produced from three combination of BA and NAA with various concentrations (0.5 mg/l; 1 mg/l; 1.5 mg/l) added in MS medium produced normal shoots (Fig.5). When the combination of concentration of 0.5 mg/l BA and 1 mg/l NAA, the number of shoots induced from each clump of embryogenic calli was significantly increased. The best medium which enabled embryogenic calli to produced the highest shoot numbers (14.38 per clump) was the MS medium supplemented with 1.0 mg/l NAA and 0.5 mg/l BA (Table 3).

Tabel 3. Effect of various NAA and BA combination on production of *T. flagelliforme* Shoots from embryogenic calli after 8 weeks of culture

Treatment	Shoots number from derived of calli
1.5 mg/l NAA + 0.5 mg/l BA	8,13[b]
1.0 mg/l NAA + 0.5 mg/l BA	14.38[a]
0.5 mg/l NAA+ 0.5 mg/l BA	4,25[b]

Means followed by the same letter are not significantly different (compared using Tukey's pairwise comparisons at P= 0.05 **)**

MS medium was the best basic medium for the in vitro production of multiple shoots of *T. flagelliforme* (Sai *et al*., 2000). Tuber is commonly used a part of plant as explants in micropropagation of rodent tuber. According Nobakht *et al*. (2009), MS medium contained 5 mg/l BAP and 1 mg/l NAA can produce the most number of shoots per explant. At a higher

concentration of NAA (0.1 – 1 mg/l) , roots were produced, but abnormally shortened and thickened and a high NAA concentration (> 0.5 uM) inhibited shoot multiplication (Sai *et al*., 2000)

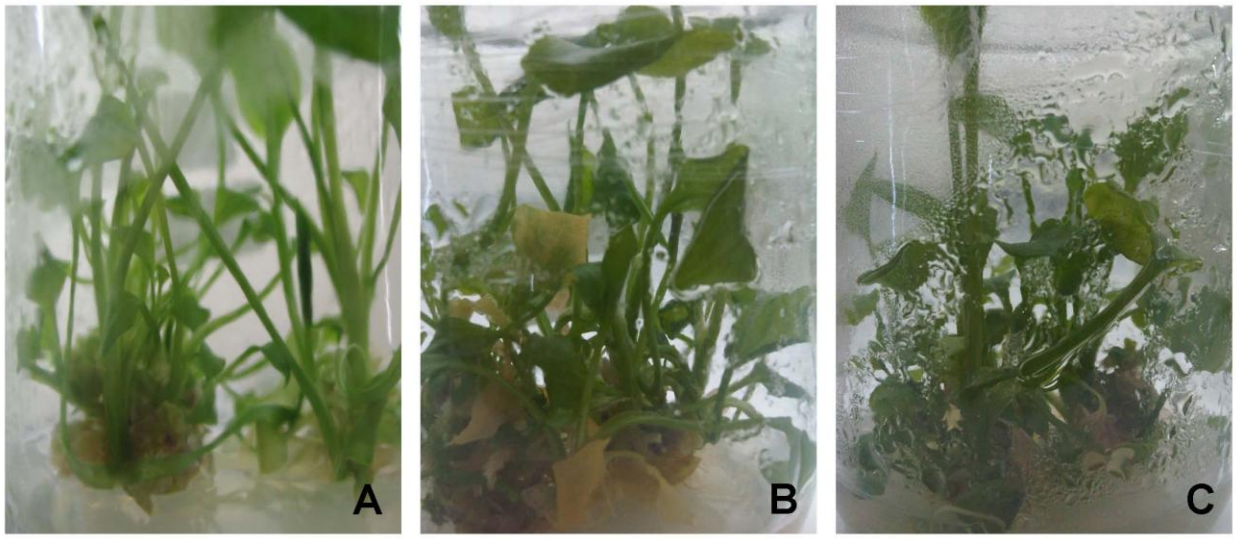

Fig.5. Induction Shoots from embryogenic Calli (A) Shoots produced in MS in the presence 1 mg/l NAA + 0.5 mg/l BA, (B) Shoots produced in MS in the presence 0.5 mg/l NAA + 0.5 mg/l BA (C) Shoots produced in MS in the presence 1.5 mg/l NAA + BA 0.5 mg/l

Conclusion

Calli induction of rodent tuber from single node can be obtained in the treatment of 1 mg/l NAA and 0.5 mg/l BA within 5-8 weeks of culture. The best proliferation of embryogenic calli on medium MS were added 1 mg/l 2,4-D and 0.3 mg/l kinetin; 0,5 mg/l 2,4-D and 0.3 mg/l kinetin. Texture of embryogenic calli were compact, friable and globular. Calour of embryogenic calli were yellownish green and light green. The embryogenic calli have been resulted up to 14.38 shoots per explant on MS basal medium and treated 1 mg/l NAA and 0.5 mg/l BAP.

Acknowlegdement

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O-MB10

Metabolite Profile of *Klebsiella* sp. Under Osmotic and Acid Shock

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ABSTRACT

A study has been conducted to determine the profile of metabolites synthesized under osmotic and acid shock in Klebsiella sp. grown in LB medium. Osmotic shock was mimicked by using NaCl, while acid shock was imposed by using aluminum sulphate. *Klebsiella sp*. was grown in LB media supplemented with NaCl, or aluminum sulphate, as a single shock-imposing substance, or by using both substances to impose a double-shock effect. Metabolite of cell was extracted using absolute methanol and was analyzed using GC/MS Rxi-5MS. The synthesised proteins indicated a different correlation pattern among shock conditions imposed. Analysis demonstrated that several metabolites were synthesized under specific shock condition. Under osmotic shock, several metabolites that are fatty acids (nonanedioic acid and tetradecanoic acid) and amides (octanamide, dodecanamid and hexanamid) were synthesized as osmoprotectant. Acid shock, on the other hand, resulted in the synthesis of metabolites of silicate anion tetramer and silicone polymer which as chelating agents aluminum. Under double-shock condition, two specific metabolites of oleic acid and stearic acid were detected. The both metabolites are a double role as osmoprotectant and aluminum chelating agents.

Keywords: osmotic shock, acid shock, *Klebsiella* sp., metabolite profile

INTRODUCTION

Klebsiella sp. are bacteria that can live in rhizosfer and able tocolonize plant roots and tolerant to environmental shock (Metting, 1993). The results of previous studies have been isolated *Klebsiella* sp. are tolerant to osmotic shock reaches 0.75 M NaCll and acidity shock $KAl_2(SO_4)_2$ reached 3000 µM in M63 mineral medium (Ikhwan *et al*., 2001).

Under conditions of environmental shock, rhizobacteria will respond physiology and affect the metabolic system (Moat & Foster, 1988). Hochachka & Somero (1984), states that, in shock conditions microbes will perform a biochemical adaptation by (1) regulation of cell macromolecular components (enzymes, isoenzyme and alloenzyme), (2) setting macromolecular cell function, and (3) setting the output macromolecular system of cells. Under conditions of osmotic shock rhizobacteria will perform three mechanisms of adaptation are: (1) osmoprotectan synthesize de novo, (2) take osmoprotectan in rhizosphere and (3) change the composition of cell walls from being damaged due to high osmotic pressure (Hartman *et al* ., 1991). According to Canovas *et al.* (1996), accumulated osmoprotectan not only to restore the turgor pressure of cell membranes, but also keep the

enzyme from inactivation by high ionic strength, and prevent damage and maintain the integrity of the cell components.

In the double shock conditions (osmotic and aluminum shock), the concentration of aluminum will affect the accumulation osmolyte cells. In conditions of high concentrations of aluminum, aluminum will close the channel K^+ so that K^+ blocked entry into the cell (Liu & Luan, 2001). Therefore K^+ ion is the major intracellular osmolyte under conditions of osmotic shock (Ohwada and Sagisaka, 1988), and the high concentration of aluminum will reduce microbial tolerance to osmotic shock. In addition, at high aluminum concentrations can occur cytoplasm aggregation (Yaganza *et al*., 2004) which would affect cell metabolism (synthesis osmoprotectan). Thus the conditions of aluminum shock, microbial tolerance to osmotic shock will decrease. On the other hand, osmotic shock will affect the inactivation of enzymes and damage to cell components (Csonka, 1989 and Canovas *et al*., 1996). This will affect the metabolism and synthesis of metabolites system cells, which can lead to accumulation of citrate decreased so that the tolerance to aluminum also decreased (Anop *et al*., 2003). Thus there is interaction between the effects of osmotic shock and shock of aluminum on cell metabolism and regulation system. Changes in metabolism and regulation of adaptive systems cause changes in the synthesized metabolites (Hochachka & Somero, 1984).

Materials and Methods

Preparation of Microorganism

Klebsiella sp. used in this research is the result of isolation and identification of a previous study. *Klebsiella* sp. grown on Luria Bertani (LB) medium (5 g yeast extract, 10 g tryptone, 5 g NaCl, 1 L H_2O) with 0.65 M NaCl as osmotic shock and acid-Al shock ($Al_2(SO_4)_3$ 1000 µM at pH 4.6) (Ayanaba *et al*. (1983).

Extraction of Metabolites

Cell harvested by cold centrifugation 4000 rpm for 15 minutes, and washed 2 times with PBS pH 7.0 solution. Pellet was resuspended in 1ml absolute methanol and sonicate in ice bucket 3 x 30 sec with repeating duty cycle 0.7. Soluble metabolites (supernatant) was separated from pellet by spin 13000 rpm for 5 min at 4 °C. The supernatant was concentrated by freeze dryer essentially as described by Christoph *et al*. (2007).

Analysis of Metabolites by GC-MS

Metabolite composition was analyzed using GC-MS (Gas Chromatography with Mass Spectrometry) Shimadzu QP2010S. The 1-*µ*L aliquots of the extracts were injected into a RXi-5ms DB5-MS capillary column (30 m × 250 *µ*m i.d.). The initial GC oven temperature was 70 °C, 5 min after injection the GC oven temperature was increased with 5 °C/min to 320 °C and held for 30 min at 320 °C. Helium was used as a carrier gas and

pressure programmed such that the helium flow was kept constant at a flow rate of 1 mL/min. Detection was achieved using MS detection in electron impact mode and full scan monitoring mode (*m*/*z* 33-600) essentially as described by Maud, *et al*. (2006).

Results and Discussion

Profile of Metabolites

The results of analysis by GC / MS, showed the number of metabolites that differ between osmotic and acid-Al shock. Metabolites were detected from each treatment were: (A) osmotic shock 23 metabolites, (B) acid-Al shock 15 metabolites and (C) the double shock (osmotic and acid-Al) 7 metabolites (figure 1). According to Hiller *et al*. (2007) microbial metabolite profiles determined by kind of growth medium and shock.

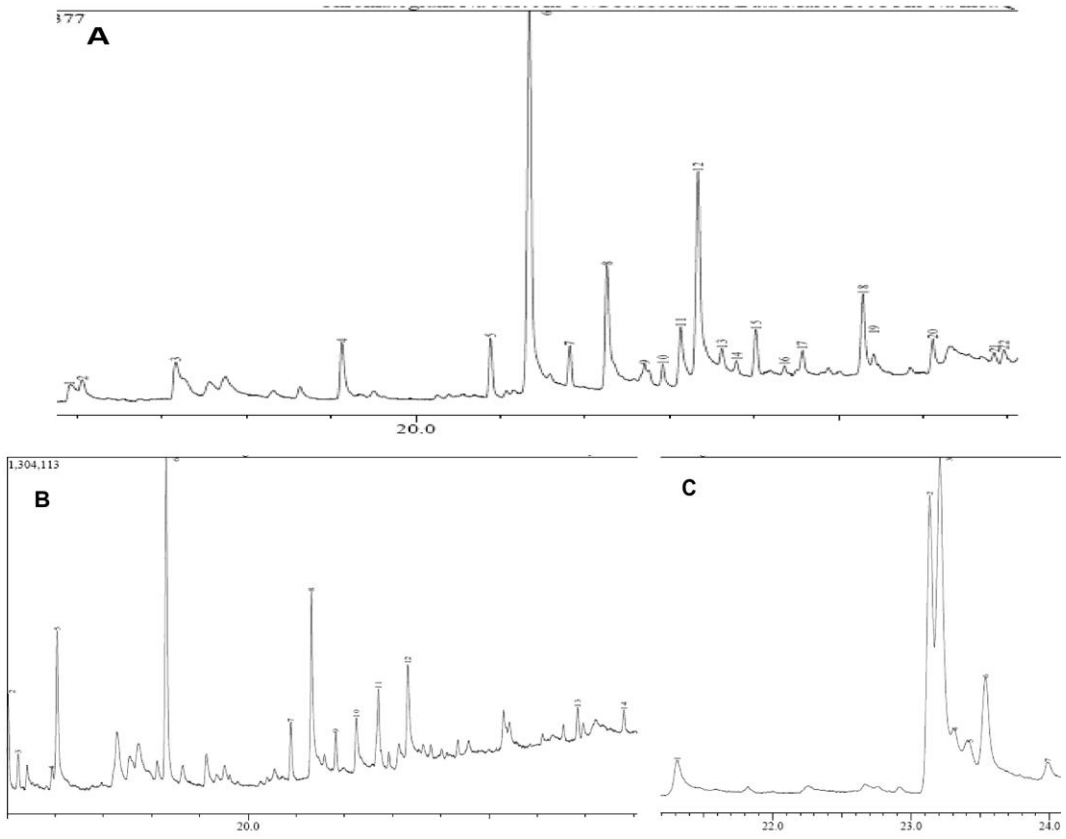

Figure 1: The GC/MS analysis of cell metabolite profile in several shock, A: osmotic shock, B: acidity shock and C: osmotic and acidity shock

Principal Component Analysis (PCA) of Metabolites

The result of principal components analysis showed that there are differences in the distribution of metabolites of each treatment on Component I (the influence of shock) and Component 2 (kind of metabolite) (Figure 2).

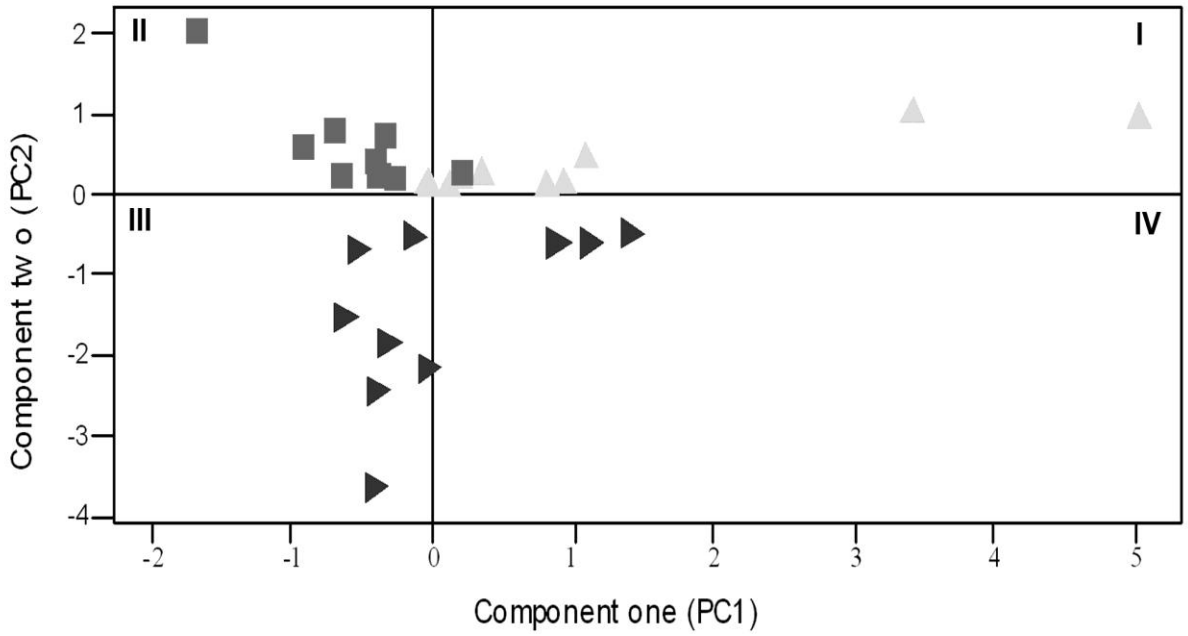

Figure 2: Principal-components analysis of metabolites *Klebsiella* sp. various treatments, ■: osmotic 0.65 M NaCl, ▲: acid-Al ($Al_2(SO_4)_3$ 1000 ppm) and ►: osmotic 0.65 M NaCl + acid-Al ($Al_2(SO_4)_3$ 1000 ppm). PC1: effect of shock and PC2: the type of the metabolites

An osmotic shock, specific metabolites synthesized tend to have negative correlation to the effects of shock (PC1) but positively correlated to the type of metabolite (PC2), in contrast to acid-Al shock tend to be positively correlated both to the effect of shock (PC1) and type of metabolites (PC2). On the other hand at double shock (osmotic and acid-Al shock), metabolites tend to negatively correlated to the effect of shock (PC1) and the kinds of metabolites (PC2). It shows that these metabolites are different metabolites that have a specific correlation both on the influence of shock and the kinds of metabolites (Dunn, 2008). Positive correlation indicates that the effect of high shock causes the synthesis of many specific metabolites, reverse the negative correlation indicates that the influence of high shock causes the less specific metabolites are synthesized.

Analysis of Specific Metabolites

Result of metabolite analysis from each treatment, there are 2 metabolites basalt, 2 non-specific metabolites and 16 specific metabolites (Table 1). The specific metabolites consisted of 5 metabolites of osmotic shock, 6 metabolites of acid shock $Al_2(SO_4)_3$ and 4 metabolites of double shock (osmotic and acid-Al shock). According to Borner *et al*. (2007), microbes will synthesize specific metabolites when responding to environmental shock.

Table 1. Analysis of metabolite specific, not specific and basalt, from each treatment shock

Retention time (menit)	Metabolite	Metabolite consentration (%)			
		Osmotik	$Al_2(SO_4)_3$	Osm+ $Al_2(SO_4)_3$	Dsicription
15.02	Alloaromadendrene	-	6.31	-	sp
16.04	Delta.-Guaiene	-	11.93	-	sp
17.16	Nonanedioic acid, dimethyl ester	2.18	-	-	sp
18.30	Patchouli alcohol	-	28.27	-	sp
19.12	Tetradecanoic acid	3.72	-	-	sp
20.88	Eicosanoic acid, methyl ester	3.18	3.9	-	nsp
21.31	Hexadecanoic acid / Palmitic acid	29.06	14.99	3.49	bsl
22.24	9-Octadecenoic acid / Oleic acid	9.27	4.36	-	nsp
22.70	9-Octadecenoic acid - methyl ester /	-	7.89	-	sp
23.13	Octanamide, N-(2-hydroxyethyl)	5.01	-	-	sp
23.15	9,12-Hexadecadienoic acid, methyl ester	-	-	29.43	sp
23.20	9,11-Octadecadienoic acid, methyl ester	-	-	40.85	sp
23.31	Octadecanoic acid / Stearic acid	19.58	9.25	7.2	bsl
23.42	9,12-Octadecadienoic acid	-	-	5,98	sp
23.62	Dodecanamide / Lauric amide	4.03	-	-	sp
23.98	Olealdehyde, dimethyl acetal	-	-	1.3	sp
24.02	Hexanamide, N-(2-hydroxyethyl)	2.57	-	-	sp
26.83	1,2-Benzenedicarboxylic acid, dioctyl ester	-	2.42	-	sp
27.79	Silicate Anion Tetramer		1.3		sp
29.20	Silicone Polymer	-	2.37	-	sp

Description: (1) sp: specific metabolite, (2) nsp: not specific metabolite, and (3) bsl: basalt metabolite,

Osmotic shock. The result of osmotic shock metabolite analysis using GC / MS obtained several fatty acids (nonanedioic acid and tetradecanoic acid) and amides (octanamide, dodecanamid and hexanamid). The Metabolites can serve as osmoprotectant under conditions of osmotic shock. According to Singh *et al.* (2002) fatty acids may function as osmoprotectant in osmotic shock conditions of high salinity. In addition, fatty acids are a major component of cell membranes, which helps maintain cell membrane transport systems and the effect on osmotic regulation system and the integrity of cell (Albert *et al.,* 1994). On the other hand amide compounds can a role in cell osmotic regulation. Nicolaus *et al*. (1989) and Csonka (1989), states that in response to changes in osmotic pressure, microorganisms will accumulate compatible solutes to adjust the osmolarity of the cell by synthesizing

osmoprotectan of inorganic cations, amino acids or amides, polyhydric alcohols and carbohydrates.

Acid-Al shock. Result analysis of metabolites in acid-Al shock was synthesized several fatty acids (oleic acid methyl ester and Benzene-dicarboxylic acid), and anion silicate (silicate anion tetramer and silicone polymer). According to Feussner & Wasternack (2002), carboxylic group of fatty acids can be negatively charged and capable of binding Al^{3+} ions. In addition, fatty acids can as a precursor of citric acid synthesis that is able to bind Al^{3+} ions, and forming a ligand system (Moat & Foster, 1988). Appana & Pierre (1996) states that, in conditions of aluminum shock, Al^{3+} ion bound by citrate in cells and secreted out of cells. On the other hand, the shock is also synthesized silicate anion tetramer and silicone polymer that can serve as chelating agents of Al^{3+} ion (Smith & March. 2001). Therefore $Al3^{+}$ ions become inactive and do not poison the cell.

Double Shock (Osmotic and acid-Al shock). Result analysis of metabolites in a double shock (osmotic and acid-Al shock) by using GC-MS are mostly fatty acid that is hexadecadienoic acid, octadecadienoic acid and linoleic acid. Fatty acids on the double shock is an efficient adaptation pattern because these fatty acids can be a double function in osmotic shock conditions as a osmoprotectant that play a role in the regulation of cell osmotic pressure and in the same time the acidity of aluminum shock conditions fatty acids may function as a chelating agent of aluminum. In addition, the fatty acid is a component of cell membranes that act to maintain cell membrane integrity in conditions of acidity and osmotic shock. According to Guerzoni *et al*. (2001), in conditions of osmotic and acidity shock affect the intracellular fatty acid composition and specific fatty acids would be synthesized in response to these shock.

Conclusion

The synthesised metabolites indicated a different correlation patern among shock conditions imposed. Analysis of metabolite demonstrated that several metabolites were synthesized under specific shock condition. Under osmotic shock, several metabolites that are fatty acids (nonanedioic acid and tetradecanoic acid) and amides (octanamide, dodecanamid and hexanamid) were synthesized as osmoprotectant. Acid shock, on the other hand, resulted in the synthesis of metabolites of silicate anion tetramer and silicone polymer which as chelating agents aluminum. Under double-shock condition (osmotic and acidity shock), two specific metabolites of oleic acid and stearic acid were detected. The both metabolites are a double role as osmoprotectant and aluminum chelating agents.

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O-MB011

INHIBITORY ACTION OF HYALURONAN-CD44 IN APOPTOSIS OF PIG GRANULOSA CELLS INVOLVES PI3K/Akt PATHWAY

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ABSTRACT

Our previous studies have revealed that Hyaluronan (HA) has ability to inhibit apoptosis in pig granulosa cells. Moreover, sufficient interaction between HA and its receptor (CD44) is necessary for cell survival signaling. The present study was aimed to elucidate relation between apoptosis inhibitory action of HA-CD44 and PI3K/Akt pathway in pig granulosa cell. Pig follicles having 3-5 mm in diameter were isolated from ovaries. Cumulus-oocyte complexes with a granulosa layer (COCG) from healthy follicles were cultured for 48 hours supplemented with FSH 50mU and various concentration of 4-MU (HA synthase inhibitor), IM7 (anti-CD44 antibody). The control group had only the medium. The protein expressions were detected using western blotting. Our data reveal that PI3K and Akt were detected in granulosa cells from healthy follicle but decreased their expression in progressing atretic follicle which most cells are apoptotic cells, indicated that PI3K/Akt pathway is involved in the cell survival and apoptosis inhibition mechanism of pig granulosa cells. PI3K and Akt were expressed in granulosa cells cultured with FSH or HA, but not in medium alone (control). Furthermore, when HA synthesis was inhibited using 4-MU, the band of PI3K and Akt also decreased, indicating that HA possibly functions through PI3K/Akt pathway. The expression of PI3K decreased after IM7 treatment, compare with FSH alone or FSH with normal rat IgG. This finding revealed that perturbation of the binding of HA-CD44 led to a decreased of PI3K expression. Hence, the binding of HA to CD44 activated the PI3K which one of survival cell pathway.

Keywords: Hyaluronan, CD44, PI3K/Akt signaling pathway, apoptosis, pig

O-MB12

Computer Aided Simulation of DNA Fingerprint Amplified Fragment Length Polymophism (AFLP) Using Suffix Tree Indexing and Data Mining

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Abstract

AFLP is one of the DNA Fingerprinting techniques which have broad application as genetic marker in various fields. Begin with the DNA sequence digestion using one or more particular restriction enzyme, ligation of the adapters to the overhanging sticky ends followed by DNA fragments amplification using PCR. The PCR reaction uses primers that match the adapter sequence and have some (1 to 3) additional "selective" bases which could be any bases, this reduces the number of bands that will be amplified. Such technique intended to increase the amplified fragments peculiarity so the polymorphism of the organism being studied could be well visualized by gel electrophoresis. The computer aided of AFLP simulation developed in this research was aimed to predict this electrophoresis result by simulate the digestion, ligation and PCR process using some pattern recognition algorithm applied to the DNA sequence from online databases. Through this simulation the researcher could determine the best combination of restriction enzyme and selective bases for their laboratory experiment. Suffix tree indexing was conducted during the exploration process of the genome sequence (in FASTA format) to find the restriction sites rapidly and create fragments of it. Data modeling enable the system draws the fragments into virtual DNA's electrophoresis pattern. Data mining accomplish the simulation by exploring overall possible virtual DNA's electrophoresis pattern and determine the best restriction enzyme and selective bases combination by calculating certain quantitative criteria.

Keywords : DNA Fingerprint, AFLP, PCR, Suffix Tree Indexing, Data Mining

I. INTRODUCTION

Since its first development in the mid-1980's, technique for DNA fingerprinting has rapidly evolved. In the field of agriculture, this technology assisted seed selection in order to acquire high quality plant such as cereals [1] and tea [2]. Many researcher suggested that Amplified Fragment Length Polymorphism (AFLP) is the best genetic marker nowadays in term of it's information quantity, reproducibility and resolution of genetic polymorphism. With this technique, DNA treated with restriction enzymes is amplified with PCR. It also allows selective amplification of restriction fragments, giving rise to large numbers of useful markers which can be located on the genome relatively quickly and reliably. Users can determine the specificity level of genetic marker by altering the restriction enzyme and sequence of bases in primer's selective bases. Unfortunately, due to the operation cost, it is

not an easy task to conduct trial and error attempt to find the best combination of restriction enzyme and selective bases. Therefore AFLP simulation program (in silico experiment) was developed in this research to help researchers simulate combinations of restriction enzymes and selective bases on virtual AFLP procedure by computational method so that they can determine the combinations that can be used to produce the desired genetic marker through in vitro experiment.

II. MATERIALS AND METHOD

Input of this computational method is DNA sequence from the online database. *Vitis Vinifera* genome sequence was taken from GenBank NCBI as an example and as much as 145 type II restriction enzymes were downloaded from the online Restriction Enzyme Database (rebase.neb.com). In order to make the simulation operational in the wet laboratory these 145 restriction enzymes were selected based on following criteria : (1) palindromic; (2) sticky end; (3) cut the DNA precisely on the restriction site; (4) no ambiguous and methylated bases on the restriction site; (5) at least one supplier available. Virtual restriction digestion then conducted by applying suffix tree algorithm as string pattern matching technique on the genome sequence. This algorithm will rapidly seek the string pattern which is match the restriction site of the enzymes being studied and then separate the genome sequence into subsequences. Hence, virtual PCR is done by exploring the compatibility between sub sequences and the primer-selective bases being studied. At the end of the simulation, exponential regression data modeling would enable the system draws the subsequences into virtual DNA's electrophoresis pattern. Data mining accomplish the simulation by exploring overall possible virtual DNA's electrophoresis pattern and determine the best possible restriction enzyme and selective bases combination by calculating certain quantitative criteria and conduct cluster analysis.

III. SYSTEM'S DESIGN

III.1 Input

DNA's genome sequence in FASTA format is required as system's raw material as well as the information of enzyme's restriction site pattern. The sequence could be store in several files (one file for each chromosome) in txt format. This FASTA sequence then considered as a text. Hence, all algorithm used in the consecutive processes should be string based algorithms.

III.2 Suffix Tree Algorithm

The first process is tracing the whole text (whole genome sequence) to find the short text (sub sequences) which is match the restriction sites of the restriction enzymes being

studied. The major computational problem when dealing with genome scale sequence is execution time due to computer's processor and memory performance limitation. It can take time up to one hour to find one short sequence along the whole genome [3]. Therefore, an effective string matching technique should be implemented to speed up the process. Hence, more restriction enzyme combination could be simulated. One popular technique to run fast string matching is suffix tree algorithm. Suffix tree are versatile data structures that can help execute short subsequences (queries) very efficiently. In fact, suffix trees are useful for solving a wide variety of string based problems [4]. For instance, the exact substring matching problem can be solved in time proportional to the length of the query, once the suffix tree is built on the database string. The example of suffix tree construction is shown in Figure 1 [5].

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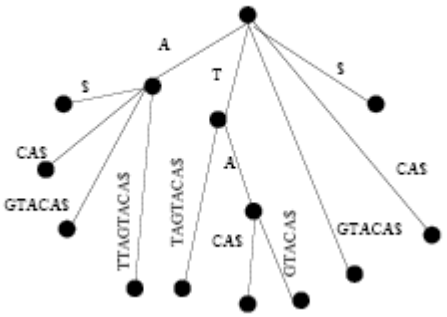


Figure 1. Suffix Tree Representation

The tree will inform every possible subsequence from a sequence as a pattern. One pattern is considered as particular path from the top node (root) to the most bottom node (leaf), for the example on the figure there are 10 possible sub sequences for the ATTAGTACA$ sequence. The $ character is added to inform the end of the sequence. There are three main function in this exploration process :

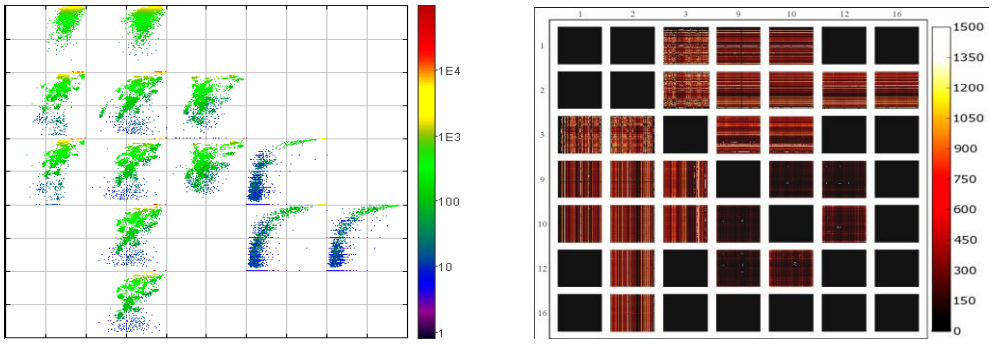
1. Build tree , construct suffix tree on the database. Every sequence (in FASTA format) subjected to the exploration should be transformed to the tree structure. Once it build, the FASTA format no longer needed so that it can be deleted and provide more space on the computer's memory.
2. Node searching, explore the tree for the queries, begin from the root (the top node) and end up at the leaf which is the most bottom node. If the query doesn't exist the system will report as "nothing". Each subsequence being found is indexed by number, represent its location on the sequence and its length (the number of the string).
3. Dispose, automatically erase the tree from the memory after it is stored on the database.

There will be 53.248 search on the *Vitis Vinifera* sequence's tree, the detail is explained in the following paragraph.

According to its restriction site, the restriction enzymes were classified into 44 groups and the simulation was conducted on 13 combinations among it. The combinations were determined as follow : (1) Three group having 4 bases of restriction site were paired with 3 group having 6 bases of restriction site, all with the most frequent match on the genome sequence; (2) The *Eco*RI and *Mse*I pair also included in the combinations although *Eco*RI do not fulfil the criteria because of there are facts that thus pair was used frequently for AFLP experiment [6,7,8,9]; (3) The restriction site of each pair do not overlap because such condition could lead bad and unpredicted restriction result. Three nucleotide selective bases were used for each subsequence's right and left hand end. Because there are 4 possible base (A,T,C,G), the total combination for selective bases should be 4^6 = 4.096. Therefore the total run for searching process on the tree is 13 x 4.096 = 53.248.

III.3 Cluster Analysis

The exploration result from the suffix tree then analyse by regarding on some criteria, which are : (1) Fragment (subsequence) length; (2) Percent of "in range" fragment, the number of fragment with the length does not exceed the polyacrylamide gel range criteria divided by the total fragment; (3) Percent of redundancy, the number of fragment with same length but different sequence divided by the total fragment. The analysis was done using multi dimension cluster analysis. The example of cluster representation is shown in Figure 2.



(a) (b)

Figure 2. Cluster Respresentation, (a) restriction enzyme and selective bases combination with their percent of "in range" fragment and percent of redundancy; (b) restriction enzyme and selective bases combination with their fragment length

III.4 The Selection Criteria

In order to find the best ten combinations of restriction enzyme and selective bases, the selection criteria should be well define. The combination will be considered good if : (1) Percent of redundancy less than 25%, too many different subsequence which have same length will reduce the polymorphism information; (2) Percent of fragment "in range" more

than 75%, too many fragment “out range” cause electrophoresis failure due to most of the fragment can not well visualize; (3) The average difference of the fragment length should be large enough so that it could be nicely separate on electrophoresis process. The selection is done by applying IF THEN rules.

III.5 Exponential Regression Model

To simulate the electrophoresis process, the system provide 1 Kbp DNA ladder from which the exponential model was developed. The exponential model between fragment size (bp) and its distance (cm) from the well is as follow:

$$\ln(\text{size}) = 10.81 - 0.736 * \text{distance}$$

IV. RESULT AND DISCUSSION

IV.1 Genome Description

The FASTA format of *Vitis Vinifera* genome sequence was separated in 19 different txt file, one file for one chromosome. Table 1 contains the description of each chromosome sequence component :

Table 1. The *Vitis Vinifera* Chromosome Sequence Description

Chromosome	Ambiguous bases per 1000 bases	GC Content (%)	Size of FASTA file (kb)
1	28	34,45	15.701
2	83	34,48	17.682
3	48	34,42	10.233
4	54	34,40	19.380
5	47	34,85	23.533
6	55	34,45	24.257
7	70	34,46	15.302
8	36	34,47	21.654
9	31	33,69	16.607
10	55	34,53	9.691
11	21	34,46	13.999
12	49	34,51	18.624
13	38	34,19	15.260
14	25	34,57	19.568
15	25	33,72	7.728
16	32	34,14	8.196
17	33	34,89	13.118
18	23	34,70	18.780
19	33	34,05	14.135
Total	**42**	**34,43**	**300.211**

IV.2 Exploration Process Performance

The main problem when facing with simulation of genome scale sequence is the operation time, but it is proven that by conducting suffix tree algorithm the operation time could be reduced significantly. Table 2 describes the time needed for suffix tree construction based on the size of the genome sequence. It is shown that the time needed increase in

linear form with the size of genome sequence, however the system could still operate in reasonable time (less than 3 minute) to handle genome sequence up to 12.1 Mbp long.. Once the suffix tree is constructed, all short pattern searching could be done in no time.

Table 2. Time for Suffix Tree Construction Based on Genome Sequence Size

Size of Genome Sequence (Mbp)	Time (second)
2,43	17
4,87	32
7,3	52
9,74	66
12,1	80

IV.2 Restriction Result Description

By conducting the data mining technique, there are several information that could be infer about the restriction result. It is known that a lot of small fragments were formed using a pair of restriction enzyme with 4 nucleotide restriction site, in the other hand just few bigger fragments were formed using a pair of restriction enzyme with 6 nucleotide restriction site. This facts were inline with the restriction digestion theory, restriction site with many nucleotide will have less probability to match the genome sequence. Therefore, the combination of restriction enzyme with 4 and 6 nucleotide of restriction site seems to be the better choice. These combinations will produce moderate number of fragments with moderate length as well.

IV.3 The Best Ten Combinations

Regarding to the selection criteria, the best ten combinations of restriction enzyme and selective bases were found. Table 3 describes thus combinations.

Table 3. The Best Ten Combinations Description

Rank	Restriction Enzyme		Selective Base		Range	Fragment in range		Redundancy	% of Restriction	% of Amplified Fragment
	1	2	1	2		Total	%			
1	AATT	ATGCAT	GCA	TAA	25-150 (126)	48	80,00%	22,92%	1,24%	0,04%
2	AATT	ATGCAT	GCA	CCA	25-150 (126)	44	80,00%	25,00%	1,24%	0,03%
3	AATT	AAGCTT	GCA	CTC	25-150 (126)	43	82,69%	23,26%	1,23%	0,03%
4	AATT	ATGCAT	GCA	TCT	25-150 (126)	42	76,36%	19,05%	1,24%	0,03%
5	AATT	ATGCAT	GCC	AAA	25-150 (126)	42	80,77%	23,81%	1,24%	0,04%
6	AATT	AAGCTT	GCA	ACA	25-150 (126)	41	77,36%	24,39%	1,23%	0,04%
7	AATT	ATGCAT	GCA	GAA	25-150 (126)	40	75,47%	20,00%	1,24%	0,03%
8	AATT	ATGCAT	GCC	TAA	25-150 (126)	38	77,55%	15,79%	1,24%	0,03%
9	AATT	ATGCAT	GCA	CGA	60-400 (341)	38	77,55%	21,05%	1,24%	0,03%
10	AATT	AAGCTT	GCA	TTT	25-150 (126)	38	77,55%	21,05%	1,23%	0,04%

Figure 3 depicts the visualization of virtual electrophoresis pattern based on the exponential regression model using 1 Kbp DNA Ladder. The blue line indicate that there is only one kind of subsequence with particular size, the green line indicate that there are two kind of subsequences with the same size, the red line indicate that there are three kind of subsequences with the same size and finally the black line indicate that there are more than three kind of subsequences with the same size. The black line should appears as the most thick and bright band in real gel electrophoresis result.

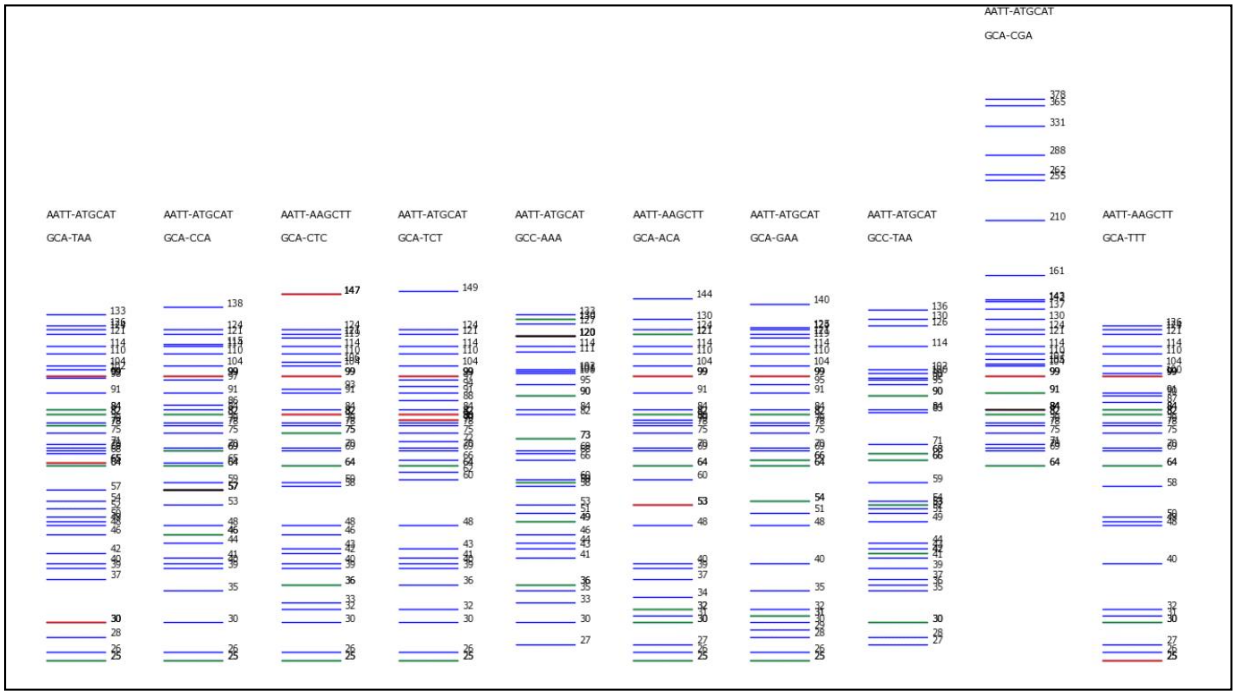


Figure 3. Visualization of The Virtual Electrophoresis Pattern

V. CONCLUSION

Like other simulation software, many factors embedded in laboratory experiment could not completely cover in this system, so that the result should be considered as recommendation (certainly with its probability of failure). However, so far the simulation result of AFLP with suffix tree indexing and data mining shows quite promising guidance for the laboratory experiment. The system developed in this research is a prototype from which more automatic and integrated system could be easily constructed. Machine learning technique such as genetic algorithm could be implemented to automate the optimization of selection criteria. At the end, laboratory conformation for this research result still could not leave behind. Therefore in the short incoming time such laboratory experiment should be conducted.

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O-MB13

Genetic Diversity of an Indigenous Balinese Bird (*Leucopsar rotschildi*) from Bali and Overseas Breeding Sites

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Abstract

Bali endemic starling or Bali mynah (*Leucopsar rotschildi*) is an endangered Balinese bird in Indonesia with their natural habitat located in Bali Barat National Park. The very exotic starlings are increasingly in demand to be raised as pets; many are stolen or poached from their natural habitat. Conservation has been performed by sending and breeding the birds overseas to American and England zoos since 1950's. After an overseas breeding process, the birds were returned to Bali Barat National Park and released into their natural habitats or either kept in captivity after passing habituation. These birds are expected to reproduce naturally to increase their natural population. For half a century breeding of birds occurs in different environment overseas, but their genetic characteristics are not known. Therefore, the aim of this study is to investigate the genetic diversity of this Balinese starling through DNA analysis. Blood and tissue samples preserved in formaldehyde were collected from Bali Barat National Park, birds bred overseas, and from semi-range habitat in the Nusa Penida Island. The result showed genetic diversity of the indigenous Balinese starling was not found to be different after many generations. However, by acknowledging that DNA samples can be analysed from deceased bird tissues preserved in formaldehyde is a novel. This result can be used as a reference of technical analysis of DNA particularly in animal samples that are exceptionally rare as in Balinese starling.

Key words: Balinese starling (*Leucopsar rotchildi*), Bali Barat National Park, DNA.

Introduction

Leucopsar rotschildi or Bali mynah is a Bali endemic bird in a status of critically endangered species. Van Balle *et al*. (2000) [1] stated that the species was highly endangered due to vastly small population, restricted to small area, illegal poaching and failing suitable habitat within its natural range.

This land bird is distinctive by bluish colour of the body and almost completely covered with white plumage, except around the eyes that is bare similar to their skin, giving the gorgeous look. These characteristics distinguished them from their relatives, *Sturnus* that have impressively distributed through out Eurasia, Africa, and the eastern Pacific but restricted to the old world [2-3].

Many studies have been carried out to evaluate the population of the bird. Strategies have been improved to maintain a captive population size to meet the educational and conservational programs using the technique so called the Bali mynah Species Survival Plan (SSPs), an association of Zoos and Aquariums (AZA) on corporative breeding and management program (http://www.aza.org/ConScience/ConScienceSSPFact/index.html; [4]).

As summarized by Earnhardt (2009) [5], most of the captive populations are small loss of genetic diversity, and the interactions of population structure, stochastic and genetic diversity. SSPs actively manage captive populations to minimize the loss of genetic diversity [6] and maintain the population size and structure[7]. In early 1980's the population size and composition of Bali mynah SSPs has met the standard of AZA's genetic and demographic objectives (Long, 2005) in Earnhardt (2009) [5] in spite of SSP participants concerned of the ability to sustain this population in the future [5].

High demand to the birds as pets and other purposes has a negative impact to the Bali mynah population, as a result the bird population decreased. Although the population of *L. rotschildi* in overseas captivity increased and considered as a self-sustaining population[9], but based on census conducted in October 1997, 12-17 individuals birds were recorded in the entire wild population of Prapat Agung in Bali Narat National Park[10]. After a temporary recovery, in 1998 the number of individuals of *L. rotschildi* was at lowest level, fewer than 15 individuals restricted to Bali Barat National Park[1, 5]. A survey conducted in March 2005 recorded of 1.000 individuals were in captivity, but only 24 were in the wild (Bird Life International Indonesian Program 2007, Bogor).

The declining number of birds in the wild is thought to be affected by extrinsic anthropogenic factors, primarily poaching for pet trade and due to the contribution of habitat loss[5, 11]. Furthermore, Earnhardt [5] states that the decreasing number of the bird population has also affected by the intrinsic population factors (e.g. stochasticity, loss of genetic diversity) that was contributed to the large fluctuations in population size.

In order to maintain the populations of the Balinese mynah in the wild, particularly in Bali Barat National Park, during late 1980's the SSP transferred the offspring to Bali. However, the supplementation had no impact on the persistence of the wild population, possibly because poaching pressure was not mitigated [5]. The SSPs anticipate for poaching to be eliminated or greatly reduced, for they will act to reintroduce the Bali mynah birds. This action necessitates the SSPs to self-sustain birds that are demographically, behaviourally and genetically appropriate for reintroductions[5]. Reintroduction of the mynah in Bali Barat Natioanl Park may have an impact to the genetic diversity of the Bali mynah; therefore this study was conducted to find out the genetic variability of the Balinese mynah that is native to Bali Barat National Park and from birds sent to Bali through the SSPs program. With regard to the conservationists and the presence number of the *L. rotschildi* in Bali Barat National Park, sample (blood and preserved muscle tissue from deceased birds) was collected in a manner to minimize the damage of the birds.

Materials and Methods

Samples were collected from bird captives in Bali Barat National Park and Nusa Penida either alive or deceased and are preserved in formaldehyde. The samples were blood from the living birds and muscle tissue samples from preserved deceased bird.

Blood samples were collected by 1ml needle in the wing or toes of the bird's vein, 150-200µl for each of 6 birds collected. Samples were then placed in a 1.5 ml ependorf tube with 150µl cell buffer lyses. Muscle tissue samples were collected from 10 deceased birds preserved in formaldehyde.

DNA was extracted with the Phenol-Chloroform methods and precipitated in ethanol following Sambrook and Russel (2001) [12] with modifications. Mixed blood sample and lyses buffer was added with 40µl SDS 10%, 40 µl 5M NaCl, 400µl – phenol pH 8.0, and 400 µl CIAA. Mixed sample homogenised for 2 hours in room temperature. Sample was then centrifuged with the speed of 5000rpm for 5 minutes. Aqueous phase DNA was moved to 1.5ml ependorf tube, then added with ethanol 2 times as much as the volume of sample. Sample was freezed for 2 hours up to one night. Frozen sample was centrifuged with the speed of 10.000rpm for 15 minutes, supernatant was removed, but the pellet was resuspended with 900µl 700% ethanol in TE solution, centrifuged with the speed of 10.000rpm for 15 min. Supernatant was removed and the pellet was dried out in room temperature or dried in vacuum drier for 30 min. Pellet was then resuspended with 50µl 80% TE and DNA sample can be used directly for DNA analyses or keep in refrigerator before the analyses.

Before the extraction of samples, the Formaldehyde preserved muscle tissue were cut in small pieces, washed with lyses buffer for many times until the formaldehyde was removed from the sample. The next procedure was similar to the blood sample.

Extracted DNA samples were amplified in PCR machine with 6 pairs microsatellite primers, those were TH3, TH6, TH12, TH15, WB2 dan WB5. The amplification was conducted in the volume of 12.5µl which contained of 10.5µl PCR platinum super mix (Invitrogen), 1µl primer mix and 1µl DNA template (sample). Amplification was carried out in the PCR that was set up in 94 ^{o}C denaturation for 45 min., annealing at 50-60^{o}C for 1.30 min. and elongation processes in 72^{o}C for 2.15 min in 30 cycles. Amplification results and DNA standard of 100bp were electrophoreses in 6% polyacrilamide gel (PAGE) for 60min. In order to visualize the DNA bands, gel was stained in silver nitrate. DNA typing was performed by comparing the distance of DNA migration and DNA standard.

Results and Discussion

The result of DNA extractions in 1% agarose gel stained in Ethidium Bromide is presented in Figure 1. Bands of DNAs migrated at similar distances from the first loading samples. Figure 1 indicates that sample in the 8th column was not visible. The DNA band in the 7th column which sample was taken from muscle tissue preserved in formaldehyde was barely visible. Less visibility of DNA bands in the gel could be as a result of poor quality of extraction or in the case of the preserve muscle tissue, the cells may yet been bound in formaldehyde, although it has been washed for many times. However, the finding that the preserved tissues can be extracted for DNA analyses is novel information.

Figure 1. Bands of DNA on agarose gel shows the speed/length of DNA migration. Samples were collected from Taman National Bali Barat (TNBB); Bali Bird Park (BBP); Nusa Penida (NS). The name of the samples (columns from left to right) are: TNBB DR 01, TNBB DR 04, TNBB DR 05, TNBB DR 06, DNA Marker, TNBB DG 06, BBP DR 10, BBP DR 12, NS 01, NS 02, NS 03. (DR = Blood; DG = Muscle).

Out of six pairs of primers, TH3, TH6, TH12, TH15, WB2 dan WB5, only two primers (TH6 and TH15) were amplified. The electrophoresis of 6% polyacrylamide gel showed that only four of 18 samples could be amplified using the two primers. This poor result could be affected not only by technical problems such as the absent of DNA due to failure during extraction, but also the failure of DNA attachment during DNA amplification. The amplification failure could be due to mutation or the primer sites changed, result in failure of primer attachment during DNA amplifications. This may be the case because during DNA extraction, there was a quantity of DNA with good quality observed and presented on agarose gel without smear.

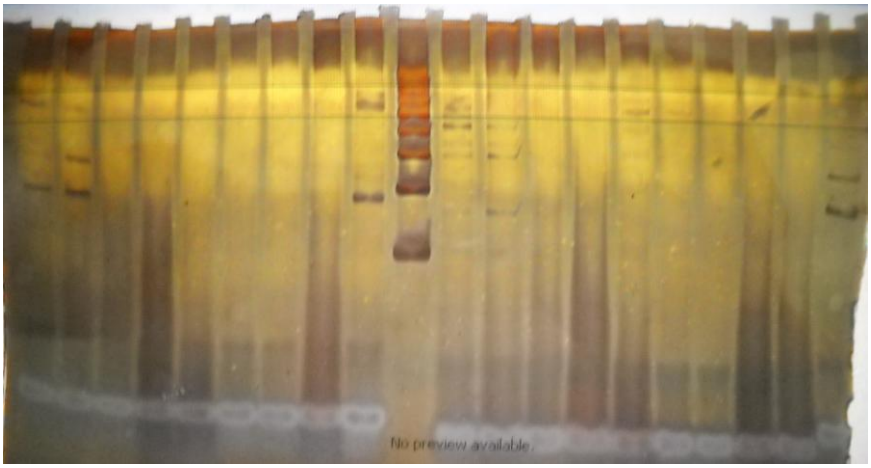

Figure 2. Bands of DNA in polyacrilamide gell. The bands shown of TH6 primer are column 1 (TNBB DR 01), 2 (TNBB DR 02), 9 (TNBB DG 04); and TH 15 primer are column 11 (TNBB DR 01), 12 (TNBB DR 02) and 20 (TNBB DG 04). Bands in column 10 is DNA ladder of 100bp.

The results also shows the length of the alleles of bird endemic to Bali Barat National Park and bird form overseas breeding (England) was not different. This means that they are a monotype genus as found by van Ballen [1] although they have been apart and bred in different condition and environment (see [1] for further historical distribution). This may also indicates that overseas breeding has similar response genetically in terms of demographic and behaviour, therefore the SSPs program on reintroducing birds bred overseas has no major impact to the genetic population of the birds in the wild. However, it is important to note that our results were only based on two alleles, therefore precaution should be taken when drawing conclusion.

The distance migration of DNA bands compared to 100bp DNA ladder, as a DNA standard, showed that each allele has similar 156bp for the TH6 primer. That means TH6 locus only has an allele (156 pb). The TH15 locus, however, has 2 alleles. One of them was allele 153bp which was found in TNBB DR. 01 and TNBB DR.02. Although the bird samples were from different source, the TNBB DR.01 from England and TNBB DR.02 from Bali Barat National Park, share similar allele. However, it should be noted that the parents of the bird from England might have been from Bali Barat National Park. The same results were found on muscle tissues from preserved deceased birds in formaldehyde.

The other allele of individual chicks from breed at Bali Barat National Park also has variations that were shown in samples of TNBB DR.01, TNBB DR02, and TNBB DG.04. This result, however, need further investigation. Other blood samples from other places (Captive bird in Nusa Penida; Figure 2: Column 18 and 19), which the parent was from England, could not confirm the findings due to the DNA that was not amplified.

Although this study has minimal results, but the novelty was the founding of the birds muscle tissues which has been preserved in formaldehyde for quite some time could be used for DNA analyses.

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O-MB14

BIOSURFACTANT CHARACTERIZATION OF BACTERIAL CONSORTIUM FROM SOIL CONTAMINATED HYDROCARBON IN CEPU AREA, CENTRAL JAVA

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ABSTRACT

Microbiology surface active agents (biosurfactant) have recently been recognize as important microbiology products with properties applicable in a number of industries and bioprocesses. Being capable of lowering surface and interfacial tension biosurfactant are today thought to be efficient replaces and possible enhancer of chemically synthesized surface active agents. Some of their superior, such as absence of toxicity, biodegrade ability and their specification, make these microbiology products both attractive for specifie industries and environtmentally acceptable. In these study, characterization of biosurfactant producing bacterial consortium were assessed by measuring surface tension, interfacial tension and emulsification activity. The result of these research showed that the surface tension was reduced to below 51 dynes/cm for bacterial consortium and the lowest interfacial tension values were obtained 10 dynes/cm, emulsification used E24 of 86,11%. The biosurfactant was a positive to contain various value of carbohydrate, protein and lipid.

Keywords : Biosurfactant, Characterization, Bacterial Consortium, Surface Tension, Emulsification Activities.

1. INTRODUCTION

Biosurfactants or microbial surfactants are surface metabolites that produced by bacteria, yeast and fungi having very different chemical structures and properties [1-2]. These biosurfactants are amphiphilic molecules consisting of hydrophobic and hydrophilic domains that find application in an extremely wide variety of industrial process involving emulsification, foaming, detergency, wetting, dispersing or solubilization [3]. Nowadays, biosurfactants are used in industries as a cosmetic and special chemical substances, food, pharmaceutics, agriculture, cleansers, enhanced oil recovery and bioremediation of oil-contaminated sites [4 -5]. They are potential alternatives of chemically synthesized surfactant in a variety of application because of their advantages such as lower toxicity, higher biodegradability, better environmental compatibility, lower critical micelle concentration, each of production, ability to be synthesized from renewable resources, higher foaming, higher selectivity, specific activity at extreme temperature, pH and salinity [2, 6]. In this recent year, the biosurfactants have been placed on the environmental impacts of chemical surfactants and new surfactants for use in any field. The aim of this study is to

characterize biosurfactant of bacterial consortium from soil contaminated hydrocarbon in Cepu Area-Central Java.

2. MATERIALS AND METHODS

a. Growth of the Biosurfactant-Producing Bacteria

The fourth strains of soil bacteria (isolate 33, isolate 34,isolate 35, isolate 38) and one bacterial consortium were obtained from a culture maintained by the Bioprocess Lab, originally isolated from a petroleum contaminated region of the Cepu Area Central Java. The strains were streaked on the surface of nutrient agar plates (Merck, Germany). After incubation at 37°C for 48 h, distinct colonies were isolated. Nutrient Broth medium was used. All of them were growth separately in a 1L Erlenmeyer flask containing 500 ml growth medium. The flask was incubated at 37°C on a shaker incubator (Pars Azma Co., type: IN07) at 200 rpm for 96 h [7].

b. Extraction of the Biosurfactants

The growth medium for each strain was centrifuged in 250 ml test tubes at 6000 rpm for 20 min at room temperature to separate the bacteria from solution. After each spin, the supernatant was collected, which was acidified to pH 2 with HCl. The biosurfactant was extracted from the supernatant using two volumes of 500 mL chloroform/ethanol (2:1) solution in a separatory funnel. The bottom layer was extracted and collected. The solvent was removed from the biosurfactant by rotary evaporation at a temperature below 40°C (Rotary Evaporator: Eyela NVC200, Tokyo Rikakikai Co,.Ltd). At heat gun was used sparingly to evaporate any remaining solvent. The weight of each product was recorded and the biosurfactants were stored in at -10°C overnight.

c. Biosurfactant Characterization

Analysis of Carbohydrate

The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test using the method of Phenol-Sulfuric Acid [8]. A volume of 0.5 ml of cell supernatant was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of sulfuric acid, and incubated for 15 minutes before measuring absorbance at 490 nm.

Analysis of Protein

The presence of protein groups in the biosurfactant molecule was assayed by Bradford Methods [9]. A volume of 100 µl of cell supernatant was mixed with 900 µl of bradford solution and incubated for 15 minutes before measuring absorbance at 595 nm.

Analysis of Lipid

The presence of lipid groups in the biosurfactant molecule was assayed by Blight and Dyer methods [10]. A volume of 1 ml of cell supernatant was extracted with 20 ml of chloroform solution and shaker during I hour. Gasses chloroform and heated in the oven and the last measure the lipid .

d. Emulsification Index (E24)

The emulsifying capacity was evaluated by an emulsification index (E24). The E24 of culture samples was determined by adding 2 ml of *Arabic Liquid Crude Oil* (ALCO) and 2 ml of the cell-free broth in test tube, vortexed at high speed for 2 min and allowed to stand for 24 h. The E24 index is given as percentage of the height of emulsified layer (cm) divided by the total height of the liquid column (cm). The percentage of emulsification index calculated by using the following equation [11,14]. $E24 = \frac{\text{Height of emulsion formed} \times 100}{\text{Total height of solution}}$

e. Surface Tension Measurement

The surface tension measurement(s) of cell free supernatant was determined in a K6 tensiometer (Krüss GmbH, Hamburg, Germany), using the du Nouy ring method. The values reported are the mean of three measurements. All measurements were made on cell-free broth obtained by centrifuging the cultures at 6000 x *g* for 25 min.

f. Interfacial Tension Measurement

Equal volume of ALCO and surfactant solution was poured into a glass beaker of diameter 4 cm and the resulting mixture used for the interfacial tension studies. The same procedure used for the surface tension measurement was used for the interfacial tension study except that the balance of the tensiometer reading for zero was checked with the platinum ring completely immersed in the ALCO phase and not in the surface or the interface of ALCO–surfactant. The platinum ring was then completely immersed in the surfactant phase before the platform was gradually adjusted until a force necessary to detach the platinum ring upward from the surfactant– oil interface was exerted (12). This experiment was repeated in triplicate at a room temperature and the average value reported.

3. RESULTS AND DISCUSSION

a. Biosurfactants Characterization

Analysis of Carbohydrate

The fourth strain (isolate 33, 34, 35, 38) and one bacterial consortium indicated their ability to produce biosurfactant that containing carbohydrate (total sugar). The results of this study shown in figure 1 below.

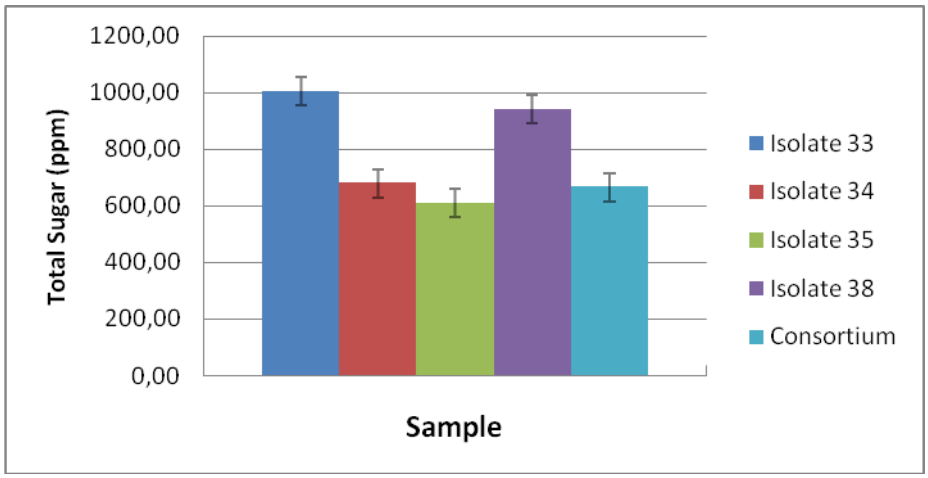

Figure 1. Quantification of total sugar for each isolate and bacterial consortium

The lowest total sugar values were obtained at isolate 35 with 608,18 ppm followed by bacterial consortium with 666,36 ppm, isolate 34 with 680 ppm, isolate 38 with 940,30 ppm and the last isolate 33 with 1006,67 ppm. It has been occurred may be due to more water solubility and availability of the substrate to the microorganism. The ester linkage in ALCO at isolate 33 and isolate 38 should be hydrolyzed to release total sugar for biosurfacant production. So, isolate 33 and isolate 38 seems to be more suitable for oxidizing ALCO component in biosurfactant production to produce carbohydarate.

Analysis of Protein

The fourth strain (isolate 33, 34, 35, 38) and one bacterial consortium indicated their ability to produce biosurfactant that containing protein. The results of this study shown in figure 2 below.

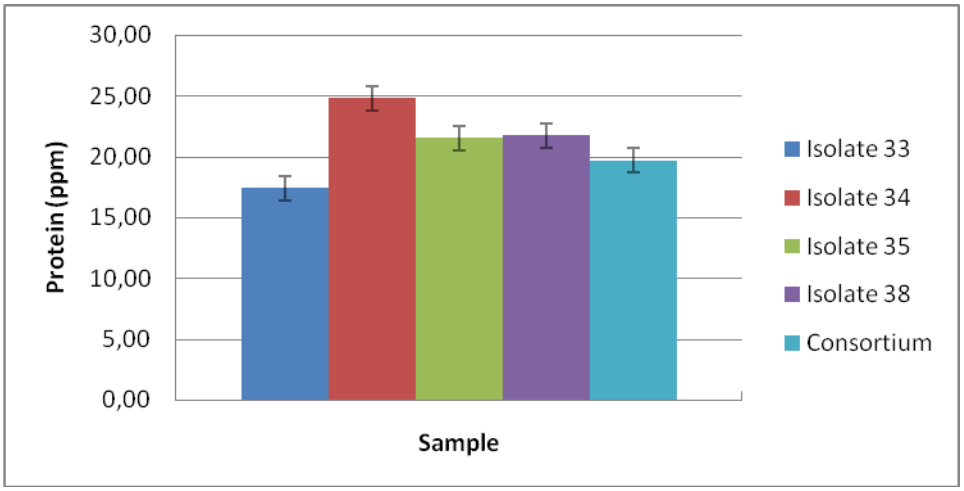

Figure 2. Quantification of protein for each isolate and bacterial consortium

The lowest protein values were obtained at isolate 33 with 17,44 ppm followed by bacterial consortium with 19,69 ppm, isolate 35 with 21,57 ppm, isolate 38 with 21,77 ppm and the last isolate 34 with 24,81 ppm. The ester linkage in ALCO at isolate 34 and isolate 38 should be hydrolyzed to release protein for biosurfacant production. So, isolate 34 and isolate 38 seems to be more suitable for oxidizing ALCO component in biosurfactant production than isolate 33, isolate 35 and bacterial consortium.

Analysis of Lipid

The fourth strain (isolate 33, 34, 35, 38) and one bacterial consortium indicated their ability to produce a small biosurfactant that containing lipid (g). The results of this study shown in figure 3 below.

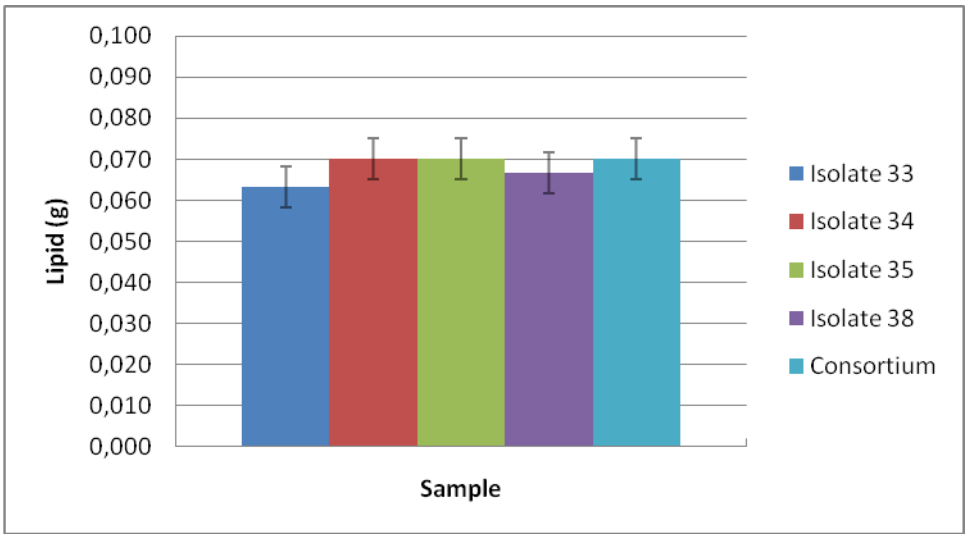

Figure 3. Quantification of lipid for each isolate and bacterial consortium

The lowest lipid values were obtained at isolate 33 with 0,063 g followed by isolate 38 with 0,067 g. Unique, bacterial consortium, isolate 34 and isolate 35 has the same value with 0,070 g each other. The ester linkage in ALCO at bacterial consortium, isolate 34 and isolate 35 should be hydrolyzed to release the same lipid for biosurfacant production. So, bacterial consortium, isolate 34 and isolate 35 seems to have the same ability for oxidizing ALCO component in biosurfactant production to produce lipid values.

b. Emulsification Activities (E24)

The emulsification activities of biosurfactant that produced by fourth isolate and one bacterial consortium were measured with triplicate treatment. The results of this study shown in figure 4 below.

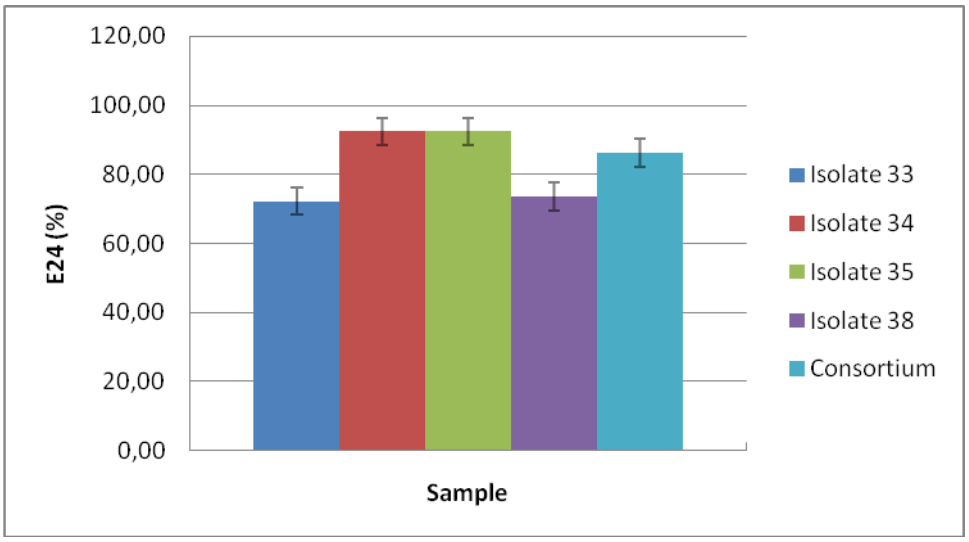

Figure 4. Prosentation of emulsification activities for each isolate and bacterial consortium

For isolate 34 and isolate 35, biosurfactant had the highest emulsification activities with each EA 92,36 % followed by bacterial consortium with 86 %, isolate 38 with EA 73,61 % and the last isolate 33 with EA 72,22 %. It is known that bacterial consortium and all of the isolates have ability to optimize uptake of insoluble hydrocarbons by producing biosurfactant that promote substrate emulsification to get into direct contact with the oil phase.

c. Surface Tension Measurement

Surfactants- reduction of air-water surface tension indicates their ability to reduce the interfacial force that holds oil and water together. The results of this study shown in figure 5 below.

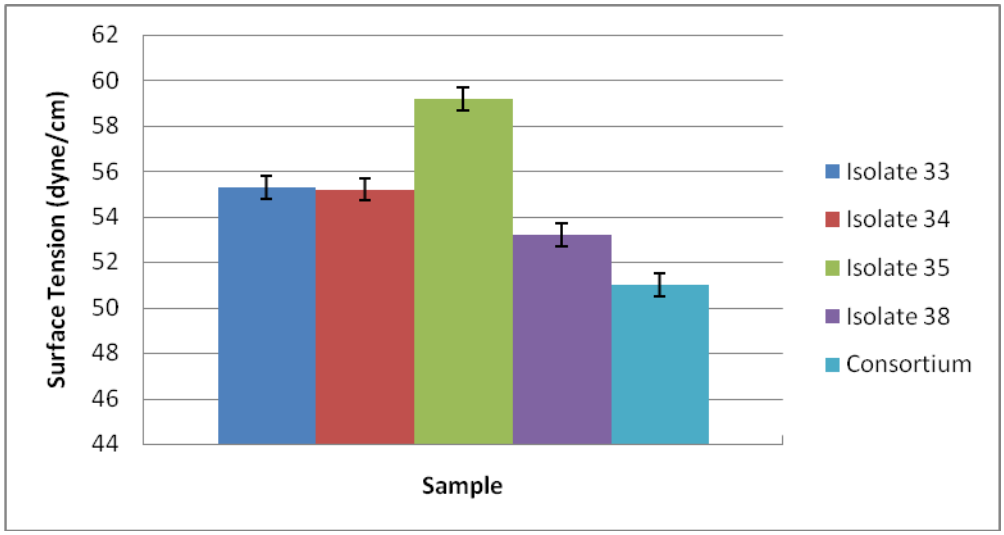

Figure 5. Surfactant activities assay results (Surface Tension)

The lowest surface tension values were obtained at bacterial consortium with 51 dynes/cm and isolate 38 with 53,2 dynes/cm. It has been occurred may be due to more water solubility and availability of the substrat to the microorganism. The ester linkage in ALCO at bacterial consortium should be hydrolyzed to release gliserol and fatty acids for biosurfacant production. So, bacterial consortium seems to be a suitable for oxidizing ALCO component in biosurfactant production.

It is known that most of bacteria optimize uptake of insoluble hydrocarbons by producing biosurfactant that promote substrate emulsification and/or solubilization and/or enhance cell hydrophobicity thus allowing the cells to get into direct contact with the oil phase (13)

d. Interfacial Tension Measurement

The interfacial tension between ALCO and distilled water was measured. The results of this study shown in figure 6 below.

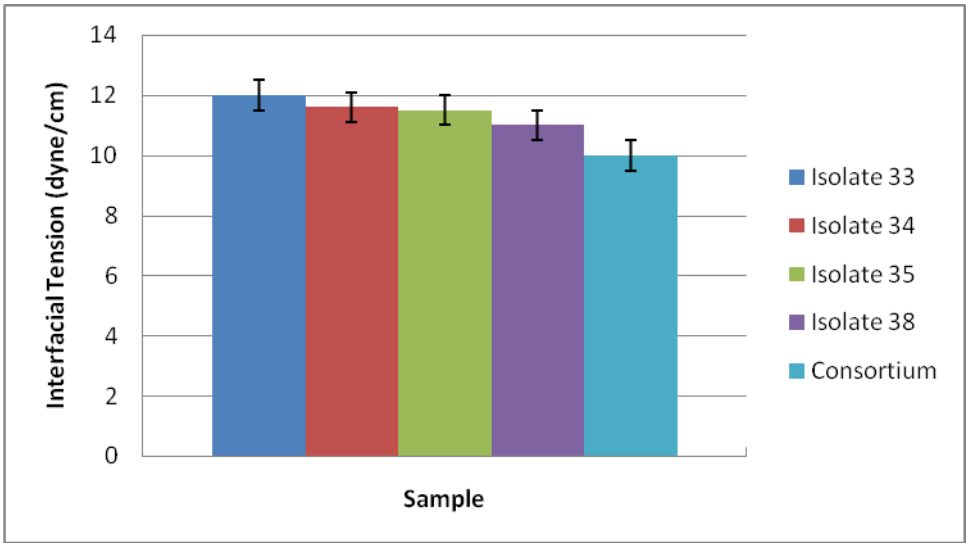

Figure 6. Surfactant activities assay results (Interfacial Tension)

The lowest interfacial tension values were obtained at bacterial consortium with 10 dynes/cm. As the surfactant solution is introduced, this value was reduced as shown in figure 6. The reduction of interfacial tension indicates the ability of surfactants to remove oil from soil. As the interfacial tension between ALCO and water is reduced, the capillary force holding the ALCO and water is equally reduced. Therefore, this reduction will increase the contact angle and the reduction of the capillary force holding ALCO and water together which result to the mobilization of oil.

4. CONCLUSIONS

The fourth strain of soil bacteria (isolate 33, 34, 35, 38) and one bacterial consortium have the capacity to use ALCO as a carbon sources. These strain and one bacterial consortium can produce biosurfacants that containing carbohydarate, protein and lipid with various values from substrates. Biosurfactant had the highest emulsification activities with each EA 92,36 % for isolate 34 and isolate 35. The lowest surface tension values were obtained at bacterial consortium with 51 dynes/cm and for interfacial tension with 10 dynes/cm.

ACKNOWLEDGEMENTS

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O-MB15

ROTTEN FRUITS AS AN ALTERNATIVE MEDIA FOR PRODUCING *NATA DE FRUITY* BY *Acetobacter xylinum*

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Abstract

Nata has been widely produced in Indonesia and it is generally made from coconut water. Stock of coconut water is limited in certain areas, therefore alternative materials are needed. Rotten fruits can be obtained in most regions in Indonesia and usually become waste, and not even sold. Most traditional fruit markets in Indonesia produce about 0,5-4 tons of rotten fruits per day. Rotten fruit has high potential as a carbon source for producing nata because it contains high carbohydrate approximately 4.7 to 22.28 %. Nata production from rotten fruits and its character were carried out at laboratory scale. The objective of the research are to utilize rotten fruits to make nata from rotten fruits juice (nata de fruity), and to explore the characters of *Acetobacter xylinum* related to biofilm formation. Rotten fruit was crushed to obtain fruit juice, then was strained, cooked and inoculated with the inocula commersial nata (*Acetobacter xylinum*), then incubated for two weeks. The biomass produced was then harvested to obtain nata. The result showed that 1 kg of rotten fruits contained approximately 53 g/L reducing sugar and produced 500-700 gr nata with 1.5-2 cm thickness. This research concludes, that a rotten fruit is a promising substrate for *Acetobacter xylinum* growth to produce nata de fruity.

Keywords: rotten fruits, nata de fruity*, Acetobacter xylinum*

INTRODUCTION

Nata has been widely produced in Indonesia and it is generally made from coconut water. Demand production of nata every month approximately 100 tons. In the dry season nata demand increased until 40 %, but can not fulfilled entirely because limitations of coconut water as raw material for nata de coco (Ardian, 2010), therefore alternative materials are needed.

Indonesia produces approximately 15.13 million ton fruit every year (BPS, 2010). Post harvest fruits abundance causes problems in Indonesia because its fruit enzymatic reaction for ripening resulted in rotten fruits and become waste. Most traditional fruit markets produce about 0,5-4 tons of rotten fruits per day (Suratmin, 2009 ; Samsiyah, 2011). Because of they contain high carbohidrat approximately 4.7 - 22.28% (Lapus, 1987, Lancasire, 2006), rotten fruits have a high potential substrate as a carbon source for bacteria to produce nata.

The objective of the research are to utilize rotten fruits to make nata from rotten fruits juice (nata de fruity), and to explore the characters of *Acetobacter xylinum* related to biofilm formation.

MATERIALS AND METHODS

Rotten fruit was crushed to obtain fruit juice, then was strained, cooked and inoculated with the inocula commersial nata (*Acetobacter xylinum*). Growth medium was used in this research content of water apple (10,81%), red apple (8,65%), mandarin apple (10,81%), green apple (7,57%), orange (27,03%), guava (29,73%) and guava water apple (5,41%). All of fruits was juiced, filtered and was made serial dilution with concentration rotten fruits (mg/100 ml) 50 %, 37,5%, 25% and 12,5%. Bacteria used in this research was *Acetobacter xylinum* which gotten from Agroprima Industries Bantul. This experiment was conducted using a complete randomized design with 4 variations extract containing different concentration of reducing sugar of rotten fruit (53 g/L, 41 g/L, 29 g/L and 18 g/L). Fruit juice was measured their reducing sugar with DNS method. Fruit juice with various concentration of reducing sugar placed in the 100 ml jam bottle and sterilized in 121°C about 15 minutes. *Acetobacter xylinum* was inoculated in the sterile fruit juice medium and incubated 12 days. Every day this medium was measured decrease of reducing sugar, spectroscopy of density of cell bacteria, thickness of nata and in the last day observation this nata was measured wet weight, net weight and fiber content.

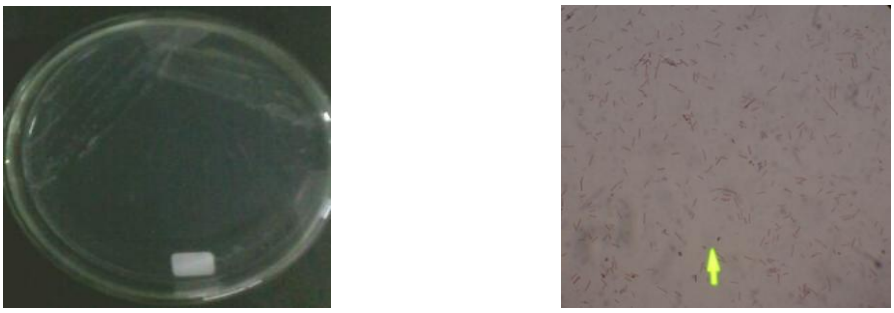
This fluid medium was added bioagar (0,5-1%), was sterized and inoculated with *Acetobacter xylinum* for observing of biofilm production. This semisolid culture of *Acetobacter xylinum* place in the sterile object glass and incubated in the room temperature and was observed biofilm production every day.

RESULTS AND DISCUSSION

The result showed that 1 kg of rotten fruits contained approximately 53 g/L reducing sugar. From the proximat test (Table 1) rotten fruit juice has hight carbohidrat content and rotten fruit juice was potentially substrat for *Acetobacter xylinum* growth to produced nata.

Table 1. content of roten fruit juice

Content	(%)
Water	97,73
Ash	0,16
Lipid	0,098
Protein	1,33
Complex carbohidrat	0,682



a b

Figure 1. (a) morfology of *Acetobacter xylinum* in the coconut water agar medium
(b) morphology of *Acetobacter xylinum* cell with gram staining 1000x magnification

Figure 1.a shows the morphology of *Acetobacter xylinum.*they are *filamentous*, *crenate, opaque,* elevation c*onvex* and white color. The microscopy observation (Figure 1.b) shows that shape of the cell is *elips* until short bacillus, they are gram negative bacteria.

Nata is indicated by the formation of Acetobacter xylinum nata on the surface of the medium. Nata is an extracellular cellulose produced by bacteria that floats on the surface of the medium due to be carried away by the gases CO_2 is trapped between the fibers of cellulose. Figure 2 below shows nata formed on a wide variety of medium after incubation for 12 days. .

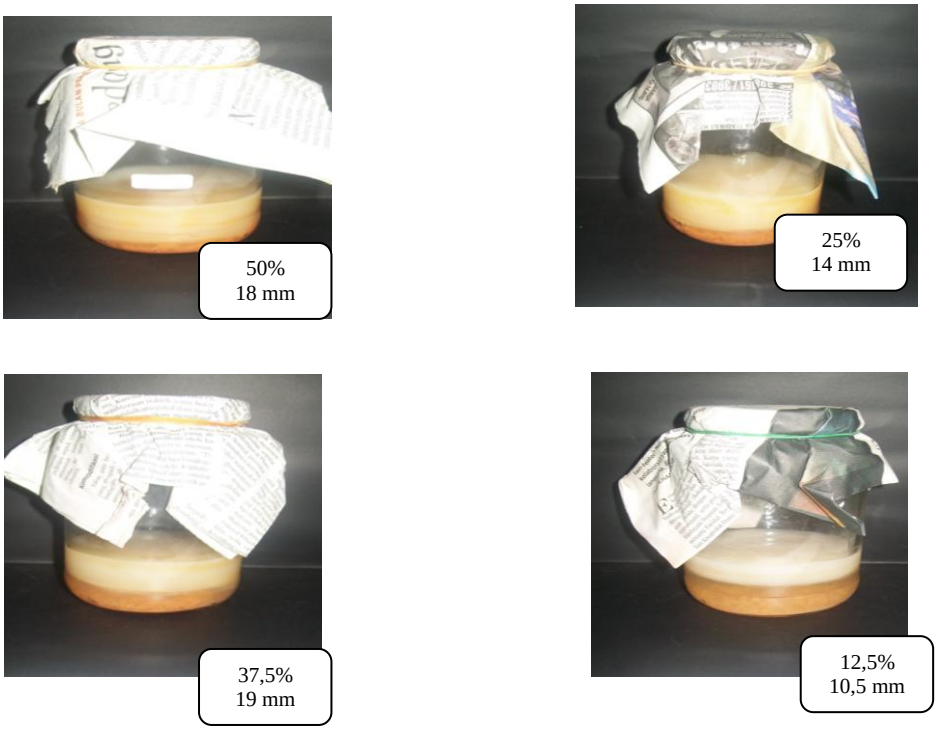


Figure 2. *Acetobacter xylinum* growth in the rotten fruit juice substrate

Acetobacter xylinum bacteria capable of growing and producing fruit nata in liquid fruit is not worth selling to different levels of reducing sugar. A variety of medium with different concentration reducing sugar shown in Table 2 below.

Table 2. reducing sugar levels at various concentrations of juice.

Concentration of Rotten Fruit	Reducing sugar (g/L)
50%	53,35
37,5%	40,97
25%	29,23
12,5%	17,85

these data indicate that 1 kg rotten fruits in 1 L of distilled water (dilution concentration 50%) reduction of sugar-containing 53.35 g / L. This reinforces that the liquid fruit is not worth selling the potential to become a growth medium in Acetobacter xylinum produce nata. Juice diluted to 37.5% sugar content reduction of 40.97 g / L. Dilution containing a concentration of 25% reduction in sugar content 29.23 g / L. while diluting the concentration of sugar-containing 12.5% reduction of 17.25 g / L. The existence of Acetobacter xylinum bacteria growth can be determined by the formation of surface nata in growth medium. Here are the results of the measured growth of Acetobacter xylinum nata formed during the incubation process.

Growth of Acetobacter xylinum in a wide range of concentrations of juice with a pH of 4 is shown by the formation of nata shown in Figure 3.

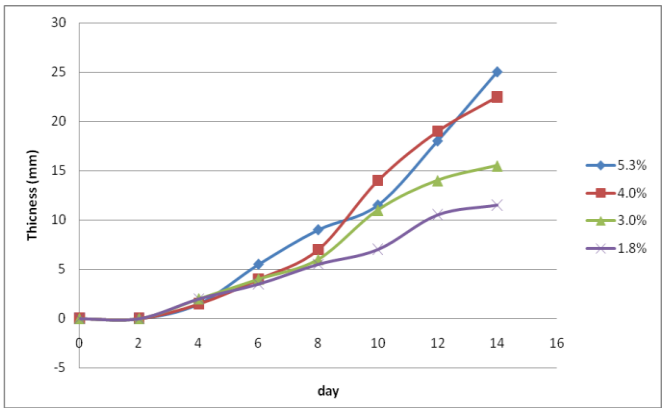


Figure 3. Nata thickness changes during incubation

Figure 3 shows the thickness of nata tended to increase in accordance with the pattern of cell growth during incubation either in medium with reduced sugar content of 18 g / L to 53 g / L. On day 0 of incubation until day two begins with the growth phase lag and not the addition of thickness. Acetobacter xylinum in the lag phase of growth will require adaptation in the media. Acetobacter xylinum began to synthesize cellulose after incubation

day 2. This is indicated by the formation of membrane floating on the surface of the medium. The results of microscopic observations showed that the cell appears enveloped extracellular cellulose which seems obvious after incubation of day 2, especially in medium with reduced sugar content of 41 g / L, 29 g / L and 18 g / L (Figure 3).

Table 3. The results of measurements of product parameters nata from 1 kg rotten fruits

Comparision between water and rotten fruit juice		Reducing sudar concentration (g/L)	wet weight (g)	net weight (g)	Thickness (cm)	Fiber content (%)	color	smell	taste
Rotten Fruite (kg)	Water (L)								
1	1	53.35	690.3^{d}	137^{c}	1.8^{d}	1,92^{c}	white	normal	normal
3	5	40.97	566^{c}	67^{b}	1.9^{d}	1,19^{b}	white	normal	normal
1	3	29.23	542.7^{bc}	23^{ab}	1.4^{d}	0,43^{a}	white	normal	normal
1	7	17.85	342.7^{a}	16^{a}	1.05^{b}	0,41^{a}	white	normal	normal

Note: Figures followed by same letter within a column indicate no real difference in the level of 5%.

Table 3. can be seen that the highest wet weight is on comparision between water and rotten fruit juice 1 kg : 1 L is 690.3 g. This suggests that the variation of sugar levels in the medium produce nata reduction with a significant difference in wet weight. The higher levels of reducing sugars in the medium, the more extracellular cellulose produced and the more water is trapped in the cellulose thereby increasing the wet weight of nata produced. Medium that produces the highest wet weight is a medium with reduced sugar content of 53 g / L.

Nata of the highest dry weight obtained from the growth of Acetobacter xylinum in medium with reduced sugar content of 53 g / L of 137 g (Table 3). The ability to grow Acetobacter xylinum in liquid medium fruit is not worth selling can be seen from the results of the analysis of sugar reduction, total acid content and pattern of growth of bacteria.

Reducing sugar content was measured by DNS method. These measurements aimed to determine changes in the substrate during the product formed. Glucose, which is one constituent sugar juice reduction as the primary substrate for growing Acetobacter xylinum cellulose formed under acidic conditions.

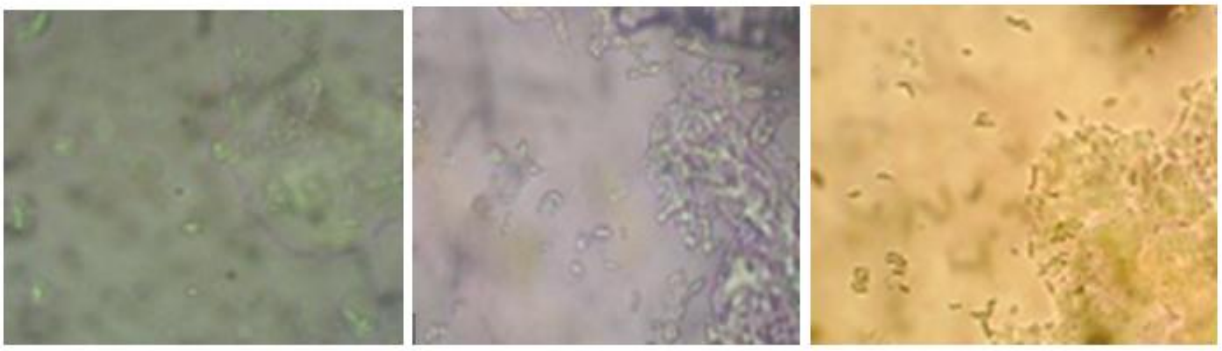


Figure 4. Biofilm (extracellular cellulose) formed by *Acetobacter xylinum* in rotten fruit medium thicker by the day.

Biofilms formed by *Acetobacter xylinum* because there glucose in the medium. Glucose is transported from outside the cell using a group translocation (fosfoenol pyruvate: fosfotransferase), where glucose chemistry changes during pass cytoplasmic membrane by the addition phosfat (Moat & Foster, 1995).

Approximately 1 kg of rotten fruits, that are not worth selling after extracted with 1 liter water can be used as an alternative growth medium Acetobacter xylinum in the manufacture of nata (nata de fruity) and produced 500-700 gr nata. Rotten fruits that contained approximately 53 g / L reducing sugar and pH 4 produce nata (nata de fruity), with 1.5-2 cm thickness, with white color, smell and taste normal and preferred. Rotten fruit is a promising substrate for *Acetobacter xylinum* growth to produce nata de fruity. Biofilm (extracellular cellulose) formed by Acetobacter xylinum in rotten fruit medium thicker by the day.

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O-MB16

In vitro Culture of *Phalaenopsis javanica* J.J. Smith (Orchidaceae) on Medium Containing Organics Materials and Benzyl Adenine (BA)

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Abstract

Phalaenopsis javanica J. J. Smith, an endemic orchid from Java, is suspected to be extinct in the wild due to high exploitation rate and intensive habitat conversion. This fact encourages Bogor Botanic Garden to provide seedlings through *in vitro* culture for mass propagation. The aim of this study is to induce the growth and development of seed derived *P. javanica* using injury treatment and media supplemented with organic materials and benzyl adenine (BA). Ten months protocorm like bodies (PLBs) were cultured on Knudson C based medium (KC) supplemented with 150 ml/l coconut water (CW) and 150 g/l green bean sprouts extract (GBSE); sweet potato juice (15 g/l), banana pulp (20 g/l), and peptone (20 g/l); and 2,5 mg/l BA. The results showed that shoots were emerged after 10 weeks on medium containing coconut water (150 ml/l) and sprout extract (150 g/l) in combination with injury treatment. The growth of shoot, leaves, and roots of PLBs after 12 weeks on this medium were also induced. This results is significantly better compared to PLBs cultured in other medium used in this experiment. The average numbers of shoot, leaves, and root of *P. javanica* after 12 weeks were 2.86, 3.17, and 1.17 respectively.

Keywords: *in vitro* culture, orchid, organic materials, *Phalaenopsis javanica*

Introduction

Phalenopsis javanica J. J. Smith, commonly known as *anggrek bulan jawa*, is an endemic orchid from West Java. It was originally found in the south of Garut. Later in 1975 it was discovered by a party from the Bogor Botanic Gardens on a mountain in West Java. Unfortunately, an Indonesian exporter of orchid species learned about the discovery and persuaded the local people to collect it to extinction from this small mountain [1,2]. Recently, this orchids is suspected to be extinct in the wild due to high exploitation rates and intensive habitat conversion. Therefore, this orchid faces a high risk to extinction if no serious conservation effort were made.

Propagation through *in vitro* culture can become an alternative management measures by providing plant stocks in large quantity, thus securing wild stock in nature [3]. Sadly, up until now there is no report or publication on the success of *P. javanica* propagation through *in vitro* or conventional methods. On the other hand, there are chances to increase success rate of *in vitro* culture, such as the addition of plant growth regulator and organic materials to stimulate growth of orchids [3]. However, the addition of growth regulators and certain organic materials on the growth of a plant species will provide a variety of influences.

These facts encourage the Bogor Botanic Gardens to provide seedlings of *P. javanica* through *in vitro* culture for mass propagation as a resort of conservation. This study aims to obtain a culture medium capable of inducing the growth and development of *P. javanica in vitro.* This study used plant growth regulators benzyl adenine (BA) and the organic materials. Coconut water, extracts of green bean sprouts, sweet potatoes, bananas pulp, and peptone were added in the Knudson C (KC) basic medium. We also tested the physical (injury) treatment that could induce the growth of explants.

Materials and Methods

The experiment was conducted at Tissue Culture Laboratory, Center for Plant Conservation, Bogor Botanic Gardens (PKT - KRB). Materials used in this study are protocorm like bodies (PLBs) of *P. javanica* from collection of Tissue Culture Laboratory, PKT - KRB (8.VIII.08). Ten months PLBs of *P. javanica* were cultured on Knudson C base medium (KC) supplemented with 150 ml/l of coconut water (CW) and 150 g/l green bean sprouts extract (GBSE); sweet potato juice (15 g/l), banana pulp (20 g/l), and peptone (20 g/l); and 2.5 mg/l BA; combined with or without injury treatments (Table 1).

The study was conducted with a Completely Randomized Design with two factors, namely the culture medium consisting of four levels and injury treatment consisting of two levels. There are eight treatments tested, each with 12 replication containing three PLBs. Furthermore, the cultures were exposed to artificial light (daylight fluorescent tubes, 36 W) with a light/dark cycle of 12/12 h at 16 ˚C. Observations were made every week until 12 weeks after planting (WAP), which includes the emergence of first shoots, leaves and roots; number of explants growing shoots, leaves, and roots (%); and number of shoots, leaves, and roots. Data from observations of explants growing shoots, leaves, and roots (%) as well as the number of shoots, leaves and roots of *P. javanica* subsequently analyzed using SPSS 13 for Anova. Significantly different values were analyzed further by Duncan test at level confidence of 0.05.

Table 1. Treatments for *in vitro* propagation of *P. javanica* on KC base medium.

Treatments	Injury treatment	Coconut water (150 ml/l) + green bean sprouts extract (150 g/l)	Sweet potatoes (15 g/l) + banana pulp (20 g/l) + peptone (20 g/l)	BA (2.5 mg/l)
P1	+	-	-	-
P2	+	+	-	-
P3	+	-	+	-
P4	+	-	-	+
P5	-	-	-	-
P6	-	+	-	-
P7	-	-	+	-
P8	-	-	-	+

Results and Discussions

Observations showed leaves, roots, and shoots of *P. javanica* first appeared at weeks 1, 3, and 10 weeks after planting (Table 2). The number of explants growing shoots at 12 WAP ranged from 0.00 to 25.00% with an average number of shoots between 0 to 2.86 (Table 3). Anova Test results show that the number of explants *P. javanica* growing shoots in eight treatments tested differ significantly. Highest germination was found in KC medium (25.00%) with the addition of organic material (coconut water and green bean sprouts extract) combined with injuries treatment on explants (P2) before planting. Values are significantly different from the percentage of explants growing shoots that were cultured on KC basic medium with or without the addition of 2.5 mg/l BA treated injuries (P1 and P4), as well as the KC media with or without the addition of organic materials and BA with or without treatment injuries before planting (P5).

The average number of shoots of *P. javanica* that grows at 12 WAP is influenced by the organic materials added into the medium and the physical treatment of injuries provided in the PLBs. ANOVA Test results show that the average number of shoots of *P. javanica* on the eighth treatment are significantly different. In the media with the addition of organic materials in combination with physical treatment (P2 and P3), the average number of shoots that appear higher than in other treatment media. The average number of shoots in both media, respectively 2.86 and 2.08 shoots (Table 3).

Table 2. Time of emergence of first shoots, leaves and roots of *P. javanica* on eight media tested.

Treatments	Time of emergence of first shoots (WAP)	Time of emergence of first leaves (WAP)	Time of emergence of first roots (WAP)
P1	0	1	3
P2	10	1	3
P3	11	1	4
P4	0	1	5
P5	0	1	4
P6	10	1	4
P7	12	1	4
P8	12	1	3

Observation showed that explants with least average leaf of 66.67% were found on treatment with the addition of KC media 2.5 mg/l BA combined with treatment of injuries (P4). These results are significantly different since in other treatments where least average leaf are much greater, even up to 100% on media treatment of KC with the addition of coconut water (150 ml/l) and extract of green bean sprouts (150 g/l) combined with treatment of injuries (P2). Leaf formation in cultures of *P. javanica* is also influenced by the organic

materials added into the medium and physical treatment of injuries provided in the PLBs prior to planting in the media. The average number of leaves of *P. javanica* on KC medium with the addition of organic materials combined with treatment of injuries (P2 and P3) were higher and differ significantly than the average number of leaves in other treatments. The average number of leaves in both media, respectively 3.17 and 3.08 leaves (Table 3). Number of explants *P. javanica* rooted at 12 WAP ranged from 11.11 to 86.11% with an average number of roots are ranging from 0.19 to 1.17 (Table 3). ANOVA also showed significant different of the number of explants growing roots and the number of roots formed within the eight different treatments.

Table 3. Growth of explants *P. javanica* at 12 weeks after planting (12 WAP).

Treatments	Injuries	Number of explants growing shoots (%)	Average number of shoots	Number of explants growing leaf (%)	Average number of leaves	Number of explants growing roots (%)	Average number of roots
P1	+	0,00^{a}	0,00^{a}	88,89^{b}	1,33^{a}	11,11^{a}	0,19^{a}
P2	+	25,00^{b}	2,86^{b}	100^{b}	3,17^{b}	86,11^{d}	1,17^{f}
P3	+	19,44^{b}	2,08^{b}	97,22^{b}	3,08^{b}	83,33^{d}	1,03^{ef}
P4	+	0,00^{a}	0,00^{a}	66,67^{a}	1,28^{a}	16,67^{a}	0,22^{ab}
P5	-	5,56^{a}	0,06^{a}	91,67^{b}	1,67^{a}	44,44^{b}	0,50^{bc}
P6	-	0,00^{a}	0,00^{a}	94,44^{b}	1,58^{a}	66,67^{cd}	0,67^{cd}
P7	-	2,78^{a}	0,03^{a}	91,67^{b}	1,72^{a}	69,44^{cd}	0,83^{de}
P8	-	0,00^{a}	0,00^{a}	86,11^{b}	1,55^{a}	52,78^{bc}	0,69^{cd}

KC medium with the addition of organic materials combined with treatment of injuries (P2 and P3) were significantly higher compared to other treatments. Therefore, we suggest that growth of explants of *P. javanica* on KC medium with the addition of organic materials and combined with injury treatment (P2 and P3) are relatively equal and better than the growth of explants on other treatments. However, based on the observation time of emergence of first shoots, leaves and roots, it appears that the growth of *P. javanica* on KC medium with the addition of coconut water (150 ml/l) and green bean sprouts extract (150 g/l) combined with physical treatment (P2) is faster than in other treatments. In addition, the stature of *P. javanica* grown on media with treatment of injuries is healthier and more firm than explants in other treatments.

The results of this study indicate that the KC media with the addition of coconut water (150 ml/l) and green bean sprouts extract (150 g/l) combined with treatment of injuries (P2) is able to induce the growth of *P. javanica* better than other treatments. All growth parameter of *P. javanica* observed are better. Currently, there are a lot of studies using coconut water

to enrich culture media, since it contains amino acids, organic acids, nucleic acids, purines, sugars, sugar alcohols, vitamins, plant growth regulators, and minerals. Additionally, coconut water also contains plant growth regulators auxin, cytokinin, gibberelin, and abscisic acid [4,5]. Auxin is responsible for cell elongation and for other developments including root initiation; cytokines is responsible for promoting cell division and morphogenesis; while gibberelin plays a role in seed germination, flowering and fruiting, as well as cell elongation; and absisic acid plays a role in ripening seeds and the process of opening and closing of stomata [6].

These results are consistent with Ishii *et al.* [7] and Amilah & Astuti [8] that used media added with coconut water or green bean extract. Ishii *et al.* [7] reported that the best callus induction and regeneration of somatic embryogenesis in *Phalaenopsis* Richard Shaffer 'Santa Cruz' PLBs are using media containing coconut water 200 ml/l. The results Amilah & Astuti [8] showed that the addition of 150 g/l in the culture media of green bean sprouts extract has given the best results in the formation of leaves and roots of *Phalaenopsis amabilis* (Bl.).

On the other hand, addition of sweet potatoes (15 g/l), banana (20 g/l), and peptone (20 g/l) on KC medium combined with treatment of injuries (P3) also gave a slightly lower response with the addition of coconut water and green bean sprouts extract combined with treatment of injuries (P2). Studies by Widiastoety *et al*. [9] showed the addition of banana is also able to induce the growth of roots and leaves on a *Phalaenopsis* orchid culture. Their results showed that the number and leaf area and the number and length of *Phalaenopsis* orchid roots on media with the addition of bananas is higher than its growth in medium without the addition of bananas.

Moreover, according to research results from Ichihashi & Islam [10], the addition of bananas and peptone can induce the growth of *Phalaenopsis* hybrid callus. Addition of basic medium with bananas will add nutrients required for growth and enlargement *P. javanica*, since hundred grams of bananas show water content (70 g), protein (1.2 g), fat (0.3 g), carbohydrate (27 g), fiber (0.5 g) and potassium (400 mg). In addition, the banana is also a source of vitamin C, B1 (thiamin), B2 (riboflavin), B3 (niacin) and B6 [11].

On the other hand, combination of peptone and sweet potato has different effect. Explants on P3 grow quite well, but shoot and root emergence were a week later than in P2. Amaki & Higuchi [12], found that peptone addition can stimulate growth of *Phalaenopsis* PLBs 'Surfrider' because peptone is a source of nitrogen; however, Ichihashi & Islam [10], found callus growth of *Phalaenopsis* hybrid can be hampered in the culture medium when added sweet potatoes.

Results are also consistent with other studies using injury treatment, where PLBs of *P. javanica* subjected to injury treatment have better growth response. Studies by Kuo *et al.* [13] showed that somatic embryos formed on the leaf pieces (the injuries) of *Phaleonopsis* 'Little Steve'. Studies by Gow *et al.* [14] on somatic embryogenesis and leaf pieces *Phalaenopsis amabilis* and *Phalaenopsis nebula* also showed that the cut areas or areas that have suffered injuries gave the best embryogenic response than other parts of the leaf. According to Kuo *et al.* [13], somatic embryos are originated from leaf epidermal cell layer. Furthermore, meristematic cells that will form a globular embryo are enlarged, which in the end, will form a mature embryo meristem and shoot apical meristem of roots.

Meanwhile, the addition of 2.5 mg/l BA does not seem to give a noticeable effect on the growth and development of the PLBs *P. javanica*. This is probably because of BA concentrations used in this study may not be sufficient to induce the growth and development of the PLBs *P. javanica*. Research by Kuo *et al* [13], showed that the highest frequency of leaf pieces embryogenesis *Phalaenopsis* 'Little Steve' found on media with the addition of 3 mg/ l BA. Other probability is the the growth PLBs in *P. javanica* requires a combination of growth regulators. Ishii *et al*. [7] reported a culture medium containing 0.01 mg/l BA and 0.1 mg/l 2,4-D is more effective in inducing callus from PLBs *Phalaenopsis* Richard Shaffer 'Santa Cruz' compared to other media. In addition, the study by Chen *et al.* [15] also showed that the callus from PLBs *P. nebula* can form in culture medium containing growth regulators 5 mg/l BA and 1 mg/l 2,4-D.

This study shows that addition of organic material using coconut water and green been sprout extract to KC basic medium along with physical injury treatment can induce faster and better growth and development of PLBs of *P. javanica*. Even so, further study is required since the number of shoots produced is still quite limited.

Acknowledgement

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ORAL - TOPIC 2

Ecology and Conservation (O-EC)

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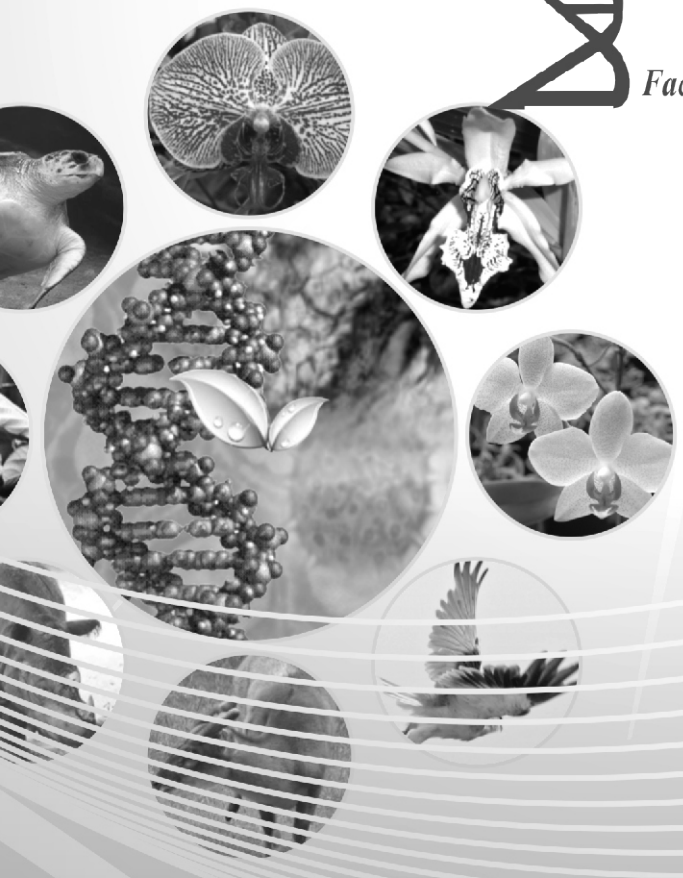

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

O-EC01

MANGROVE FOREST ASSESSMENT IN SEMBILANG NATIONAL PARK BANYUASIN SOUTH SUMATERA USING LANDSAT TM/ETM IMAGERY

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ABSTRACT
Mangrove ecosystem has become one of the key factors in considering the global warming issue and thus mangrove ecosystem becoming increasingly important. It is well-known that the mangrove ecosystem plays important roles in coastal regions by its functions including, supplying food and fuel wood for humans and natural protection against erosion. This papers present a mangrove forest assessment from 2003 to 2007 of the Sembilang National Park, Banyuasin South Sumatera. The aims of this research are to describe the plant composition and structure of mangrove forest in the study area, and to evaluate the deforestation level and its amplitude by means of a retrospective analysis of the cover and distribution area of mangrove using Landsat TM/ETM imagery. Cover area and distribution of mangrove in the study area were mapped using Landsat ETM+ (acquired 2003) and TM (acquired 2007). A supervised classification was applied using the maximum likehood algorithm, considering five initial classes. This classification was evaluated by obtaining a classification error matrix and by assessing its accuracy. The results showed that the mangrove forest area declined from initial estimate of 88,944.65 hectare in 2003 to 86,762.42 hectare in 2007 which represent a decrease of 2,182.23 ha in a 4-yr period.

Keywords: Mangrove forest, Landsat TM/ETM+, Sembilang National Park

1. Introduction

Indonesia, as an archipelago country, is well known for rich and diverse natural resources. Among the resources is mangrove forest which form a very unique ecosystem in the coastal area. One of province in Indonesia which have mangrove forest area that is South Sumatera Province. The total extent of the forests is 363.424 ha (1). They are distributed in two regencies, namely Banyuasin and Ogan Komering Ilir Regency. Most of mangrove forest in Banyuasin regency reside in Sembilang National Park.

The Mangrove forest are the dominant ecosystem along the sheltered shoreline of Banyuasin Coast. They are a vital resources that serves the inhabitat of coastal areas and it is important in stabilizing coastal ecosystem. Mangroves provide wood products for house construction, firewood, and other non-wood forest products such as tannins and medicine (2). At the ecosystem level, mangroves serve as habitat and breeding areas for many commercially important fish and crustaceans, provide detritus for offshore fisheries, control coastal erosion as well as maintaining water quality. Mangrove ecosystems provide protective habitat for fish, crabs, shrimps and nesting grounds for bird species (3). A strong

correlation has been shown to exist between the presence of mangrove ecosystem and the productivity of fish and marine lie in coastal areas (4).

Despite these benefits, mangrove forest vegetation is threatened by human and natural factors. Along the coastal areas in most developing countries especially Indonesia, populations pressure has led to the conversation of many mangrove areas to other uses, including infrastructure development, aquaculture, rice production, and shrimp ponds (3 dan 5). Over the years, mangroves forest have been subjected to ever-increasing human population and economic pressure that has led to their degradation (6).

The major problem facing the management of mangrove forests in Banyuasin is the lack of management plan. Owing to lack of reliable and up-to-date comprehensive vegetation maps, mangrove managers do not have access to information on the present forest condition and also on the changes that have occurred in the forest cover over period of time. Accurate mangrove vegetation maps with details of distribution and abundance are essential for monitoring forest changes over time, for estimating mangrove production and for investigating linkages with other ecological system that rely on them either directly or indirectly. A comprehensive database, including the information on distribution and extent on mangrove areas and forest structure is a prerequisite for the development of mangrove management plans. Essentially, change detection involves the ability to quantify temporal effects using multitemporal data sets. One of the major applications of remotely sensed data obtained from Orbiting Satellites is change detection because of repetitive coverage at short intervals and consistent image quality (7).

2. Materials and methods

2.1 Description of the studi area

Administratively, Sembilang National Park is located in Banyuasin district and has been a national park since March 19th 2003. It is located at 1.3^{o} – 4^{o} South latitude and 104^{o}40' – 105^{o} 15' East longitude (Figure 1). The total area of park is ± 202.896,31 ha (including its waters area). It is a natural coastal wetland area with various forest ecosystems of peat moss swamps, freshwater swamps, mangrove forests and mud flats. Banyuasin regency has 80% of the flat topography of the land to tidal marsh and lowland swamp, while a 20% longer, wavy to undulating form of dryland with an altitude range 00-40 meters above sea level. It has type B1 according to the classification Oldemand climate with an average temperature of 26.1^{o} to 27.4^{o} Celsius and average humidity and relative humidity 69.4% - 85.5% with an average rainfall 2723 mm / year (8).

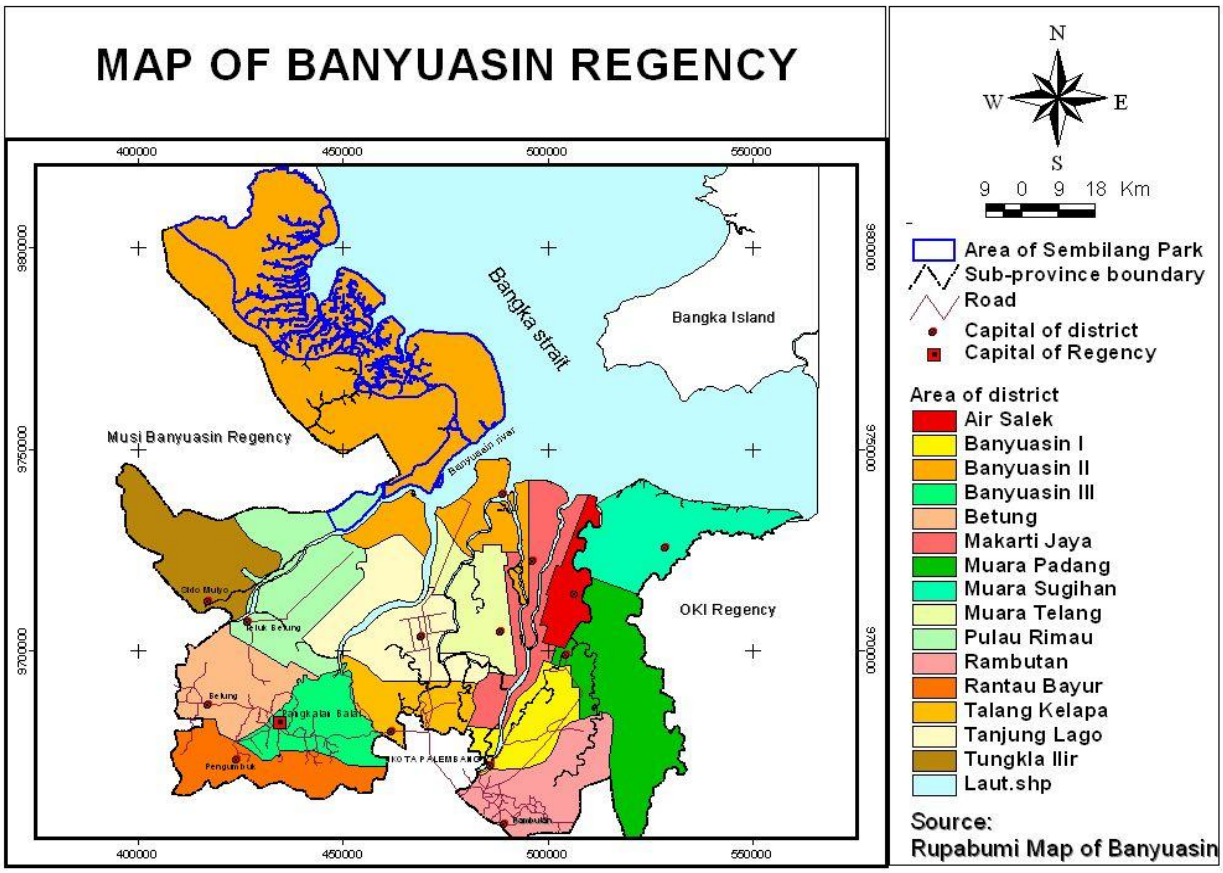

Figure 1. Banyuasin regency map, Province of South Sumatera

2.2 Data Acqusition and Processing

The Landsat satellite image data employed in this study were acquired in 2003 and 2007. The Landsat ETM+ and TM satellite data were processed using ENVI 4.4 image processing software. The images were imported into ENVI format. Two steps were taken to process the data. In the first step, the image were imported into ENVI standard image format (.hdr). Since the images were in single bands, they were stacked together using the layer stack technique to form a floating scene. A subset of the images was obtained from the floating scene to identify the study area and then, later displayed as false-color composites using a band combination.These data were geometrically corrected, overlain, and precision rectified to ground control points were selected from a series of topographic maps. In order to assure the map accuracy in terms of the relationship between specific pixels and map co-ordinates, the images were geometrically corrected using first order polynomial transformation. The final positional accuracy (RMSE) for the transformation was within < 0.5 pixel (9). The images were later resampled using the nearest neighbor techniques in order to preserve as much of the original details in the images as possible. The images were later enhanced using histogram equalization techniques, and classified using an supervised classification technique to identify land cover features within the study area.

2.3 Sampling Procedurs

An intensive field survey was conducted in Oktober 2009. The ground-truthing objective was to correlate field features with the result of satellite imagery classification. In addition, essential ecological data, as well as botanical and phonological features were collected. Transects were selected and made to run from the sea ward or channel bank inward across the types already marked out on the image, the length of each transect depending on the locality and the extent of the types. Sampling units were 30 X 30 m^2 quadrat (adapted for landsat resolution). Sampling for adult trees was restricted to stems with diameter $\geq$ 10cm. Within the quadrat, individual trees were identified and counted. Vegetation measurements included tree height and diameter of the stem at breast height (DBH), The impotance value (IV) of each species was calculated by summing its relative density, relative frequency and relative dominance (10).

3. Results and discussion

The digital supervised classification methods applied on the 2003 ETM+ and 2007 TM image resulted in 12 classes, and mergered to be 7 main classes. For this analysis, the ocean and rivers were merged into one class. The classification result of the 2003 and 2007 images are provided in Figures 2 and 3, and Table 1.

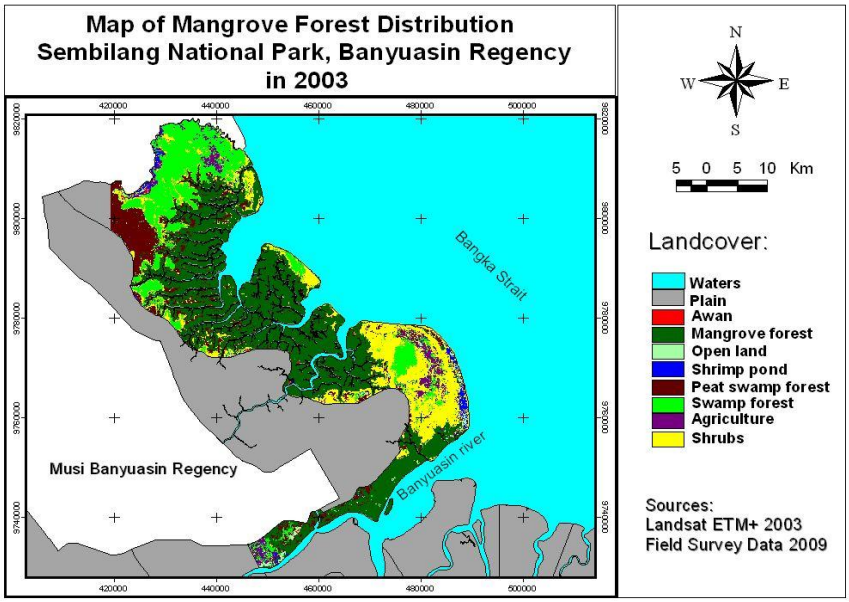

Figure 2. Map of mangrove forest distribution in Sembilang National Park, result of supervised classification (2003)

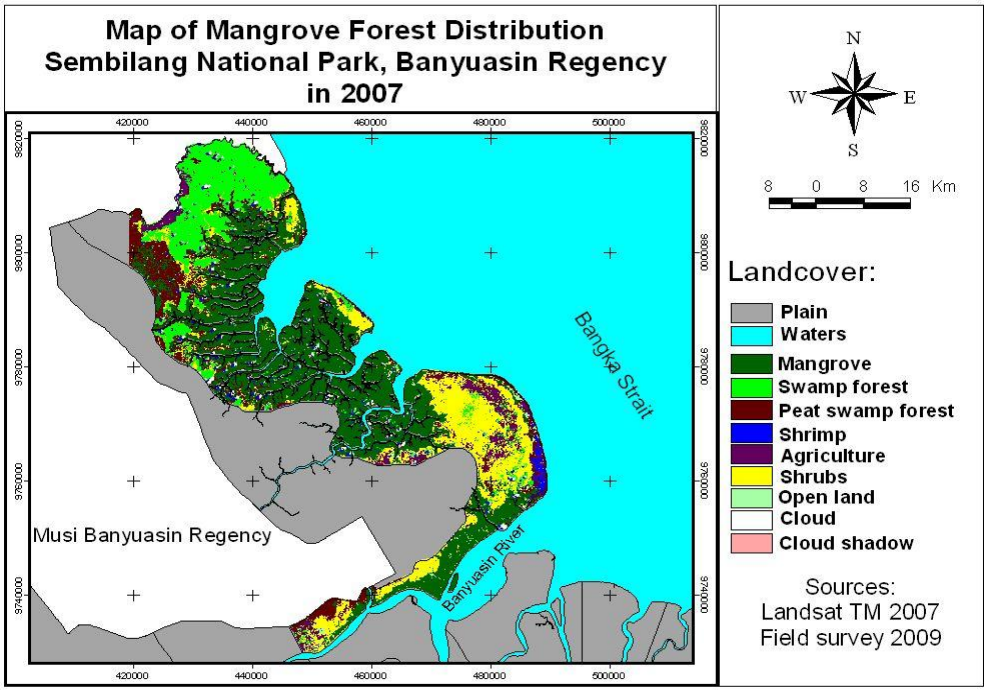

Figure 3. Map of mangrove forest distribution in Sembilang National Park, result of supervised classification (2007)

The results were compared to the ground truth data which provided an overall accuracy of 80 and 85% for the 2003 and 2007 images, respectively. Mangrove declined from an initial estimate of 88,944.65 hectare (ha) in 2003 to 86,762.42 ha in 2007 representing an overall decrease of 2.45 percent (Table 1). There was a significant decrease in areas covered by open land, Agriculture and Shrimp. While there were decline in mangrove, agriculture activities and shrimp in the area were increasing. For instance, between 2003 and 2007, agricultural activities increased from 5,905 ha to 8,597.61 ha representing a change of 45.6 percent. Shrimp posted an increase of 92.5 percent from 2,042.64 in 2003 to 3,932.19 in 2007. Table 1. Results of the classified 2003 and 2007 images.

Table 1. Changing of Landcover Class in 2003 to 2007

No.	Classes	Area (ha) in 2003	Area (ha) in 2007	% change (2003-2007)
1.	Mangrove	88,944.65	86,762.42	-2.45
2.	Swamp forest	31,658.55	28,951.92	-8.85
3.	Peat Swamp forest	19,871.19	20,487.69	+3.1
4.	Open land	3,169.17	1,694,43	-46.53
5.	Agriculture	5,905	8,597.61	+45.6
6.	Shrimp	2,042.64	3,932.19	+92.5
7.	Shrubs	29,552.67	30,509	+3.24

The decrease in mangrove and swamp forest may be partly due to the economic activities along the coast. Also, the increase in the agriculture activities in this area may be attributed to the reduction in the mangrove, swamp forest, and open land.

Community characteristics

Structural attributes like basal area, density and species composition that were recorded from sample plots were used to characterize mangrove community of Sembilang National Park. In general, there are eight mangrove species that occur in the study area. Based on the highest, the principal species are *Rhizhopora mucronata* (IV = 132.25%) , *Bruguiera gymnorrhiza* (IV=90.49%). Others are *Rhizophora apiculata, Avicennia marina, Bruguiera spp, Xylocarpus granatum, Sonneratia alba, Excoecaria agallocha* and *ceriop tagal.*

4. Conclusion

Have happened degradation of mangrove forest in Sembilang National Park. This study has also shown that the most significant factors affecting mangrove degradation are shrimp pond and agricultural activities. This calls for the need of government of Banyuasin regency to integrate the local population into the decision making process. There is need for the government to integrate remote sensing data into the management planning process. This may provide baseline data for planning of mangrove resources and show existing spatial patterns of the area. Incorporation of remote sensing data may also assist the inventories of coastal resources over time including mangroves resources.

5. References

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O-EC02

MANGROVE MAPPING AND MONITORING AS PART OF SUSTAINABLE COASTAL ZONE MANAGEMENT

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not presented

O-EC03

Profile of mangrove forest at abandoned shrimp pond in Segara Anakan, Cilacap

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ABSTRACT

Mangrove of Segara Anakan is the largest area of remnant mangrove in Java. Between the 1996 to 1997 a large of mangrove forest was cleared and converted to intensive shrimp ponds at alarming rate. However, after one to two years these shrimp ponds failed and were abandoned. These abandoned shrimp ponds left large areas of canopy gaps, which was occupied by shrub and liana mangrove. Beside that, this mangrove area also experienced heavy siltation and tree cutting. The purpose of this research was to study the forest profile at the abandoned shrimp pond. Data vegetation was collected from 2 quadrate plots of 50mx50m using Oldeman methods. The results revealed that the abandoned shrimp ponds was composed of two layers od forest. The canopy gaps triggered the pioneer species of mangrove shrubs and liana, *Acanthus ilicifolius* and *Derris heterophylla* which dominated 100% of the mangrove forest floor. The mangrove trees were consisted of natural and planted tree species. The natural-tree species were composed of *Sonneratia alba*, *Avicennia alba*, and *Aegiceras corniculatum*. The planted tree was *Rhizophora apiculata*, 42.29%, at the island of the pond. The *A. alba* was the natural tree which clumped in the shrimp pond canal. The levy of the ponds was dominated by *Wedelia marina*. The forest floor vegetation both *Acanthus ilicifolius* and *Derris heterophylla* prevented the mangrove tree propagules to grow, and they characterized this abandoned shrimp pond.

Key words : canopy-gap, mangrove tree, *Acanthus*, *Derris*

O-EC04

THE STUDY OF FRESHWATER FISH DIVERSITY IN RANU PAKIS, KLAKAH DISTRICT, LUMAJANG AS THE FIRST STEP OF FISH CONSERVATION

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ABSTRACT

Ranu Pakis is one of three lakes located in Lumajang region, which are used as fresh-water aquaculture. The damage of the water bodies might cause the deterioration of fish diversity. The purpose of this research is to study the diversity of fresh water fishes living in Ranu Pakis District Klakah, Lumajang as the first step of fish conservation. This research used a descriptive explorative approach. Sampling was done using 30x15 cm fish-net (Mesh 0.1 mm). Fishes caught then identified based on morphological characteristics. The diversity was analysed using Shannon-wiener index, eveness and richness were also counted. The result shows that there are six species of fishes living in Ranu Klakah, those are: *Gobionella shufeldti*, *Oreochromis mosambicus*, *Oreochormis niloticus*, *Clarias batracus*, *Rasbora argyrotaenia*, and *Gambusia affinis* with species diversity low to medium (0.51 to 1.31), evenness hight (0 to 0.73), and species richness hight (1.25 to 1.28).

Keywords: fish diversity, Ranu Pakis, conservation.

Introduction

Ranu Pakis is one of the three largest lakes in Lumajang which is used by local community for fresh-water aquaculture of *Oreochromis mossambicus* and Tilapia using floating net system. In addition, they also use the lake for washing, bathing, irrigation, and recreation.

Fish feeding which is given at regular intervals to support fish life might harm other organisms which depend on water resources[1)]. The damage of water bodies can lead to damage and even loss of fish diversity, especially in Ranu Pakis. In daily base, the local community Ranu Pakis doing fishing outside the cage area. They left behind the consideration for the natural preservation which might put more danger to the environtment. Recent data from the Department of Marine and Fisheries (2008) states that most areas in Indonesia have experience overfishing and already been in critical condition[2)].

According to the World Bank (1998) Indonesia is rich of freshwater ecosystems with high biodeversity, yet less the attention in resources management[3)]. Various developmental activities threaten the preservation of freshwater biota. Various kinds of information required for biodiversity conservation efforts, including the benefits for humans, distribution, status, trends of disrupting threat, and ecological relationships[4)]. The determination of protected

areas will not hit the target if do not accompanied by good knowledge about the distribution of species and conservation priority determination of particular areas[5).]

Material and Method

Target organism

The target organisms are spesies of fish which are living in Ranu Pakis

Method

The study was conducted in Ranu Pakis District Klakah, Lumajang, from February to March 2011. To study the diversity of fish we catch the fish using 30x15cm (0.1 mm mesh) fish net in three replications. The observed areas are including inlet, middle, and outlet of water body (Fig. 1). This data is supported by interview data with the local community and ecological parameters.

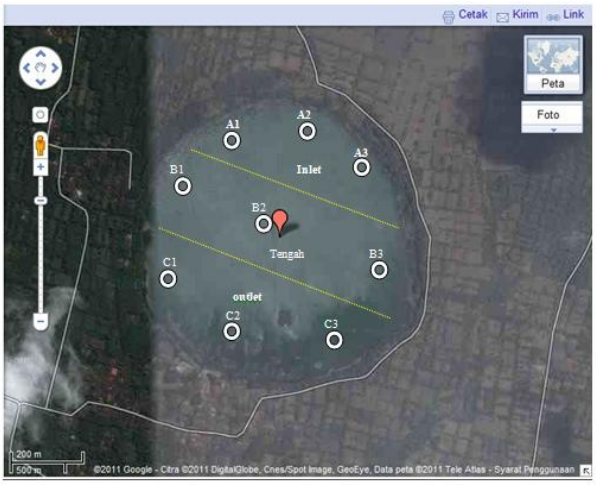

Fig 1. Point Sampling Fish in Ranu Pakis District Klakah, Lumajang. A1, A2, A3, B1, B2, B3, C1, C2, and C3 are sampling points.

Data Analysis

To find out the diversity of the fishes, the data was analyzed using Shannon-Wiener formula, counted the Eveness, and Richness index, as well.

Result and Discussion

Ecological parameter

During the observation, we found that the ecological parameters of Ranu Pakis showing a neutral condition (Table 1).

Table 1. Physico-chemical parameters of Ranu Pakis during Februari-March 2011

No	Parameter	Value
1	pH	7,5 - 8,7
2	DO	8,03 - 11,12
3	Temperature (°C)	26,7 - 28,3
4	Sachie Disc Depth (m)	0 - 1,3
5	Turbidity (mg/l)	4,6 - 5,7
6	Brightness (Lux) x100	693,7 - 800,1

The Fish diversity

Based on the morphological characteristics, we found six species of fish *Gobionella shufeldti*, *Oreochromis mosambicus*, *Oreochormis niloticus*, *Clarias batracus*, *Rasbora argyrotaenia*, and *Gambusia affinis* (Fig. 2). Diversity index of fishes living in Ranu pakis is low up to medium, with evenness is hight, and richness hight (Table 2).

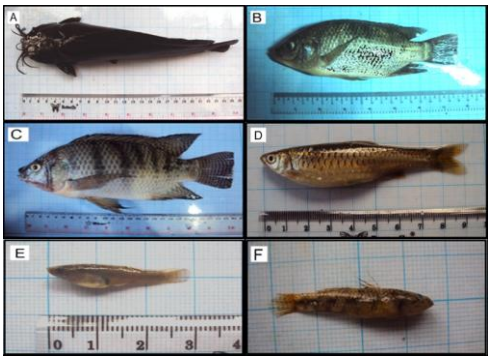

Fig 2. Species of fishes lining in Ranu Pakis A. *Clarias batracus*, B. *Oreochromis mossambicus* C. *Oreochromis niloticus* D. *Rasbora argyrotaenia*, E. *Gambusia affinis*, F. *Gobionella shufeldti*.

Table 2. Summary of Diversity Index, Evenness and Species richness in Ranu Pakis

Station	H'	E	R
Inlet	1.05	0.59	1.25
Midle	1.31	0.73	1.28
Outlet	0.51	0.29	1.28

Quotation: H' Shannon-Wiener index; E veness index; R Richness index

Fish diversity in Ranu Pakis is low to medium. The diversity can be categorized low when 0 <H' <2.30. A community have a high diversity when there are many species with a number of individuals of each species evenly distributed are relatively[6]. Thus, when a community consists of only a few species with an uneven number of individuals, communities can't be said to have a high diversity[7].

Evenness index show high value, it indicate that the location where the fish were found have nearly the same tolerance limits of abiotic conditions and the availability of existing resources. Evenness index was used to view the evenness of the distribution of individuals among species[8].

The richness index show high value, it caused by many different types which living in these habitats. It can be happened if only the food chain is long and complex which gives a greater opportunities for every component to interact. The high richness index is influenced by various environmental factors, such as the type of natural food supplies for fish, heterogeneousity, and complexity of environmental conditions and the pH.

According to local community there are two types of Tillapia found in Ranu Pakis, those are *Oreochromis mossambicus*, locally named Nila, and *Oreochromis niloticus* locally named Mujair. Other fishess found, following the local name are Gatul fish (*Gambusia affinis*), Goby fish or Cakul fish (*Gobionella shufeldti*), Lele fish (*Clarias batracus*), and Gatul fish (*Rasbora argyrotaenia).* In 2008 Subarijanti reported those are six species that found in Ranu Pakis, including Tawes (*Puntius javanicus*), Nila (Oreochromis mosambicus), Cork (*Ophiocepalus striatus*), Lele (*Clariasbatracus*), Black Tilapia (Oreochormis niloticus) and Tombro (Cyprinus carpio L)[9]. From this research we found that there are three species have no more exist; those are Cork (*Ophiocepalus striatus*), Tawes (*Puntius javanicus*) and Tombro (Cyprinus carpio L). From these research we found three new species, there are Wader (*Rasbora argyrotaenia*), Gatul (*Gambusia affinis*), and Goby (*Gobionella shufeldti*). We suggest that the enviromental changing which occur in Ranu Pakis has caused the missing of those three species.

The finding of new species in Ranu Pakis might be caused by the entrace of invasive fish through the inlet flow of Ranu Pakis, or by the introduct by local community which develop the aqua-culture system there. Further identication is required to find out what spesies are living in these lake, along with those six we found.

Fish that are caught, and who had obtained can be influenced by the invasive fish from the inlet and the activities of fish cages. Further inventaritation to find out what the fish that living in these waters are considered necessary to know what kind of fish that living in these lake.

From the interview with local community we found that the produktivity of Ranu Pakis is decreasing by five fold; recently the people could only catch 5kg, compared to 25kg (10 years ago). Beside, there is changing in clarity of Ranu Pakis water as an impact of cage-system aqua-culture. Capture, it cause a suddent death of fishes in particular system. This decreasing maight be influenced by fishing method they apply daily catching they do and or the tools they use such as gill net which may the overfishing[10)].

Based on the interview we suggest that fish sudden-death in Ranu Pakis is caused by the increasing population of algae. The impact of this phenomenon the changing of water color which becomes dark green and have very bad smell. The population explosions of algae the ecological impact of eutrophication. If algae die, it can settle and accumulate at the bottom of the lake[11)]. Decay of algae by bacteria resulted in decreased concentrations of dissolved oxygen in the bottom of the lake which, if is last long it will arrive to a very low level under the ability to support fish life, the situation, often referred to as *fish kills*.

Conclussion

Based on the morphological characteristics, we found six species of fish *Gobionella shufeldti*, *Oreochromis mosambicus*, *Oreochormis niloticus*, *Clarias batracus*, *Rasbora argyrotaenia*, and *Gambusia affinis*. In 2008 those are six species that found in Ranu Pakis, including Tawes (*Puntius javanicus*), Nila (Oreochromis mosambicus), Cork (*Ophiocepalus striatus*), Lele (*Clariasbatracus*), Black Tilapia (Oreochormis niloticus) and Tombro (Cyprinus carpio L). From this research we found that there are three species have no more exist; those are Cork (*Ophiocepalus striatus*), Tawes (*Puntius javanicus*) and Tombro (Cyprinus carpio L). From these research we found three new species, there are Wader (*Rasbora argyrotaenia*), Gatul (*Gambusia affinis*), and Goby (*Gobionella shufeldti*).Diversity index of fishes living in Ranu pakis is low up to medium, with evenness is hight, and richness hight.

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O-EC05

THE EFFECT OF FISH CULTIVATION USING FISH KARAMBA TOWARDS THE QUALITY OF THE RANU PAKIS WATERS, KLAKAH SUB-DISTRICT, LUMAJANG REGENCY

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ABSTRACT

The raising of fish in fish karamba can affect the physical and chemical parameters of water. Continued cultivation of fish in fish karamba can also cause environmental degradation, which is marked by the decrease in water quality. Therefore research is needed to obtain information on the effect of fish cultivation in fish karamba towards the quality of water in Ranu Pakis, Klakah sub-district, Lumajang regency. This research was carried out by measuring parameters which are physical, chemical, and biological; the result was then compared with the standard environmental quality of the East Java province. Results shows that from eleven environmental parameters that were tested, smell, water temperature, conductivity and turbidity did not show significant differences. Meanwhile, for DO, TSS, pH, BOD_5, Nitrate, the sum of *Coliform* and *Fecal coliform* bacteria showed significant differences, where the area near the fish karamba had lower qualities. This is caused by the excessive addition of fish feed and the presence of fish feces from metabolic processes, making the amount of organic materials in the water rise. This causes water quality to decrease.

Keywords: fish cages, water quality, physical parameters, chemical parameters, biological parameters.

INTRODUCTION

Indonesia is one country that most of its territory is water. One of the water resources which are found in Indonesia is the lake. The waters of the lake is one form of freshwater ecosystems that exist on the surface of the earth. According Haryani (2004) the lake is a natural water body which is always inundated throughout the year and has a particular water quality, varied from one lake to another lake. Quality of water between the lake with each other lakes differ depending on the content of pollutants into the waters of the lake, and ingredients derived from the lake itself. Lake ecosystems, including freshwater habitats that have the calm waters are characterized by the presence of a very slow stream of about 0.1 to 1 cm / sec or no current at all.

Triangle Lake is the name for Pakis lake, Klakah lake and Bedali lake. Triangle Lake is a lake formed by volcanism, which is the lake formed due to volcanic activity. Triangle Lake is located in District Klakah, Lumajang. Agriculture and fisheries are the main

commodities in Klakah District. Types of fish produced, among others, Tilapia and fish Oreochromis mossambicus. Pisciculture in the district Klakah done by the method of keramba. Keramba placed in rows and along the banks of moderate Ranu Pakis. Installation of keramba starting from a distance of about 50 m from the shore to 150 m to the center of the lake. Rearing fish in keramba have an impact on the quality of these waters. Rearing fish in keramba can affect the physical and chemical factors such waters (Sari, 2007).

At the time the amount exceeds a certain limit can lead to high sedimentation process of accumulation of food remains in the bottom waters, the waste will cause a decrease in water quality (reduction of oxygen supply and pollution of lake water) which in turn affects the animals are kept. Food remains and metabolism of fish in cages maintenance activities as well as domestic wastes from agricultural activities of household waste as well as a major cause of declining lake ecosystems function ended on pollution of the lake, ranging from eutrophication caused the explosion (blooming) of phytoplankton and water weeds such as water hyacinth (Eichornia crassipes), and others that can result in aquatic organisms (mainly fish farming).

Sari, (2007) describes the activities of fish culture in keramba constantly also cause environmental degradation, which is characterized by declining water quality. Environmental constraints faced in farming activities such as territory or spatial arrangement of the development of cultivation that do not pay attention to the environmental carrying capacity due to improper management, causing environmental problems with all aspects of its complications in the long period of time. Organic waste is always produced each cultivation cycle will cause problems on water quality and soil condition of the lake bottom, so the decline in water quality easily occur. These conditions led to abiotic factors such as dissolved oxygen and pH often fluctuates, this will also lead to the decline in fish production.

MATERIALS AND METHODS

This study is a descriptive exploratory study. The study was conducted by measuring the parameters of physics, chemistry and aquatic biology. Parameters include odor, temperature, conductivity, turbidity, pH, DO, TSS, BOD_5, Nitrite, Total *Coliform* and *fecal coliform* total. The measurement results are then compared with the quality standard according to its designation, based on Government Regulation no. 82 of 2001 on water quality management. For the waters of the lake that used standard quality standard that is the quality standard of quality class I and class II standard. Tools and materials used in this research that DO meter, digital pH meters, Turbidity meters and konduktifitimeter, incubator, autoclave, LAF (Laminar Air Flow), spectrophotometer, analytical balance, stoves (heating), desiccator and oven. The materials used are water samples, aquades, beef extract,

peptone, lactose, BGLB, aluminum foil, cotton, wrapping paper, label paper, rope, 70% alcohol, matches, filter paper.

RESULT AND DISCUSSION

Results of measurements of physical parameters, chemical and biological water environment Pakis lake, District Klakah, Lumajang compared with the value of the Environmental Quality Standard According to Government Regulation no. 82 of 2001 on Water Quality Management.

Parameter	Unit	Baku mutu		Sample Location			
		Gol I	Gol II	Inlet	Centre	Outlet	Around fish keramba
Smell	-	-	-	Tidak	Tidak	Amis (+++)	Amis (+)
Temperature	^{0}C	-	-	28,3	28,2	28,2	27,6
Konduktivity	µs/cm	-	-	299	311	319	316
Turbidity	mg/l	-	-	9	6	6	5
TSS	mg/l	50	50	401	254	294	352
pH	mg O_2/l	6-9	6-9	7,90	7,83	8,46	8,31
DO	mg/l	6	4	7,55	8,27	7,41	6,87
BOD_5	mg/l	2	3	5,06	6,00	6,55	7,60
Nitrit (NO_2-N)	mg/l	0,05	0,05	0,49	0,39	0,49	0,50
Total *Coliform*	MPN/100 ml	1000	5000	30	6	673	240
Total *Fecal coliform*	MPN/100 ml	100	1000	23	0	283	23

Keterangan:
(+) : Smell sting
(++) : Smell more sting
(+++) : Smell most sting

1. Smell

Rearing fish in keramba affects the intensity of the odor of the waters in Pakis lake, District Klakah, Lumajang. The pungency is shown in the inlet, middle, outlet and around fish cages vary from one to another smell that smell is the result of decomposition of organic substances contained in water. Decomposition of organic substances is performed by microbes present in waters. Residual organic material derived from fish feed and fish excrement. According Warlina (2004), the smell of the waters is the result of the decomposition of organic material by microbes waters. Organic substances in the waters described by the bacteria produce NH_3 and amin. Amin is what causes the fishy smell in the waters.

2. Temperature

Rearing fish in keramba did not affect the temperature of the waters in Pakis lake, District Klakah, Lumajang. Water temperature is affected by the radiation of sunlight, air temperature, weather and location. Solar radiation is the main factor affecting the rise and fall of water temperature. Sunlight causes the hot water on the surface more quickly than

deeper water bodies. Water density decreased with the increase of temperature so that the surface and deeper water can not be mixed perfectly. This will cause the temperature stratification (stratification themal) in water bodies (Sasi, 2007). Suwono (2010) mentions that a stable temperature will greatly affect the circulation and water mass stratification, it can affect the distribution of organisms. Based on the results of measurement is known that the temperature of waters in the inlet, middle, outlet and around the fish keramba and it ranged fairly constant (27-29)^{0}C.

3. Conductivity

Rearing fish in keramba did not affect the conductivity of the waters in Pakis lake, District Klakah, Lumajang, this is indicated from the results of measurements show that the conductivity at each sampling site was relatively similar. Conductivity indicates that in these waters contained dissolved chemicals such as NaCl. Conductivity value depends on the total concentration of ions in water. From the results of conductivity measurements it appears that the conductivity at the inlet area of 299 µs / cm, was 311 µs / cm, outlet 319 µs / cm and about 316 fish keramba 316 µs / cm. Conductivity in a water proportional to the temperature rise. The temperature rise in the waters of the conductivity will also rise. Mahanal (1998) mentions that the electrical conductivity is also influenced by temperature, temperature rise of 1º C will increase the electrical conductivity of 1.9%. Conductivity associated with salinity, the higher the salinity the higher the conductivity. According to Boyd (1990) is the salinity levels of the ions dissolved in water.

4. Turbidity

Rearing fish in keramba did not affect water turbidity in Pakis lake, District Klakah, Lumajang. Turbidity is the intensity of the darkness in the water caused by materials that float. Turbidity waters generally caused by the suspension of particles such as clay, silt, dissolved organic materials, bacteria, plankton and other organisms Mahida (1993). Of the four sampling sites, the highest turbidity was located in the inlet area equal to 9 mg / l, while for the middle of 6 mg / l, outlet 6 mg / l and around fish keramba 5 mg / l. Differences in levels of turbidity can be caused by organic and inorganic substances contained in water. Organic component consists of phytoplankton, zooplankton, bacteria and other microscopic organisms. While the inorganic component consists of detritus, inorganic particles and sludge particles (Supono, 2008).

5. TSS (Total Suspended Solid)

Rearing fish in keramba did not affect water turbidity in Pakis lake, District Klakah, Lumajang. TSS consists of silt and fine sand as well as the bodies of microorganisms mainly caused by scraping the ground or carried by erosion into water bodies. The highest TSS content is located in the inlet 401 mg / l and TSS second highest in the area around

the fish keramba 352 mg / l. TSS in the middle of 254 mg / l and an outlet region of 294 mg / l. TSS at Pakis lake waters caused by the content of the sludge particles carried by water flow and also from the remnants of water-borne organic material derived from domestic waste and the community. The remaining fish feed given to fish in keramba is also a major factor causing high suspended solids. Suspended solids can be either mineral or organic material derived from soil erosion, industrial, sewage and waste that can be found in surface water. Suspended solids can be toxic if excess oxidized by the organism so that it can lower the DO concentration to cause death in fish (Sasi, 2007).

6. pH

Rearing fish in keramba affect the pH of the waters in Pakis lake, District Klakah, Lumajang. PH value indicates the degree of acidity or alkalinity of a water that can affect the lives of plants and aquatic animals (Setyobudianti, 1997). water pH increased along with the sampling stations. pH 7.9 at the inlet area mgO2 / l, the middle region 7.83 mgO2/ l, the area around the fish keramba of 8.31 mgO2 / l and pH too high is located in the outlet area of 8.46 mgO2 / l. Mahida (1993) states that the waste industry and household waste can affect water pH value. PH value can affect the speciation and toxicity of chemical compounds from microscopic elements contained in the waters, for example toxic H_2S is mostly found in polluted waters and waters with low pH values. In addition, pH also affects the value of BOD_5, phosphate, nitrogen and other nutrients. pH of water affects the level of fertility because it affects aquatic organisms living there. Sasi (2007) says the more the number of fish cages will increase the amount of dissolved organic material and causes the pH value decreased, due to increasing CO_2 concentrations due to microbial activity in deciphering organic material.

7. DO (*Disolve Oxigent*)

Rearing fish in keramba affect aquatic DO in Pakis lake, District Klakah, Lumajang. Source of dissolved oxygen in water comes from the diffusion of oxygen in the atmosphere, currents or the flow of water through rain water and the activities of photosynthesis by aquatic plants and phytoplankton. Boyd (1990) describes the oxygen can diffuse directly from the atmosphere after the contact occurs between the surface of the water with air containing 21% oxygen. Of the four sampling sites appears that daerak around fish keramba has the lowest oxygen content of 6.87 mg / l compared to the inlet 7.55 mg / l, was 8.27 mg / l. and outlet of 7.41 mg / l. This occurs due to organic matter content of feed derived from fish described by microbes and the microbes in the decomposition process that requires oxygen for energy. In addition the number of fish that many causes oxygen content of the smaller fish around the keramba. Fish in keramba do respiration so that the amount of DO in water is reduced (Sasi, 2007). Oxygen demand for fish is relatively quiet

compared to fewer fish while on the move and spawn. The main cause of reduced levels of dissolved oxygen in the water due to contaminants that can consume oxygen. Contaminants are mainly composed of organic materials and anorganic derived from sources, such as manure (animal and human), organic waste, waste materials from industry and households. According to Connell and Miller (1995), most of the contaminants that cause decreased dissolved oxygen is organic waste.

8. BOD_5 (*Biological Oxigent Dimand*)

Rearing fish in keramba affect aquatic BOD_5 Pakis lake, District Klakah, Lumajang. BOD is defined as the amount of oxygen required by organisms at the breakdown of organic material under aerobic conditions. Breakdown of organic materials used by organisms as food and energy derived from oxidation processes (Salmin, 2005). Based on research results from the four sampling sites appear highest BOD_5 content is located in the vicinity of fish keramba of 7.60 mg / l, while that for the inlet area of 5.06 mg / l, was 6.00 mg / l, and the area outlet 6.55 mg / l. BOD5 content of which is due to the high content of organic material around the fish keramba is quite high. The content of organic material is derived from the rest of the feed given to fish in keramba. Sukimin (1990) describes the organic wastes that pollute the waters of the lake, based on the origin can be differentiated into organic waste originating from outside the lake and is derived from activities in the lake water body. Waste originating from outside the lake in the form of industrial waste, domestic, and agriculture, while those derived from activities in the waters of the lake is the remnant body pellets of fish farming activity in the keramba (cage Floating Net).

9. Nitrite

Rearing fish in keramba affect aquatic Nitrite Pakis lake, District Klakah, Lumajang. Based on the results of measurements of nitrite in the vicinity of fish keramba in the amount of 0.50 mg / l, while the inlet and outlet have the same nitrite content that is equal to 0.49 mg / l, and the regional center by 0.39 mg / l. A high content of nitrite caused by the content of pollutants originating from a given fish feed. The remaining fish feed and fish feces are organic materials with high protein content. Excess feeding causes the accumulation of organic material to accumulate. Acucumulation organic material is followed by a decay process that utilizes oxygen from the water and the decomposition of anorganic material which is a fertilizer for phytoplankton. The process of decomposition of organic materials was carried out by Nitrosomonas and Nitrobacter bacteria. High content of nitrites which can also be caused by high pH, where the content of the pH around the fish keramba is more alkaline than the other locations so that the nitrification process can run optimally. Low content of DO also cause nitrification processes can run optimally.

10. Total *Coliform* and *Fecal coliform*

Rearing fish in keramba affect the total amount of total *Coliform* and *Fecal coliform* waters Pakis lake, District Klakah, Lumajang. Aquatic environments easily contaminated by pathogenic microorganisms (harmful) coming from various sources such as settlements, agriculture and animal husbandry. Based on the test results it appears that many Coliform bacteria content in the outlet area of 673 MPN/100 ml. This shows that the outlet has been contaminated by the feces both human and animal waste manure. Outlet region is an area that is often used by people around for everyday purposes such as bathing, washing and even toilets are also performed at the outlet of lake, other than that the outlet is also the area closest to the fish keramba. *Fecal coliform* bacteria is an indication of fecal contamination in the most efficient, because the *Fecal coliform* only and always present in human feces (Effendi, 2003).

CONCLUSION

The conclusion of organic matter and nutrients that come from outside and from aquaculture keramba would affect the availability of oxygen and water carrying capacity. Carrying capacity is the ability of the waters in the receiving waters, dilute and assimilate the load without causing changes in water quality or pollution. Oxygen reserves in the waters of the lake is very limited. In the end the excessive feeding of fish on fish KJA system become the main cause of decline in lake ecosystems function ended on pollution of the lake, ranging from eutrophication caused the explosion (blooming) of phytoplankton and water weeds like water hyacinth (*Eichornia crassipes*), and so another that can cause death in aquatic organisms (mainly fish farming) and ending with more thickening layer of sediment in the lake water body.

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O-EC06

Morphological Characters of Suspension Feeder Bivalve *Potamocorbula faba* (Bivalvia: Corbulidae)

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ABSTRACT

Corbulidae bivalves are well-known as resistant species on habitats that are experienced environmental disturbances. In some areas, corbulid are widely exploited for consumption as well as animals feeding. The objective of this research was to study the morphological characters of *Potamocorbula faba* that are very abundant in the estuarine regions of Sidoarjo coastal water. Specimens collected from Sidoarjo Coastal Water, East Java. Specimens were relaxated by using $MgCl_2$ 7% in sea water and fixed in 10% formalin in sea water. Finally, specimens were preserved in 70% ethanol. Observation was done on the internal morphology of all specimens. Morphometric measurements were done on the length, height, and width of shells, length and width of ctenidium and labial palp. The results of this research showed that the diagnostic characters of *P. faba* were the exterior and interior characters of the shell, form and the type of siphon, and the form of foot. Ratio length: height of the shell=1: 0,6; ratio length= width of the shell: 1: 0.4, ratio height: width of the shell= 1: 0.6. The ctenidium of *P. faba* is relatively bigger than its labial palp, the ratio of ctenidium: labial palp is 1: 0.9. Based on the morphometric measurements of ctenidium and labial palp, *P. faba* can be categorized as suspension feeder.

Keywords: *Potamocorbula faba*, Corbulidae, Bivalvia, morphological character of bivalve, suspension feeder

INTRODUCTION

Corbulidae bivalves are well-known as resistant species on habitats that are experienced environmental disturbances. Some research revealed that Corbulid bivalves could live in abundance in polluted water.[1,2] Hrs-Brenko[2] proposed that the nature of the shell has contribution to maintain their survival on disharmonic environment.

Corbulid bivalve, namely *kupang putih* (*Potamocorbula faba*) was reported abundant in costal water of Sidoarjo and Surabaya. This bivalve dominated the estuarine region and has been fished by local people for commercial traditional food called *lontong kupang*, raw material of *krupuk* and *petis*, as well as for animal feed. Although this bivalve is popular among the local people, the biological studies of this species are still limited. Previous studies focused on the pollution and heavy metal content of bivalve, such as *kupang putih*.[3-6]

Therefore, we interested to describe the habitat as well as the morphological characters of *Potamocorbula faba* as the database of this species.

MATERIALS AND METHODS

Bivalve samples were collected from Sidoarjo coastal water, East Java Indonesia. Specimens were collected by using vertical core sampler. Some habitat parameters, namely type of substratum, acidity of the substrat, as well as turbidity and salinity of water were observed and measured.

Specimens were relaxed by using $MgCl_2.6H_2O$ 7% in sea water, and fixed by using formalin 10% in sea water. Finally, specimens fixed in Alcohol 70%. Morphometric measurements were done to 50 specimens by using caliper, including length, height, and width of the shell, and length and the width of ctenidium (gill) and labial palp. Internal morphology of the specimens was observed carefully under magnifier lamp. These morphometric data were analyzed by using regression analysis.

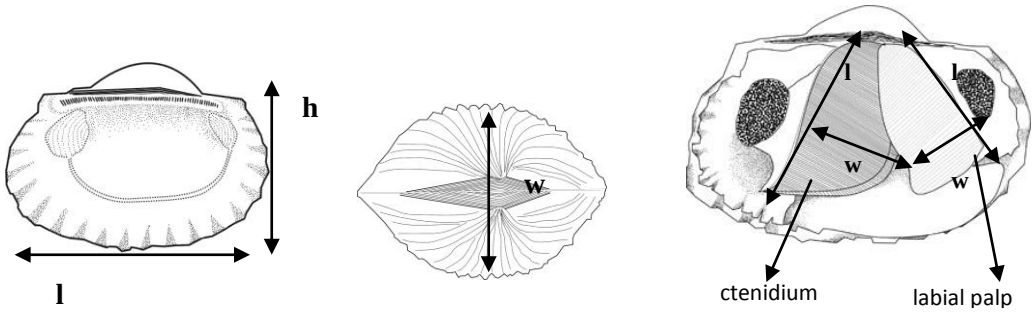

Figure 1. Morphometry of shell, ctenidium, and labial palp; l: length, h: height, w: width

RESULTS AND DISCUSSION

Diagnostics characters of *Potamocorbula faba.* Relatively small (10.5–14.40 mm). Trignonal shell, truncate posteriorly. White shell. Periostracum tick, glossy and brownish grey. Inequivalve, right valve bigger than left valve at ventroanterior region. Trigonal chondrophore located at left valve. Right valve with big and prominent cardinal tooth.

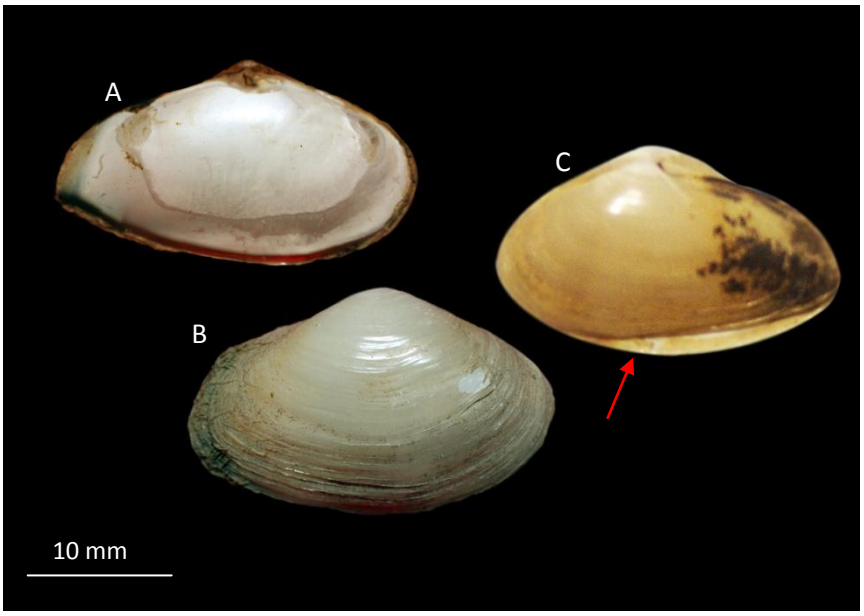

Figure 2. Morphology of *Potamocorbula faba*; A: interior of left valve; B: exterior of right valve; C: inequivalve shells, right valve bigger than left valve

Description. Elongate trigonal shell**.** Exterior shell is white, covered by relatively tick periostracum. Periostracum is brownish grey. Smooth exterior shell with concentric striae. Anteriodorsal shell with stronger concentric striae and covered by ticker periostracum. Dorsal of the shell flattened and narrowed posteriorly. Lunule and eschuteon not clear. Right valve is bigger and more convex than left valve (Figure 2).

Heterodont hinge teeth. Left valve has two big cardinal teeth and one small cardinal tooth. Chondrophore is located between these two cardinal teeth. Left valve with adductor scars. Anterior adductor scar is almost equal with posterior adductor scar. Posterior adductor scar located at posteriodorsal of the shell, while anterior adductor scar located at anteriodorsal of the shell. Palial line entire, sinus poorly developed (Figure 3).

Flesh yellowish white. Mantle attached ventrally, pedal gape located at ventroanterior. Foot is axe-like, relatively big, with ventral byssal groove. In some specimens, in these part can be found one byssal thread, yellowish white. Eulamelibrach gill type. Gill located anterior–posterior. Outer demibranchia is much smaller than inner demibranchia. One pair of labial palp located at anterior of gill. Labial palp is wide. Anterior and posterior adductor muscles are equal and rounded. Anterior adductor muscle attach to anteriodorsal of the shell. Posterior adductor muscle attach to posteriodorsal of the shell. Anterior pedal retractor muscle attach to interior shells, at dorsal region of anterior adductor muscle. Posterior pedal retractor muscle attach to interior shells, at dorsal region of posterior adductor muscle. Posterior pedal retractor muscle is bigger than anterior pedal retractor muscle. Siphon very short, can be distinguished into inhalans and exhalans siphon.

Exhalans siphon located at dorsal region of inhalans siphon. Exhalans siphon is pinkish, with short tentacles on its tip. Inhalans siphon is bigger than exhalans siphon, with longer tentacles. Inhalans siphon is brown (Figure 4).

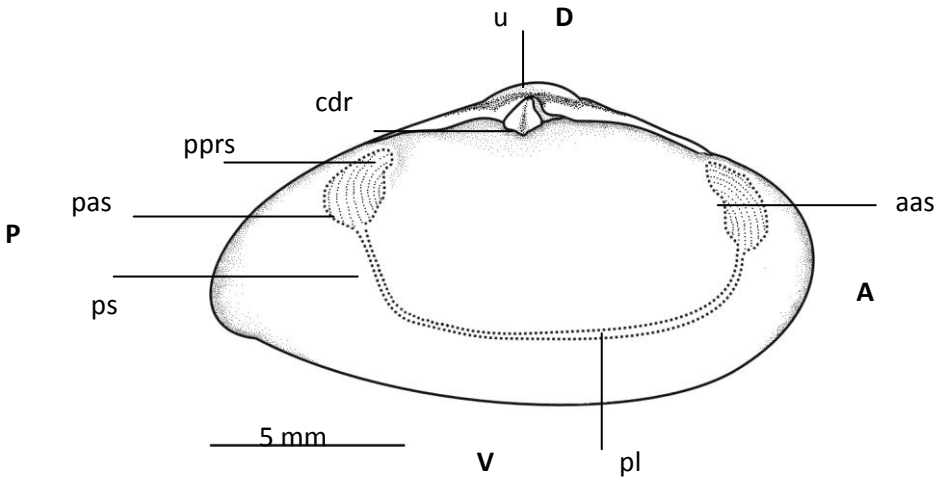

Figure 4. Interior of shell of *Potamocorbula faba;* A: anterior, P: posterior; D: dorsal; V: ventral; aas: anterior adductor scar; pas: posterior adductor scar; pl: palial line; ps: palial sinus; lig: ligament, u: umbo

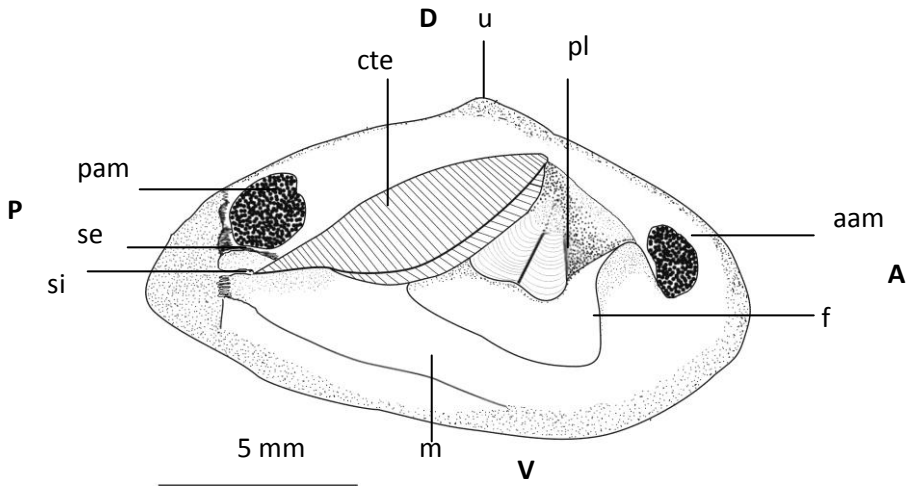

Figure 4. Internal anatomy of *Potamocorbula faba* A: anterior, P: posterior; D: dorsal; V: ventral; aam: anterior adductor muscle; pam: posterior adductor muscle; u: umbo; cte: ctenidium; lp: labial palp; si: siphon inhalans; se: siphon exhalans; m: mantle; f: foot.

Kupang putih [*Potamocorbula faba* (Hinds, 1843)] is the only corbulid bivalve that can be found in the coastal water of Sidoarjo. Previously, *Potamocorbula faba* was classified

under genus *Corbula* Bruguiere 1797, however it was revised and classified under genus *Potamocorbula* Habe 1955 (Willan, 2010; personal communication). Collection of *Potamocorbula faba* at Museum Zoologicum Bogoriense, i.e. MZB Pel.1643, was also determined as *Corbula faba*. Some members of genus *Potamocorbula* have been also classified under genus *Corbula* before finally revised, namely *Potamocorbula amurensis* (Schrenck, 1867), *P. laevis* (Hinds, 1843), and *P. ustulata* (Reeve, 1844).[7]

Important characters that can be used to distinguish are as follows: Shell is relatively small (10.5–14.40 mm); trignonal shell, truncate posteriorly; white shell. Periostracum is tick, glossy and brownish grey. Inequivalve shell: right valve is bigger than left valve at ventroanterior region. Trigonal chondrophore is located at left valve. Right valve has big and prominent cardinal tooth.

The valve of *Potamocorbula faba* is elongate trigonal with the ratio of length and height of the shell = 1: 0.6; ratio length and width of the shell = 1: 0.4 and ratio of height and width of the shell = 1: 0.6. The ratio of morphometry of the shell reveals that the shells of *Potamocorbula faba* tend to elongate and flat. The pattern of relationship of the length and height of the shells can be shown by regression equation Y=-0.24+0.626X, R^2= 0.764; the length and the width of the shells by Y=0.156+0.576X, R^2= 0.764; while the height and width of the shells by Y=0.577+0.549X, R^2= 0.626 (Figure 5). These equations also reveal that the form of the shells of *P. faba* tend to vary. This can happen due to the influence of environment.

The results of regression analysis also reveal the pattern of shells growth. The growth of the height and width of *P. faba* are negative allometric against its length because b<1. This shows that the shells grow faster in their length compare to height as well as width of the shells. The pattern of the growth of height against the width is also negative allometric. It means that the shells grow faster in their height than their width.

Morphological characters of *Potamocorbula faba* are very suitable to their mode of life. *Potamocorbula faba* can be catagorized as *shallow infauna* which burrow in 0–3 cm of the soft bottom substratum. This bivalve burrows on vertical position, hence the tip of posterior end of the shells can be shown on the upper layer of the substratum, while the foot is extended to the substratum through ventroanterior pedal gape. Carlton *et al.*[7] reported that *P. amurensis* also burrow in the sediment and half up to two three of the shells can be shown on the upper layer of the sediment. The siphon of *Potamocorbula faba* is very short, hence this bivalve is only able to have shallow burrow. Hrs-Brenko[2] stated that *Corbula gibba*, the other member of Corbulidae, have short siphon and burrows in 0–5 cm. Lamprell *et al*.[1] also stated that Corbulid bivalves have short and retractile siphon.

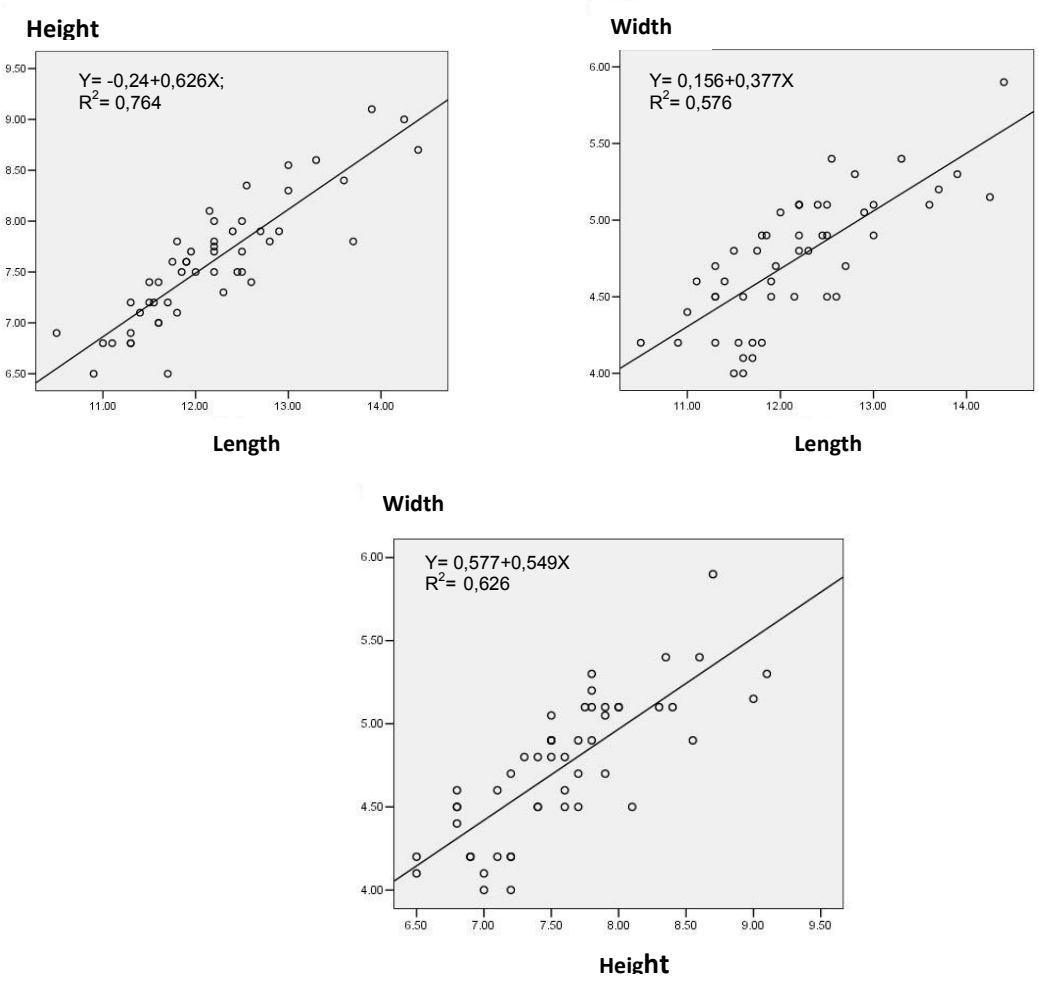

Figure 5. Pattern of morphometry of *Potamocorbula faba* shell based on linear regression.

The results show that *Potamocorbula faba* has eulamelibranchia gill, and the *outer demibranchia* of the gill is smaller than the inner *demibranchia*. The labial palp is relatively smaller than the gill; the ratio of gill and labial palp insang dan palpus labialis = 1: 0,9. This ratio reveals that *Potamocorbula faba* is more active as *suspension feeder* because the gill is more developed and big in size to support its function in pumping the water. Compton *et al.*[8] stated that *suspension feeder* has big gill and small labial palp to support the function of gill to pump the water. The finding of this research supports the results of other research. Lamprell *et al*.[1] stated that Corbulid bivalves are *ciliary suspension feeder*. In addition, Hrs-Brenko[2] also reported that *Corbula gibba*, which belongs to Corbulidae, is *suspension feeder*. *Potamocorbula faba* is dominant species in the estuary of Kepetingan River. Moreover, this bivalve is also found in abundance in the coastal water of Pulo Dem, the estuarine region of Porong River. Both of estuaries of Kepetingan River and Porong River have relatively higher content of sand compare to other region of Sidoarjo Coastal water,

the sand content is 3,72–51,87%. Lamprell *et al.*[1] also reported that Corbulid bivalve can be found at sandy bottom as well as muddy sandy bottom.

Potamocorbula faba can be found not only at subtidal, but also at lower intertidal. This bivalve occupies habitat with wide range of water salinity, namely 12–30‰, water temperature: 29–32°C, range of substratum acidity: 6,6–7, and range of turbidity: 11,4–56,7 FTU. Carlton *et al*. (1990) reported that *P. amurensis,* which is native species of China, Japan, and Korea, are able to survive and live in abundance in San Fransisco Bay. *Potamocorbula amurensis* are able to live in water salinity <1‰–32,6‰ and extrem temperature range, namely 8,0–23°C.

During the sampling period, *Potamocorbula faba* was the only living bivalve that could be found, but in extremely high density, namely 3549–10000 individu/m^2. Some factors may influence this condition. First of all, the estuarine region of Kepetingan River was in disharmonic condition due to organic and inorganic pollution. In polluted ecosystem, only resistant organisms can survive and multiply in number. Lamprell *et al*.[1] mentioned that Corbulid bivalves have high degree of tolerance of environment degradation and some of them can be found abundantly in polluted environment. Hrs-Brenko[2] also reported that *Corbula gibba* (Corbulidae) are living abundantly in polluted beach, harbour, and bay. *Potamocorbula amurensis* were found in abundance, and reached almost 10.000 individu/m^2.[7] The dominancy of this species can change the benthic community, hence *P. amurensis* was categorized as “pest” by National Introduced Marine Pest Information System-Australia.[9]

The unique morphological characters of *P. faba* support their survival on extreme environment. The equivalve shells enable the valves closed tightly, hence they can protect their selves during the extreme conditions such as extreme salinity. Their develop eulamelibranchia gill also support their feeding as suspension feeder. The presence of retractile siphon supports the activities of shallow burrower, which also increase their survival rate.

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O-EC07

A Preliminary study of forest vegetation and mammal species in Unipa Natural Forest Education Area, Manokwari-West Papua

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O-EC08

Sumatran Butterflies Conservation in Mount Betung, Lampung

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ABSTRACT

Conservation for Sumatran Butterflies had been done in 4 hectares forest area (secondary forest) at 460m above sea level. Since 1998, the method was habitat enginered to achieve a supportive microhabitat for butterflies diversity. Water system and planted plants rewarded had been succeed to attract butterflies, visit and reproduced in forest area that had been recovered. Butterflies population was rising as the availability of larval food plants and nectar plants. Nowadays, there were 160 Sumatran Butterflies species had been identified.

Keywords: Sumatran Butterfly, habitat engineering

Introduction

Biodiversity of butterfly is a potential nature resource in Indonesia which have not been use optimally. Butterfly has beautiful color of wings, living as pollinator and giving economic value for supporting ecotourism.

Butterfly biodiversity is determined by food plant availability. That condition is giving barrier on butterfly life cycle. In addition, Soekardi (2000) some species are supported by only one specific plany. For example, *Troides helena* larvae only eat *Aristolochia tagala* leaves, *Appias lybithea* only eat *Cleome rutidosperma* leaves, *Appias lybithea* only eat *Cleome rutidosperma* leaves. During butterfly, need nectar from flowers as its food. The drinking nectar activity made butterfly's role as pollinator.

Mount Betung Lampung (1.240 m above sea level) are protected forest area with 22.224 ha and located facing Lampung Bay. It has a critical condition (Anonim, 1997) because of the land conversion become cultivated area. These condition became threat for butterflies species, the existence in natural environment were having pressure, mainly caused by disturbance in ecosystem and food plants for larva were shrinking (Soekardi, 2000). Because of that, it is the time to do butterfly conservation.

The problem in conserving butterflies is how to make a sufficient microhabitat that support butterfly life cycle in certain location.

Methodology

Open garden type butterfly park was built on 4 ha area, 400 m above sea level on Mount Betung, Lampung. The early condition of the area was dominated by *Pterocarpus indicus, Coffea Arabica* and *Imperata Cylindrica*. There were no food plants as larva food plant neither nectar plant, unless food plant for butterfly grass (*Lycaenidae* and *Hesperidae* family).

Habitat enrichment is chosen as the main methodology in this research with adding the plants resources that is needed for butterflies to thrive. The long continuous research started in June 1999 until Juni 2001. Since June 1999, there were 25 species food plants was planted with 200-800 plants each species. Those plants were collected from Mount Betung Lampung in > 700 m above sea level and several places in Lampung Province and other province in Sumatra.

The planted food plants are *Aristolochia tagala, Clausena excavate, Apama tomentosa, Citrus aurantifolia, Michelia champaca, Asystasia intrusa, A. coromandeliana, Persea Americana, Graptophyllum pictum, Piper aduncum, Cassia siamea, Cassia alata, C. biflora, Evodia malayana, Triphasia trifolia, Annona muricata, A. squamosa, Synedrella nodiflora, Fleurya interrupta, Flacourtia rukam, Pseuderanthemum reticulatum, Murraya koenigii, Clotnopis gigantean, Cleome rutidosperma, Loranthus sp.*.

Nectaring plants which is planted in research area was based on observation in field on type flowers which often visited by butterflies. This type of plant also chosen by the height of flower tube, the longer tube will be visited by bigger butterfly with longer proboscis while the short tube will be visited by much smaller butterfly with shorter proboscis. The planted nectar plants are *Clerodrum paniculatum, Ixora javanica, Lantana camara, Stachytarpheta indica, Celosia argentea, C. surinamensis, Calliantra callothrsus, Tithonia rotundifolia,* and *Cuphea hysopifolia.* Each was planted 200-800 plants.

Research on butterfly visitation to nectar producing plants and food plants was conducted after habitat enrichment is done using survey method.

Results and Discussion

Sumatran butterfly conservation in Mount Betung , Lampung has been successfully invite 160 species of Sumatran butterfly from the surrounding area (See: Table 1). Soekardi (2009) stated that butterfly conservation model in Mount Betung, Lampung with habitat engineering and enrichment had cause various butterfly populate and breed in the area.

This is mainly cause by various species of food plants and nectar producing plants available to the butterflies which are selected through continuous research according to butterfly needs and preferences (Soekardi, 2009).

There is a clear result that when there is an increase in biodiversity of plants, it is also followed by the increase of butterfly's biodiversity (See Figure 1). We engineered the type of plants with continuous plant type additions that closely related to butterfly needs.

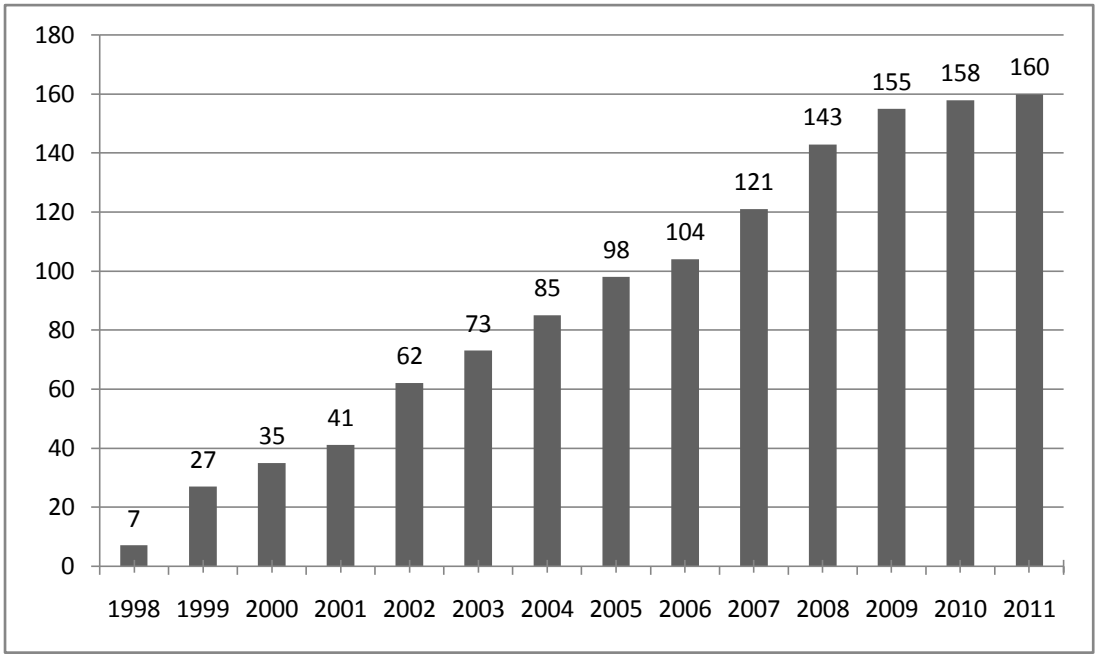

Figure 1. Species Trends in Butterfly Park Mount Betung, Lampung

Table 1. Sumatran Butterflies Species that Populate the Butterfly Park (2011 Survey)

No.	**Family**/ Species
	Papilionidae
1	*Atrophaneura coon*
2	*Atrophaneura nox*
3	*Graphium agamemnon*
4	*Graphium doson*
5	*Graphium sarpedon*
6	*Maendrusa payeni*
7	*Pachliopta aristolochiae*
8	*Papilio demoleus*
9	*Papilio demolion*
10	*Papilio helenus*
11	*Papilio iswara*
12	*Papilio memnon*
	Papilio memnon agenor
13	*Papilio nephelus*
14	*Papilio palinurus*
15	*Papilio peranthus*
16	*Papilio polytes cyrus*
	Papilio polytes romulus
17	*Pathysa antiphates itamputi*
18	*Troides helena cerberus*
	Pieridae
19	*Appias libythea*
20	*Appias lyncida*
21	*Appias indra*
22	*Catopsilia pomona*
23	*Catopsilia pyranthe*
24	*Catopsilia scylla*
25	*Delias hyparete*
26	*Delias pasithoe*
27	*Eurema blanda*
28	*Eurema brigitta*
29	*Eurema hecabe*
30	*Eurema sari*
31	*Eurema simulatrix*
32	*Hebomoia glaucippe*
33	*Leptosia nina*
34	*Pareronia valeria*
35	*Saletara liberia*
	Nymphalidae
36	*Acraea violae*
37	*Amathusia phidippus*
38	*Amathuxidia amythaon*
39	*Ariadne ariadne*
40	*Cethosia hypsea*
41	*Cethosia penthesilea*
42	*Chersonesia rahria*
43	*Cirrochroa surya*
44	*Cupha erymanthis*
45	*Danaus chrysippus*
46	*Danaus genutia*
47	*Discophora sondaica*
48	*Doleschallia bisaltide*
49	*Dophla evelina*
50	*Elymnias hypermnestra*
51	*Elymnias nesaea*
52	*Elymnias panthera*
53	*Euripus nyctelius euploeoides*
54	*Euploea core*
55	*Euploea eunice*
56	*Euploea mulciber*
57	*Euploea tulliolus*
58	*Euthalia aconthea*
59	*Euthalia adonia*
60	*Euthalia ipona*
61	*Faunis canens arcesilas*
62	*Hypolimnas anomala*
63	*Hypolimnas bolina*
64	*Hypolimnas missipus*

65	*Ideopsis(Radana) juventa*
66	*Junonia hedonia*
67	*Junonia(Precis) atlites*
68	*Junonia(Precis) iphita*
69	*Junonia(Precis) orythia*
70	*Lebadea martha*
71	*Lethe europa*
72	*Lethe minerva*
73	*Lethe vindhya*
74	*Lexias pardalis*
75	*Melanitis leda*
76	*Modusa procris*
77	*Mycalesis anaphita*
78	*Mycalesis janardana*
79	*Mycalesis meneus*
80	*Mycalesis perseoides*
81	*Mycalesis visala*
82	*Neptis clinia*
83	*Neptis clinioides*
84	*Neptis harita*
85	*Neptis hylas*
86	*Orsotriaena medus*
87	*Parantica aspasia*
88	*Phalanta phalantha*
89	*Polyura hebe*
90	*Symbrenthia lilaea*
91	*Tanaecia iapis*
92	*Tanaecia pelea*
93	*Tirumala septentrionis*
94	*Xanthotaenia busiris*
95	*Ypthima baldus*
	Lycaenidae
96	*Allotinus falax*
97	*Anthene emolus goberus*
98	*Arhopala aurelia*
99	*Arhopala pseudocentaurus*
100	*Caleta roxus*
101	*Catochrysops strabo*
102	*Chilades pandava*
103	*Deudorix epijarbas cinnabarus*
104	*Everes lacturnus*
105	*Flos apidanus*
106	*Hypolycaena erylus*
107	*Hypolycaena thecloides*
108	*Hypolycaena(Zeltus) amasa*
109	*Jamides alecto*
110	*Jamides celeno*
111	*Jamides elpis*
112	*Jamides malaccanus*
113	*Leptotes plinius*
114	*Manto hypoleuca*
115	*Miletus biggsii*
116	*Miletus gopara*
117	*Pratapa deva*
118	*Pratapa icetas*
119	*Prosotas dubiosa*
120	*Prosotas nora*
121	*Rachana jalindra*
122	*Rapala iarbus*
123	*Rapala pheretima*
124	*Sinthusa nasaka*
125	*Spalgis epius*
126	*Spindasis seliga*
127	*Surendra vivarna*
128	*Tajuria albiplaga*
129	*Zizina otis*
	Riodinidae
130	*Zemeros flegyas*
	Hesperiidae
131	*Ampittia dioscorides camertes*

132	*Ancistroides gemmifer*
133	*Badamia exclamationis*
134	*Borbo cinnara*
135	*Celaenorrhinus asmara*
136	*Charmion ladana*
137	*Choaspes subcaudatus*
138	*Coladenia dan*
139	*Gangara thyrsis*
140	*Hasora schoenherr chuza*
141	*Hasora taminatus*
142	*Hidari irava*
143	*Hyarotis adrastus praba*
144	*Koruthaialos sindu*
145	*Lotongus avesta*
146	*Matapa aria*

147	*Matapa cresta*
148	*Notocrypta paralysos*
149	*Oriens gola*
150	*Pintara pinwilli*
151	*Pirdana hyela*
152	*Plastingia naga*
153	*Plastingia pellonia*
154	*Potanthus flavum*
155	*Pseudocoladenia dan*
156	*Suarda swerga*
157	*Suastus gremius*
158	*Tagiades japetus*
159	*Telicota augias*
160	*Udaspes folus*

If we do the grouping per family we found that Papilionidae family has 18 species, Pieridae family has 17 species, Nymphalidae species has 60 species, Lycaenidae has 34 species, Riodinidae has only one species, while Hesperiidae has 30 species (All totaling 160 species).

In this research butterfly family with the highest biodiversity is Nymphalidae. According to Smart (1975), Nymphalidae family is one of the largest butterfly family; it is represented in all world regions which contains of several thousand species. The lowest biodiversity is held by Riodinidae family. This family was belong to the Lycaenidae family as sub family (Riodininae) that just recently being promoted to family level.

Conclusion

1. Butterfly conservation can be done with habitat enrichment, that is closely related with diversity food plants and nectar plants conservation.
2. Open garden type butterfly park has been succeeded inviting 160 species butterflies from 6 families to visit and reproduce.
3. Open garden type butterfly park in Mount Betung can be model in-situ butterfly conservation.

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O-EC09

The functions of chickens to livelihood: an initial study to support the conservation design of indigenous chickens in West Java

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ABSTRACT

Some studies showed that the population of indigenous chickens in Indonesia have been decreasing. To maintain their population and sustainable use, it is important to design conservation programmes which take into account the functions of the chickens to household economics. This is to ensure that the genetic traits those are important for fulfilment these functions can be incorporated in the conservation scheme.

A study to determine the functions of indigenous chickens for farmers' livelihood was conducted in two districts in West Java. The study employed survey method using face to face interview to the farmers who have been rearing two indigenous chickens; Pelung chicken in Cianjur District and Sentul chicken in Ciamis District.

The result showed that the functions of the chickens are as income source, insurance and saving as well as social integration. Income source is the main functions of Sentul chickens because the farmers have regular money for their daily activities by rearing these chickens. The main function of Pelung is as insurance and or saving which means that by keeping the chickens, the farmers can have some money to provide some expenditure such as school fee and for saving against unexpected events. Another function for both chickens is as social integration which means that raising chickens is considered as a means of networking and by rearing these chickens, the farmers can have satisfaction. Further study should be exploring genetic traits which are important for fulfilment these functions.

Keywords : livelihood, conservation design, indigenous chickens, West Java

Introduction

Animal Genetic Resources (AnGR) contributes significantly to humankind through the production of food and non-food items. Approximately 30 to 40 percent of the total value of global food and agricultural production is derived from animal products (Igrassia et al. 2005). AnGR is also important for rural livelihood by contributing to the livelihood of 70% of the world's rural poor (LID 1999). In addition, AnGR is closely linked to the religious and socio-cultural aspects of life of people in developing countries (Branckaert & Gueyé 1999).

Currently, FAO (2007) reported that there are more than 7,500 breeds in the world of which about 5,500 are mammalian breeds and 2000 are avian breeds. A total of 1,487

breeds (or 20 percent) is classified as being 'at risk'[1]. The number of breeds at risk is 607 breeds for avian species among which, chickens have by far the highest number of breeds at risk on the world scale.

A recent study pointed out that Indonesia is one of chicken domestication centres in the world (Sundari et al 2007). However, studies which investigate population numbers are limited. Some of those have been executed by Susanti et al (2007; 2008) who concluded that population numbers of indigenous chicken in West Java is decreasing. For instance, Sentul chicken has only 100 – 1000 female adults while there are 5,000 – 10,000 Pelung chicken. In addition, Wareng and Ciparage chickens are nearly extinct. Based on FAO risk classification Sentul, Wareng and Ciparage can be classified as 'at risk' while Pelung is not at risk. However, Pelung chicken and other indigenous chicken breeds in Indonesia are predicted to decrease because of Avian Influenza outbreaks (Diwyanto & Prijono 2007).

FAO (2007) indicated that the rapid spread of large-scale intensive production; inappropriate development policies and management strategies; disease outbreaks and control programmes and various types of disasters and emergencies were important threats to AnGR. Importantly, all these threats occur in the poultry sector and conservation action may be necessary in this sector (Hoffman 2009).

Conservation can take different forms, depending on need and resources (Wolliams et al. 2008). Nevertheless, conservation methods are broadly grouped into in situ in vivo, ex situ in vivo and in vitro (Gibson et al. 2005). In-situ conservation through the community based approach is likely the best strategy for conservation of Indonesian indigenous chickens. This is because most chickens have been kept by certain communities for generations and have become important for the livelihood of these communities. AnGR are household assets for farmers because they have multiple livelihood functions, including income, non-income and socio-culture purpose (Dorward et al. 2001). Rege (2001) argued that local communities are the most appropriate people to conserve particular breeds since they depend on their livelihood to these breeds and they will be loss if the breeds are decreasing.

Methods

The study to determine the asset functions of indigenous chickens for farmers' livelihood in West Java was conducted in December 2009 and February 2010. The purposive sampling technique was used to determined locations of study in two districts in West Java; Cianjur and Ciamis districts. In Cianjur, Buni Kasih Village in Warung Kondang

[1] A breed that has been classified as either critical, critical-maintained, endangered, or endangered-maintained (FAO 2007)

sub district was chosen because it has been acknowledged as an area in which Pelung was firstly developed. Benteng and Cigembor Villages in Ciamis District were chosen because there are no farmer groups in Ciamis which rearing Sentul chickens except in these villages. This study employed face to face interview to the 20 farmers in Cianjur and 21 farmers in Ciamis.

Result and Discussion

From an economic perspective, environmental resources, including AnGR as part of natural capital, together with physical, human, social and financial capital (Barbier, 2003; Farrington et al., 1999) are important economic assets. The importance of the indigenous chicken breeds to the livelihoods of farmers in West Java was assessed through a method called Asset Function Framework approach which was developed by Dorward et al (2001; 2004).

Asset Function Framework approach

This approach was based on a sustainable livelihoods approach applied to livestock production system settings. The conceptual framework brings together the relationships between the different livelihood functions of assets. Asset functions can be broadly classified according to contributions to production, savings, buffering, insurance, consumption and social integration Dorward et al (2004). *Regular cash income* may be achieved from sales of products such as eggs, meat and manure or of live animals. Many products and services that are sold are also used for domestic *consumption* or for production of other commodities which themselves may be consumed or sold.

AnGR may contribute to livelihood as variety of savings type functions such as buffering which means that investments are made during periods when production or income exceeds consumption needs and then these investments are drawn upon later in the season when lower production and income are not sufficient to support consumption needs. In terms of *saving*, AnGR may provide some major expenditure such as a major purchase or investment, or expenditure on school fees. In addition, AnGR may contribute to some *insurance* against unexpected events that either reduce income or make extra expenditure demands. Another form of saving is the process of *accumulation*. By this process AnGR inventory of a household is built up over time as consumption needs and sales do not exceed the reproductive capacity of the livestock kept.

The last function, AnGR may take part in *social integration* functions for society and culture. Ownership may confer status or animals may need to be exchanged or provided in certain traditional ceremonies or relationships which are important for people's position in local society.

The functions of indigenous chickens

Pelung chickens are regarded local to Cianjur District (West Java). The farmers in this area kept these chickens as singing chickens. There are regular contests for Pelung in Cianjur and other districts in West Java held by government and non government organizations. On the other hand, Sentul chickens are originated from Ciamis District in West Java. Some studies indicated that this chicken is a good egg producer. However, the result from this study showed that the farmers are kept these chickens for meat production. The chickens are capable to gain 900 gram (sale weight for indigenous/local chicken) within 2,5 months.

Table 1 reveals that indigenous chickens fulfil income and several non-income functions including savings and social integration. Income function and consumption functions were less important. The majority function of chickens in Cianjur is as social integration. This function is higher in Cianjur (56%) than that in Ciamis (33%). Most of farmers in Cianjur regard Pelung as a local heritage that should be maintained for its existence. This is because most of the farmers believe that Pelung is originated from their village and they have an obligation to take care this chicken. In addition, by keeping these chickens, the farmers can have important position if their chickens winning singing contests.

Table 1. The functions of the indigenous chickens

Functions	Sentul	Pelung
	Percentage (%)	
Income	24	25
Saving and insurance	30	19
Social Integration	33	56
Consumption	13	0

In Ciamis, in terms of social integration function, raising chicken is considered as an effort to change condition from poor to a better condition (successful people) as a result the community will pay more respect to the farmers. The similarity in the two locations is that the activity of keeping Pelung in Cianjur or Sentul in Ciamis is regarded as a means of networking. By rearing these chickens, the farmers are able to connect people and or organization outside their community.

The number of farmers who regard chickens as income function is quiet similar in both locations; Cianjur (24%) and Ciamis (25%). This function is derived from sales of chicken products including meat, egg and live chickens. By keeping chickens, the farmers

are able to support daily need such as buying rice and cooking oil and also to cover daily cost mostly for children needs. Another function of chickens is saving including another form of saving such as insurance. This function is lower in Cianjur (19%) than that of Ciamis (33%). By raising chickens, the farmers store of assets which instead of being used immediately are set aside for use in the future. The money from selling of chickens is able to provide some expenditure such as school fee, renovating house and or purchasing resources such as land for crop activity. The money from the chikens is also able to be saved for the life in the future (elderly period).

The least function of chickens is as consumption in which only Sentul contributes such function to the farmers in Ciamis (13%). The farmers can eat meat and eggs from Sentul for their families' protein need. This is contrary with Cianjur, in which the farmers are not use to eat Pelung and its products.

Discussion

A conservation design, mainly in situ conservation, should take into consideration the functions of the chickens to farmers' livelihoods. This is to ensure the sustainability of the programme by maintaining the chickens as a household asset for the farmers. Therefore, livelihood improvement could be the objective of conservation of AnGR (Anderson 2003).

Once breeds are put in an in situ conservation programme, it is necessary to manage them in particular breeding programmes. The first step in planning any breeding system is to define what the breeding objectives are. The result indicated that the breeding objectives for these two chickens may be different. Chicken breeding programmes traditionally focus on the genetic improvement of production and reproduction traits that have a clear economic value. This may be addressed for developing breeding objectives of Sentul chickens since these chickens are reared for meat production. The production traits which may be used as breeding objectives are growth rate, meat percent and feed efficiency.

On the other hand, Pelung chickens are kept for their sound; consequently, the breeding objective should be more focus on non production traits. However, the non-production important traits may have an economic and a noneconomic value. Even though Pelung are reared for their beautiful sound and the farmers kept these chickens as an obligation to maintain local heritage, the chickens which win contests have high prices that can give significant economic value to the farmers. Further study should be exploring genetic traits which are important for fulfilment these functions.

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A preliminary study on human and long-tailed macaque conflict in Karst Habitat of Gunungkidul: Public Opinion

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ABSTRACT

Homo sapiens have historically coexisted with long-tailed macaques (*Macaca fascicularis*) throughout South and Southeast Asia.this species is the most wide distributed among primates because of its capabilities to adapt in a wide variety of habitats both in and outside its natural range, including secondary and disturbed forest. In their outside natural habitat they have close association with human. This close association has developed into a complex relationship where both species positively and negatively impact each other. For example, human and long-tailed macaque conflict had been reported from Indonesia, Malaysia,Singapore,Thailand and India.

Public opinion plays important role in the planning and management of wildlife. This survey using semi-structured interview taken from 17 participant from 4 villages. These 4 areas was reported crop raiding by long-tailed macaques. This survey conducted on the intensity of man-monkey conflict, as proposed by Chauhan and Pirta (2010), and pest behaviour of long-tailed macaques.

It aims to measure the intensity of man-monkey conflict dan seeking possibilities to solve the problems from public opinion.

In karst habitat of Gunungkidul, long-tailed macaques activites damaged to crops. Long-tailed macaques alsa attack livelihood and taken chicken egg at Duwet Village. At Gesing and Sundak Village there is report about monkey pest behaviour. Public opinion tend to be negative because lost of potential harvested crop. This attack always occurs every dry season in different intensities. Public opinion tend to negative to long-tailed macaques and clasified into pest animal.

Keywords: long-tailed macaques, conflict

INTRODUCTION

Kabupaten Gunungkidul sebagian besar wilayahnya terdiri dari kawasan karst yaitu sekitar 13.000km^2. Kawasan karst terbentuk dari pelarutan batuan kapur memiki bentang alam kompleks dengan keanekaregaman habitat yang unik (Infield, 2004 dalam Ismanto et al, 2008). Fenomena permukaan (*eksokarst*) meliputi bentukan bukit-bukit kecil berbentuk kerucut dengan jumlah mencapai 40.000 buah. Karena adanya bentukan-bentukan bukit kecil ini maka kawasan karst Gunungkidul dikenal sebagai kawasan Gunungsewu. Bentang alam karst Gunungsewu sendiri meliputi Kabupaten Gunungkidul, Wonogiri dan pacitan.

Dari sudut pandang ekologi kawasan karst juga menyimpan banyak fenomena menarik. Sifat batuan kapur yang tidak mengikat air mengakibatkan kondiri

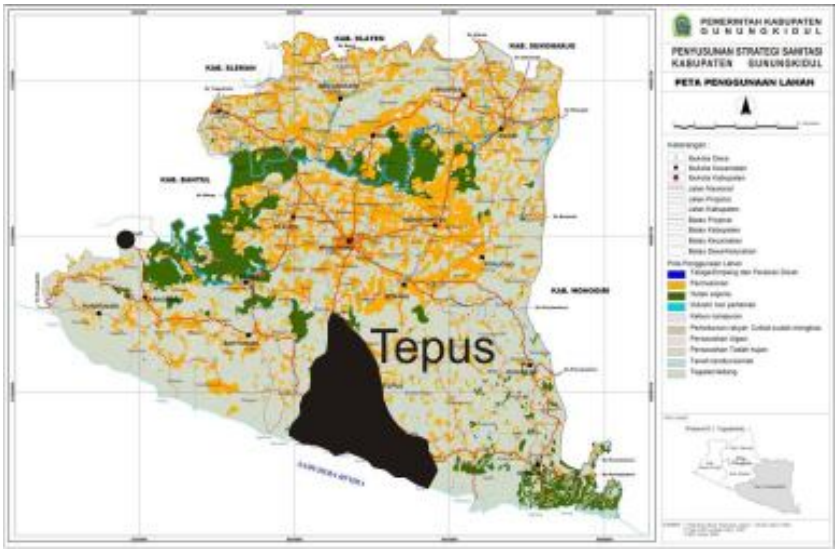
kekeringan di daerah karst. Zona selatan Kabupaten Gunungkidul yang meliputi Kecamatan Tepus, Rongkop, Girisubo, Saptosari,Paliyan dan Panggang mengalami kekeringan pada musim kemarau.

Kondisi kekeringan menekan keberadaan biota termasuk vegetasi dan hewan-hewan vertebrata dalam kehidupannya. Seperti disebutkan oleh Krebs (2009) bahwa masalah dasar dalam ekologi adalah menentukan distribusi dan kemelimpahan suatu organisme. Kondisi kawasan karst yang terbatas sumber daya airnya menekan organisme yang hidup di dalamnya. Di daerah Gunungkidul bagian selatan tanaman keras yang mendominasi adalah jati dan akasia. Pohon jati memiliki fenomena ekologis yaitu mengugurkan daun selama musim kemarau (Hasanbasri et al., 1996). jenis-jenis ketela,jagung,dan kacang.

Satwa liar seperti monyet ekor panjang (*Macaca fascicularis* [Rafles,1821]) banyak ditemui di zona selatan karst Gunungkidul. Monyet ekor panjang merupakan salah satu jenis primata yang mampu hidup pada berbagi jenis habitat (Hasanbahri *et al*, 1996) dan memiliki sebaran paling luas diantara primata lainya di Asia (Gumert,2008). Secara alami keberadaan monyet tidak meresahkan masyarakat apabila mereka hidup pada habitat alaminya dan relatif tidak berdampingan dengan manusia (Djuwantoko *et al*., 2008).

Sebaran populasi monyet di Indonesia cukup luas, dari kawasan barat sampai Nusa Tenggara Timur (Djuwantoko et al., 2008) termasuk di kawasan karst Gunungkidul. Konflik monyet dengan masyarakat lokal banyak dilaporkan oleh media massa (http://nationalgeographic.co.id, 13/7/11). Di daerah karst Gunungkidul konflik ini disebabkan karena kerusakan habitat monyet (Setiawan et al., 2008) dan perluasan lahan pertanian (Marchall dan Hill, 2009) sehingga monyet dianggap sebagai hama karena sifatnya yang invasif, kemampuan adaptasi yang tinggi pada berbagai habitat yang telah terganggu (Iskandar et al., 2008).

Di Indonesia, monyet ini telah banyak dilaporkan memiliki perilaku sebagai hama diantaranya terjadi di lahan pertanian di Jawa, Sumatera dan Kalimantan. Berdasarkan observasi satwa ini menyukai hutan sekunder yang berbatasan dengan lahan pertanian, bahkan seringkali mencari makan di lahan tersebut. Hal ini tentu tidak diharapkan karena mengakibatkan kerugian yang signifikan bagi petani. Serangan dari monyet pernah dilaporkan dari taman rekreasi Cisarua dan Cagar Alam Gunung SImpang di Jawa Barat, Desa Nyemani, Jogjakarta, dan Situbondo Jawa Timur. Monyet ini tidak hanya memakan dan merusak tanaman pangan seperti padi, pisang, jagung tetapi juga dilaporkan mengambil telur ayam di Situbondo. Dari permasalahan tersebut nampak ada kebutuhan kritis mengenai pengaturan populasi monyet untuk memastikan konservasi populasi alaminya dan meminimalkan dampak negatif terhadap lingkungan dan ekonomi (Iskandar *et al*, 2008).



Koleksi data

Gambar 1. Lokasi Penelitian

Dalam penelitian ini dilakukan wawancara semi terstruktur terhadap petani di Kecamatan Tepus Gunungkidul yang meliputi 6 Dusun. Hasil penelitian diharapkan bisa memberikan gambaran awal mengenai tingkat dan pola serangan monyet dalam kaitanya dengan aktivitas pertanian masyarakat.

BAHAN DAN METODE

Lokasi penelitian adalah Kecamatan Tepus Kabupaten Gunungkidul yang meliputi 6 Dusun dengan luasan kira-kira 5 km^2. Secara administratif dusun-dusun tersebut masuk dalam Desa Tepus dan Purwodadi. Dusun-dusun tersebut terletak di sepanjang Pantai Selatan Gunungkidul yang merupakan habitat monyet.

Tabel 1. Nama-nama Dusun yang disurvei

No	Nama dusun	Desa
1.	Pule gundes	Tepus
2.	Sundak	Tepus
3.	Trosari	Tepus
4.	Gesing,	Tepus
5.	Ngandong	Purwodadi
6.	Duwet	Purwodadi

Koleksi data dilakukan dengan wawancara semi terstruktur yang dilakukan terhadap para petani yang sedang beraktivitas di lahan pertanian. Wawancara dilakukan pada minggu terakhir bulan Agustus 2011 menggunakan bahasa Jawa dilakukan pada jam-jam istirahat saat aktivitas berladang (09.00-12.00).Penggunaan bahasa Jawa ini karena dinilai lebih komunikatif. Responden yang diwawancarai adalah petani dijumpai pada waktu pengambilan data. Waktu yang diperlukan untuk tiap-tiap wawancara kurang lebih 20-30 menit difokuskan untuk menggali informasi mengenai tingkat serangan monyet terhadap tanaman pertanian dan usaha yang telah dilakukan. Pewawancara membuat catatan yang diperlukan terhadap informasi yang diberikan oleh responden.

Pada panduan wawancara perilaku monyet dikategorikan ke dalam 3 kelompok (Djuwantoko,2008) yaitu perilaku dengan ancaman ringan, mendekati tapi tanpa kontak fisik, dan dengan kontak fisik. Jawaban masing-masing pertanyaan tersebut berkisar dari sangat sering, sering jarang dan tidak pernah.

Tabel 2. Panduan Wawancara

No	Pertanyaan
1.	Berapakah umur Anda?
2.	Berapa lama Anda tinggal di desa ini ?
3.	Apakah pekerjaan Anda?
4.	Tanaman apa saja yang ditanam di ladang?
5.	Seberapa sering anda menjumpai monyet di ladang atau rumah?
6.	Seberapa sering monyet tampak mengancam Anda?
7.	Apakah Anda pernah digigit atau dicakar monyet?
8.	Pernahkah monyet mengganggu atau menyerang hewan ternak? Hewan ternak apa saja yang diserang
9.	Apakah Anda pernah melihat/mendengar monyet menyerang dan merusak tanaman di ladang?
10.	Apakah Anda pernah melihat/mendengar monyet masuk ke rumah warga?
11.	Apakah Anda pernah melihat/mendengar monyet merusak bahan makanan di rumah warga?
12.	Pernahkah Anda melihat/mendengar warga menembak/menjebak monyet?

Diadaptasi dari Marchal dan Hill,C. (2009)

HASIL DAN PEMBAHASAN

Jumlah responden yang berhasi ditemui di ladang selama 2 hari survey adalah 17 orang yang terdiri dari 9 orang pria dewasa (53%) dan 8 wanita dewasa (47%) dengan kisaran umur 17.7% dibawah 30 tahun, 47.1% berumur antara 30 – 50 selebihnya 35.3% berumur lebih dari 50 tahun. Hampir semua responden mata pencaharianya petani (15 orang; 88.2%) hanya 2 orang (11.7%) bekerja sebagai pegawai tetapi melakukan aktivitas bertani.

Tabel 3. Kisaran umur responden

Umur		%
- 30 tahun	3	17.7
30 - 50	8	47.1
50 -	6	35.3
N	17	100

Semua responden telah menempati desanya selama lebih dari 10 tahun.

Lebih dari separuh responden (77%) sering bertemu monyet di ladang dan ketika bertemu monyet menunjukkan perilaku mengancam seperti menyeringai (70.5%).

Tabel 4. Frekuensi Bertemu Monyet di ladang

		%
Sangat sering	8	47.06
Sering	5	29.41
Jarang	4	23.53
Tidak pernah	0	0
N	17	

Tabel 5. Perilaku Monyet mengancam ringan (menyeringai) ketika bertemu orang

		%
Sangat sering	3	17.65
Sering	9	52.94
Jarang	5	29.41
Tidak pernah	0	0
N	17	

Walaupun demikian tidak ditemui laporan adanya kontak fisik seperti kejadian dicakar atau digigit oleh monyet.

Tabel 6. Pernah melihat kejadian kontak fisik (digigit atau dicakar) moyet

		%
Sangat sering	0	0
Sering	0	0
Jarang	0	0
Tidak pernah	17	100
N	17	

Sementara itu kejadian serangan terhadap tanaman di ladang frekuensinya hampir sama di tiap dusun yaitu 29.41%. terdapat dua dusun yang tidak ada laporan mengenai serangan monyet ini.

Tabel 7. Pernah melihat monyet yang merusak tanaman di ladang

		%
Sangat sering	5	29.41
Sering	5	29.41
Jarang	5	29.41
Tidak pernah	2	11.76
N	17	

Kejadian monyet yang menyerang hewan ternak adalah 11.76% dan 35.29% sementara yang menyatakan jarang dan tidak pernah ada 29.41% dan 23.53%.

Tabel 8. Pernah melihat monyet yang menyerang hewan ternak

		%
Sangat sering	2	11.76
Sering	6	35.29
Jarang	5	29.41
Tidak pernah	4	23.53
N	17	

Separuh lebih responden menyatakan pernah melihat monyet masuk rumah warga (53%) sementara 47% yang lain menyatakan jarang atau tidak pernah.

Tabel 9. Pernah melihat monyet masuk ke rumah warga

		%
Sangat sering	2	11.76
Sering	7	41.18
Jarang	3	17.65
Tidak pernah	5	29.41
N	17	

Sebanyak 47% dan 23.53% responden menyatakan tidak pernah dan jarang melihat monyet yang mengambil bahan makanan di rumah warga. Sementara itu sebanyak 23.53% dan 5.89% menyatakan pernah melihat monyet mengambil makananan di rumah warga.

Tabel 10. Pernah melihat monyet mengambil bahan makanan di rumah warga

		%
Sangat sering	4	23.53
Sering	1	5.882
Jarang	4	23.53
Tidak pernah	8	47.06
N	17	

Laporan kejadian mengenai monyet yang masuk ke rumah warga hanya terjadi di Dusun Duwet walaupun kejadian tersebut jika dibandingkan dengan keseluruhan masih berimbang (tabel 9). Pola gangguan monyet di Dusun Duwet ini cukup menarik. Monyet di Dusun Duwet tidak merusak tanaman di ladang tapi menyerang hewan ternak di rumah dan mengambil bahan makanan berupa telur ayam. Monyet yang masuk rumah warga ini berjumlah 2-5 ekor. Kejadian serupa di Jawa Timur dilaporkan ole Iskandar et al. . (2008).

Hewan yang diserang monyet di Dusun Duwet adalah kambing jantan maupun betina. Menurut informasi warga pola serangan berkisar dari gigitan pada daun telinga, ekor sampai dengan perilaku kawin terhadap kambing jantan. Sementara menurut Setiawan (2002) monyet betina yang sedang birahi menjadi lebih aktif dengan menyerang betina lainya. Adanya perilaku monyet jantan yang mengawini kambing perlu diamati lebih jauh untuk membuktikan informasi dari responden. Walaupun Dusun Duwet dan Ngandong terletak bersebelahan, kejadian monyet masuk rumah dan serangan terhadap ternak tidak terjadi di Dusun Ngandong.

Serangan monyet ke rumah-rumah warga di Dusun Duwet ini menunjukkan ada perubahan perilaku. Perubahan perilaku bisa diakibatkan oleh tekanan dari struktur populasi yang tidak seimbang antara jantan dan betina.Responden juga menginformasikan bahwa monyet tidak takut dengan wanita. Di ladang monyet hanya mau pergi bila diusir oleh pria.

Monyet yang merusak tanaman (*crop raiding)* di ladang adalah kawanan di Dusun Pule Gundes dan Trosari yang memiliki batasan langsung dengan pantai. Tanaman yang diserang pada akhir musim penghujan adalah kacang tanah, sedangkan pada musim kemarau monyet menyerang ubi jalar. Hal ini merupakan indikasi berkurangnya pakan alami monyet saat memasuki musim kemarau. Monyet menyerang secara berkelompok besar (puluhan) sehingga bisa menghabiskan seluruh panenan di ladang. Sementara di Dusun Sundak yang memiliki kawasan pantai justru tidak didapat informasi mengenai monyet yang menyerang tanaman di ladang ataupun masuk ke rumah warga. Dusun Gesing yang tidak berbatasan secara langsung dengan pantai juga tidak didapat informasi kejadian monyet yang menyerang tanaman di ladang.

Usaha yang dilakukan warga untuk mengatasi serangan ini dilakukan secara sporadis saja yaitu saat kawanan moyet ditemui di ladang atau dirumah. Cara yang dilakukan baru antara lain dengan melempar batu atau menggunakan anjing. Tapi penggunaan anjing ini juga kurang efektif karena monyet-monyet dewasa cukup kuat untuk menyerang anjing. Untuk untuk menangkap dengan senjata belum dilakukan karena keterbatasan peralatan. Sementara itu usaha dengan dengan meracuni monyet pernah dilakukan tapi dihentikan karena berkaitan dengan mitos. Secara umum warga bersikap pasrah dengan kejadian serangan ini.

KESIMPULAN DAN SARAN

Dari hasil penelitian dapat disimpulkan bahwa :

1. Kejadian monyet yang masuk rumah warga hanya di Dusun Duwet dimana monyet menyerang kambing dan mengambil telur.
2. Di Dusun Duwet tidak didapat informasi mengenai serangan monyet di ladang warga.
3. Di Dusun Pule Gundes dan Trosari monyet hanya menyerang tanaman di ladang tanpa ada kejadian masuk rumah warga.
4. Belum ada usaha optimal dari warga untuk meminimalkan serangan monyet ke ladang warga.

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O-EC11

Eastern Indonesian Field Intensive: a collaborative model for international education

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ABSTRACT

This paper describes a collaborative model for international education in natural resources management and rural development. International education can take the form of materials from a “developed” to a “developing” context, in order to address a perceived “deficit”. This paper outlines an alternative model built upon respectful partnership and mutual capacity building for long-term teaching and learning collaboration. UNDANA, UKSW and CDUhave collaboratively developed curriculum for a field intensive in eastern Indonesia, and now with UGM, attended by staff and students from each institution.

Students and staff visit the village of Linamnutu, TTS, West Timor during a 2 week field intensive, to investigate issues associated with water and food resources, relevant to village concerns. The teaching and learning is cross-cultural and cross-institutional. Students take the role of research associates on a research project which is lead by academic and research staff from the partner institutions. Thus the learning is situated, work-integrated and positioned at the research-teaching nexus.

The purpose of the field intensive is to prepare senior undergraduate students, master students and junior staff with skills and understandings required to face regionally relevant problems. The field intensive provides opportunities for the development of technical skills, research skills, collaboration skills, cross-cultural confidence, language skills and firm, long-term relationships among staff, students and village participants.

The success of the field intensive has underpinned the importance of long-term, trusting relationships among the participating university staff, residents of Desa Linamnutu and District Government.

O-EC12

Ecology, management and use of native rices *Oryza meridionalis* and *O. rufipogon* on northern Australian floodplains

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ABSTRACT

This paper describes the ecology, management and potential use of native Australian rices *Oryza meridionalis* and *O. rufipogon* of northern Australia. These native grasses are widespread and abundant, are of significant conservation value and underpin the vertebrate food-chain on the tropical monsoonal floodplains. Significantly there is no domestic rice cultivation in northern Australia, apart from short-lived trials in the 1970s. Consequently, these populations have not suffered the genetic erosion of populations elsewhere in SE Asia where cultivated rice is grown.

Further, rural Indigenous communities in northern Australia are focussing on the development of small enterprises based on wild harvest of native foods for sale as “bush tucker” products in the local tourism and restaurant industries. Wild rice may be a suitable plant for inclusion in such an enterprise.

However, native rice populations are under threat from weeds, including in the World Heritage listed Kakadu National Park. In some areas they have suffered complete displacement by exotic perennial pasture grasses introduced from central America to support cattle grazing for export to Indonesia, the Philippines and elsewhere.

This paper describes phenology, seed biology and selected nutritional properties of native Australian rices, and their potential as a “bush tucker” product, as well as issues for floodplain management associated with the invasion of floodplains by introduced grasses and their displacement of native rices.

O-EC13

Understanding Tree Preservation with Sacred Black-White Clothe: The Balinese Initiative for Plant Conservation

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Abstract. The Balinese believed that on the big tree in sacred places occupied by big tree occupied by the supra-power. One of it is called *banaspati*, and every body afraid to disturb the trees. The trees preserved through black–white clothe that become sacred. The objective of study to determine the species tree was preserved and it distribution at the main temples. Data was obtained by observation, especially in the temple, which placed near the main road at four regencies.

There are six species of trees were preserved. These trees included; *kepuh (Bombax ceiba L),kepah (Sterculea foetida),beringin (Ficus benjamina), pole (Alyxia reinwardtii), ancak (Ficus religiosa),* and *aa (Ficus sp).*These plants were found at the three main temples in each village. The majority big trees were found at funeral areas, because this area most widest and it located at outside of villages. Plant conservation provides insight into the relationship between using black-white clothe and plants preservation. The plants were distributed not only in the village but also at central of city. More than 67 trunks of tree were closed by black-white fabric and the age of plants are varied from 50 years until 200 years. There were two main factors underlying the existence of plants distribution. First, more people closed big tree groups when they growths on the sacred area. The second, people build small temple when they found big tree in sacred area.

Keywords: Tree Preservation, Sacred black-white clothe, Local initiative

INTRODUCTION

Human beliefs about the nature of ecology are the distinctive contribution of our species to the ecology itself. Religious beliefs, especially those concerning the nature of powers that create and animate, become an effective part of ecological systems (Grim, 2001,p: x). Religious worldviews are unique because they draw the world of nature into wholly other kind of universe. Religion distinguishes the human species from all others, just as human presence on earth distinguishes the ecology of our planet from other places in the known universe. Religious life and the earth's ecology are inextricably linked, organically related.between human belief and practice mark the earth

The Balinese believed that on the big tree occupied by the supra-power. One of it is called *banaspati*, and it caused every body afraid disturb the trees without certain ceremony. According to Balinese Hinduism black is symbolize the power of Visnu, the preserve (*sihiti*). White symbolize of Siva, the power of recycled (*pralina*). Black and white-color mean preserve or recycled that the trees become sacred. The Balinese very conscious that their life and death depending on the tree. No tree no live, this is meaning of black-white color. They people build shrine or *palinggih* under some of big tree (Nala, 2009, p: 12).

Using black-white clothe the Balinese try to protected this tree and they prayer at bellow of sacred big tree. They pray to the God to thank that He created trees, and asked Him in order to protect the people in different way. Lord Himself is *vanaspati*, the Master of Forests, which sustain our life (Rgveda. Samhita.V.1.p: 32).

Bali is small island is undergoing rapid change and there many land was transformed to build many building. Globalization and rapidly development of tourism, has been changed the traditional Balinese orientation into treating land only has functional and materialistically valuable. The conversion of agricultural land to development rose drastically in Badung regency and in Denpasar regency. The temple landscape is only one as reserved land who people afraid to sell and these areal for plant conservation.

The objective of study is to determine the kind of tree was preservation and its distribution and it knowledge's of Balinese peoples.

MATERIALS AND METHODS

The study area are located at the four regencies (*kabupaten*); that regency are Tabanan, Badung,Gianyar, and Denpasar. These regencies were selected by purposive sampling. The number tree species were marked by Black-White Clothe and it distribution was obtained by observation, especially plants in landscape main temple where was placed at the main road. A *pura* (temple) is the Balinese Hindu sacred place to worship God with all of His manifestations Balinese village at least stands three type of temple. First *Pura Puseh*-the temple of Creation, second; *Pura Desa* which is community temples. The third is the *Pura Dalem*, the temples of Death, and it temple mostly widest landscape area.

The information Balinese knowledge's was obtained by unstructured interviewing from the key persons. The key persons both two groups of *pendeta* (monk), and experts Balinese Language, at Udayana University. The study was carried out during three time periods: January 2010, June 2010 and October 2010. Based on the information and data obtained the vernacular of plants were noted. Extensive surveys were therefore carried out in it four Regencies. A series informal interviews which to determine to determine the knowledge Balinese peoples.

RESULTH AND DISCUSSIONS

1. Protected Tree with Black-White Clothe

There are six species of trees were preserved in all location. These trees are;; *kepuh (Bombax ceiba L), kepah (Sterculea foetida), beringin (Ficus benjamina), pole (Alyxia reinwardtii), ancak (Ficus religiosa),* and *aa (Ficus sp).* More than 120 trunks of tree were closed by black-white clothe and the plants age are varied from 50 until 200 years (Figure 1)

Figure 1. The Plants Preserve with Black-White Clothe in Temple Areas
From top left fo right: *Ancak, Aa, Pole,Kepuh, Kepah, Bingin*

The plant exists almost in groups, although some of it grows solitary. The largest groups were found in cemetery area, where the landscape area are widest. The village temples consist of three temples (Pitana, 2001, p: 119). Balinese village at least stands three type of temple. First *Pura Puseh* the temple of Creation, second; *Pura Desa* which is community temples. The third is the *Pura Dalem*, the temples of Death, which is used for cemetery and cremation ceremonies. The combinations of plants groups consist of two trunks until five trunks. Especially in pura Dalem Abian Semal village that were found five species of tree and it were clothed by black –white clothes. These plants are *Kepuh, beringin, pole, kepah,* which amount 25 trees were located in half hectare. The existence of these plants in each temple area were supported by applied *Tri Hita Karana* principles, Eismen,1990,p: 190, Nala, 2010, p:5). There were two main factors underlying the existence of plants at the sacred areas. First, using ancient doctrine they prohibit cutting big tree, especially the sacred tree on the temple, except for specific purposed

The second, people whose will build small temple when they found big tree and it become sacred area. Traditionally, the Balinese establish a temple in every village. Balinese cosmology seeks to design their villages, that is in physical, environmental, and organizational harmony with the human being. Man is considered in Hindu thought to be a

microcosm of the universe at large, which has tree parts (Eisemen, 1990, p: 190, Somvir, 1995). What makes the Hindus special is that they honor the whole of Creation, see the presence of God in everything. *Vanaspati* – the master of forest and *Kalpha talaveta* – the tree of live (*Kalphataru*)

. The cemetery areas dedicated to Siva the recycler of life. Human beliefs about the nature of ecology are the distinctive contribution of our species to the ecology itself. Religious beliefs, especially those concerning the nature of powers that create and animate, become an effective part of ecological systems (Grim, 2001: p: 9). The holy place and all big tree in the temple area is very important to conserve it plant. According the holy place, Watson declare (1991, p: 95); all have become holy place- recognized by shaman and wizards; visited by bards and witches; settled by hermits and meditating mystics. Their chains of influence remain intact, their messages transmitted through time in some basic biological way that makes them perceptible and valuable of all kinds in all ages.

2. Plant Distribution

There big tree that closed by white-black cloth were found at the three main temples in each village. The majority big trees were found at funeral areas or cemetery where the *pura dalem* is located. *Pura Dalem* dedicated to *Dewi Durga* – the dissolver and recycler of life. Plant conservation provides insight into the relationship between using black-white clothe and plants preservation (Table 1).

Table 1. Plant Distribution that Preserved with Black-White Clothe

No	Name of Regency	Number of Temple	Number of Plant Preserved with Black-White Clothe	Average of Tall and tree Diameter	Average Canopy
1	Gianyar	33	41	18.4 meters 1,1 meter	18,3 m2
2	Tabanan	38	48	20,3 meter 1,3 meter	20,2 m2
3	Badung	28	19	19,2 meter 1,1 meter	20,1 m2
4	Denpasar	14	12	17.3 meter 0,9 meter	18 m2
	Total Average	113	120	18,1 meter 1.1 meter	19,2 m2

The tree groups were found in all regency and it was disperse in every village. Total of temple where its plant disperse 113, the number of plant were preserved with Black-White Clothe 120 trunks, tall average 18,1 meter, diameter 1,1 meter and canopy average 19,2 m2. It plants disperse in temple where it average 125 meter square.

The groups these trees doesn't disturbed by human activities and it mostly life like in natural ecosystem. These plants were spread more than five hundreds of temples where each

village has at least three temples. Those plants were conserved is spread at all island, that disperse like mosaic principles (Figure 2) (Suryadarma, 2005, p:45).

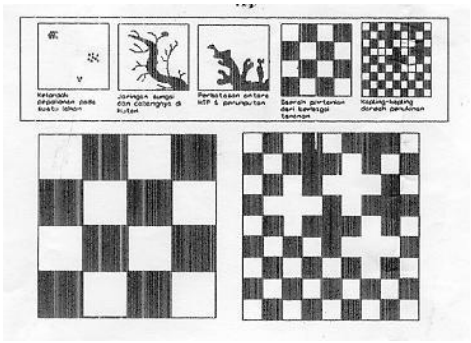

Figure 2. The Mosaic Principles Plants Distribution Based on Existence of Temple

Their cultural activities are important to play a key role in the use of natural resources and conservation biological diversity. Over the last few decades, there has been a growing recognition regarding the role and responsibilities of indigenous and tribal peoples in managing and conserving the often complex ecosystem inhabitant. According to WWF, 1996, indigenous and tribal peoples inhabit mainly in areas where they have lived for thousand years, making them therefore the earth's most important stewards of natural resources (UNESCO, 2009, p: xix). Balinese is unique to a culture or society whose people looking everything is connected. It is like hologram or whole record because every part contains the whole (Watson, 1991). The existence a group of big tree where it spread in each the temple is a symbol of *vanaspat*i, that power of God at the plants. The people will get *vanaspati* power in their cultural landscape it like mosaic principles, These activities is linkage between cultural activities and biological diversity. It is the most relevant with sacred site and conservation. To pay attention of cultural landscape and sacred site is a part of strategies biodiversity conservation.

Regency Denpasar where the capital of Balinese Province at least were found fourteen temples and twelve trees. These tree are *kepuh (Bombax ceiba L),beringin (Ficus benjamina), pole (Alyxia reinwardtii), ancak (Ficus religiosa)*. The *kepuh* tree is located near the central of office Province Bali and big banyan tree near the office of Denpasar Regency. The tall of tree 18 meter, diameter 1.7 meter and it canopy 225 m2 square. The four banyan trees were found in the around the office of Gianyar regency, where the age of tree more than one hundred years. The banyan tree as the symbol of *Kalpha talaveta or Asvattah* --- the indestructible *Pipal Tree* (*Ficus religiosa*). It form is not perceived here as such neither its end, nor its foundation. This tree having its roots above and branches bellow, whose leaves are the Vedas (Bhagavad Gita, XV). Below and above are spread its branches, nourished by the *Guna*; senses-objects are its buds; and below in the world of

men, stretch forth the roots, originating action. The root at below direction as a symbol of basic need and the up direction is a symbol of spirit of life. The preserved of tree with Black-White Clothe which based on their belief that are equal with the principles of biodiversity conservation. Conservation based on cultural activities and it initiative by local peoples Biodiversity conservation has gained prominent place on international agendas, and as a result, protected area have been set aside to conserve the world biological richness. Many attempts have been made to involve local people in management on those areas and the involvement of farmers in conservation activities is remaining crucial. This is due in part to complex relationships which exist between farmers and their environment relationship which are often not sufficiently understood by conservationist (Toledo, 1990).

For the Balinese there is no dividing line between himself and the God. He, who sees Me everywhere, and sees everything in Me, he never gets separated from Me nor do I get separated from him (Bhagavad Gita, VI). The concept of harmony is vital. Those who live in close touch with their surroundings do not seek to control the environment They emphasis is always placed on effort to connect between man and nature (Watson, 1991, p: 95).

4. The People Knowledge

The Balinese people whose did not know the meaning of why it plant preserved with Black-White Clothe. They do what must they done, it does not asked why. They are very practical in their religious activities that based on karma law - the law of return. Whatever we take, we must return, or natured demands a return for every gift received. Every object must responsibly for its replacement. Nature has extreme penalties for those who break such law, and for descendant and neighbors. If we want pleasure in live, than we should preserve the life around us (Mollison,1986, p :10). Even in the inanimate world we are dealing with a life force, and our act are of great effect, like thermodynamic law, that is concern with “closed system”. The reaction of the earth is to restore equilibrium and balance. If we overload, deform, deflect natural system and process, then we will get a reactions, and this reaction may have a long-term consequences

The study cultural landscape, sacred site has become highly relevant and increasingly necessary search for biodiversity conservation. Balinese appreciate nature very much, even to the extent that cutting a tree down is considered a crime (Swellengrebl in Budihardjo, 1986,p 43, Suryadarma, 2008, p: 12). Balinese, too hold a certain view about reality of the world and even beyond, i. e. the interconnection between the reality of the world and metaphysical world. The connection between human and a tree, especially a tree in sacred areas is our believed. Bali although small island is filled with sacred landscape areas and wisdoms of conservation. According to key person information; people is just follows the

Balinese calendar. It calendar declared a set aside days and ceremonies to honor plants, animals, and even inanimate objects to have of sacredness. Some other objects place and objects like forests, rivers, big tree and unusual landmarks we consider to be guarded by grotesque and fearsome spirits, thus we treat them with caution as not to offend the spirits. Consequently, trees and animals living in those areas are protected (Panji, 2001, p: 23). Overall, most Balinese still hold a strong sense of the sacred, and sense of the place, i.e. an affinity toward homes, family, community and land, which in principals can serve as a foundation for a more holistic approach of living, both for present and the future generation

There are many different ways looking of environment and the interrelationship between humans and their social and biophysical surroundings (Toledo, 1992, p: 6).

The exploration how nature is seen by hunans groups through a screen of beliefes, knowledge and purposes, and how in term of their images humans use, manage and appropriate natural resources, has been a central task of most ecologically oriented research conducted by anthrophologists, agronomists, human ecologists

The relationship between Balinese cultures and their natural environment is highly elaborated. It involves a complex series of adaptation over time both religion/culture and nature. The plant preservation with Black-White Clothe is the result of a series of conscious and unconscious human intervention over time. That is a symbol and our identity. It was universally agreed that “every community needs a symbols of its existence” (Walker, 1961 in Budihardjo, 1986, p: 7). These activities can be regarded as the result of active management of the natural environment defined by the historical setting, need, and lifestyle of their peoples. The key person agreed that using white-Black Cloth as a sacrifice is fundamental to establish it plant with symbolic system. The peoples learn more from belief rather than science and how to literate it into science?

There are two ideal models knowledge of conservation, that based on science and wisdom or wisdom and science. Preserved tree is a mixed objectives knowledge and subjected of beliefs’ that are useful for an appropriate plant conservation.

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O-EC14

A Heteromycotrophic Orchid *Didymoplexis pallens* Griff.: Life Pattern and Genetic Approach

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not presented

O-EC15

Preying Behavior of Land Planarian

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ABSTRACT

As one of the countries with megabiodiversity, Indonesia has a wide variety of species which have yet been investigated, including land planarians. These animals live in a cool and moist area with low intensity of light. In the ecosystem these animals play an important role in controlling the population of earthworms. This paper reports the observation of prey-finding and feeding behavior of land planarians. These observations were made directly on their natural habitat in Tidar Villa Estate area, Malang, Indonesia and Biology Laboratory of State University of Malang. Land planarians rely on chemoreceptor in their head to find their prey. Once it is catch preys are immobilized by concentrated liquid secreted by this planarian. After the prey is paralyzed, land planarian covers it with their body and secretes a proteolytic enzyme to soften the body of their prey. The softened prey body then being ingested by the esophagus protruded from their ventral body.

Keywords: land planarian, preying behavior.

INTRODUCTION

Indonesia is known as a country with megabiodiversity [(1)]. This high diversity of almost all kingdoms is supported by the tropical climate and adequate moisture of its nature. However, many species have yet been investigated, one of which is land planarian [(2)].

Land planarians are member of flat-worm (Platyhelminthes). Their habitat are areas which have sufficient moisture, darkness, cold, and wet under the rocks, wood, debris or wreckage, under shrubs, and on the ground with sufficient rainfall. Land planarians is photonegative, their activities, including eating, mostly are nocturnal or in early morning under the shade. Land planarians can survive in drought only when loss of water in his body does not exceed 45 percent of their body weight. Land planarians are able to utilize their body tissues, such as reproductive tissue and which being digest by them selves when they run out of food reserves[(3)].

Land planarian prey on small invertebrates include earthworm, insect larvae, insects, snails, slugs, and prey on one another (cannibalistic). Earthworm is the main prey of land planarians. Due to their preying habit land planarians has become a threat to farmers. The decline in earthworm populations due to land planarians assault causing reducing levels of soil fertility [(4)].

To detect their prey land planarians depend on the chemoreceptor located in a single ciliated pit under the head or in a ciliated ventral groove. Their dependence on the

chemoreceptor is caused by their lack of eyes. Because of that reason, land planarian always located on the wet soil. Land planarian is secreting mucus to help the movement of their body. They attack the prey using unknown toxin which paralyzes them. After the prey immobilized, the next process will begin [4].

The group to which planarians belong is distinguished by a digestive cavity that has three main branches [5]. They have a pharynx which functions as an digestive organ. It varies in structure from a simple, ciliated tube to a complex organ developed from the folding of muscle layers. In the latter, the free end of the tube lies in a pharyngeal sheath and can be projected out of the mouth during the feeding [6].

MATERIAL AND METHODS

Animal

Land planarians found in Tidar Villa Estate Malang area.

Observation

In-situ observation had been done in Tidar Villa Estate where the animals were found. Ex-situ observation had been done in Biology Laboratory of Biology Department, State University of Malang. For *ex-situ* observation animals were carried out from its natural habitat and maintain in a terrarium. Similar type of soil was provided for them, and the humidity had been maintained by adding a certain amount of tap water. They are feed once a week with fresh earthworm at any size.

The observation was focused only on their preying behavior including: the way they search and detect, catch, and digest the pray. Pocket digital camera was used to record still photos and videos. A raw video was edited using Movie Maker software in order to compile every step of preying processes. Both photos and videos are kept as its origin; no editing in color, lighting, size, or background, have been done.

Data was analyzed carefully through recorded photos and videos.

RESULT

In this study we found a fan-shape head land planarian. Concerning the limited time available, we omit the detail identification of land planarians we found, except the size which is ranging from 1 cm up to over 20 cm in length. We suggest the short one is juveniles while the longer one is adults. These animals display two distinct black lines on dorsal side along their body separated by a wider creamy line in the middle. The head is flexible flat, more fan-shape, instead of arrow-shape (Fig. 1). In this paper, we do not attempt to present the taxonomic identification, since the data has not yet complete.

Fig. 1: Land Planaria found in Tidar Villa Estate area. Red arrow: fan-shaped head; Yellow arrow: creamy middle line; White arrow: black line.

From our preliminary observation we found that land planarian eats once a week, so we feed them with newly caught earthworm once a week. There are three steps of their preying behavior, started with detecting the prey, capturing the prey, and the last is digesting the prey, respectively.

Detecting the prey

In this step, land planarians use their head. Their flat fan-shaped head move on the dump soil elaborately in all directions until they detect the trace of earthworm. Once they find the trace they will follow it and eventually enter into the worm hole. Normally, we found that worm run out from the other end of the hole in a hurry soon after the planarian enters it. In this case, land planarian will run after that prey until they catch it. We suggest that land planarians posses a highly sensitive receptor in their head, since they show their ability to detect the worm trace even it has been far, around 3-4 meters, away compared to their body length (Fig. 2; Movie 1, separately supplemented).

Fig. 2. A land planarian is hunting its prey. This picture shows a bigger (longer/older) planarian pursuing a target earthworm. Yellow arrow: fan-shaped head; green arrow: tail tip.

Normally, it is not easy for planarian to catch the earthworm, this prey will fight back and move away first it is caught. We also observed that the part worm body which was touched by the planarian becomes rigid and does not move anymore. A study reported that planarian secrets a sort of toxin to paralyze the prey [(2)].

Capturing the prey

Planarian keeps pursuing the earthworm until it is captured. Soon after being captured the worm will show a weaken movement until it is completely paralyzed. Planarian then flattened their body to cover whole body of the victim (Fig. 3). This stage occurs up to several minutes. In this stage we found white sticky mucus secreted by ventral part of planarian body. This mucus spread over the body of earthworm (Movie 2, separately supplemented).

Fig. 3: Capturing the prey. Pink arrow: flattened planarian body; yellow arrow: captured earthworm; blue arrow: secreted mucus.

Digesting the prey

At the time of grasping its prey, we suggest that proteolytic enzyme, might contained in the mucus; chemically crush the body of earthworms. It can be seen by the texture of the remnant (Fig. 4A). We also found that a victim might be shared among several planarians. In our observation we found four young planarians sharing a breakfast (Fig. 4B). The softened body of the prey is absorbed by oesophagus protruded from the hole in the mid-ventral region of planarian body. We can examine that the food is accumulated inside, in the middle (antero-posterior) part of the planarian body (Fig. 4C).

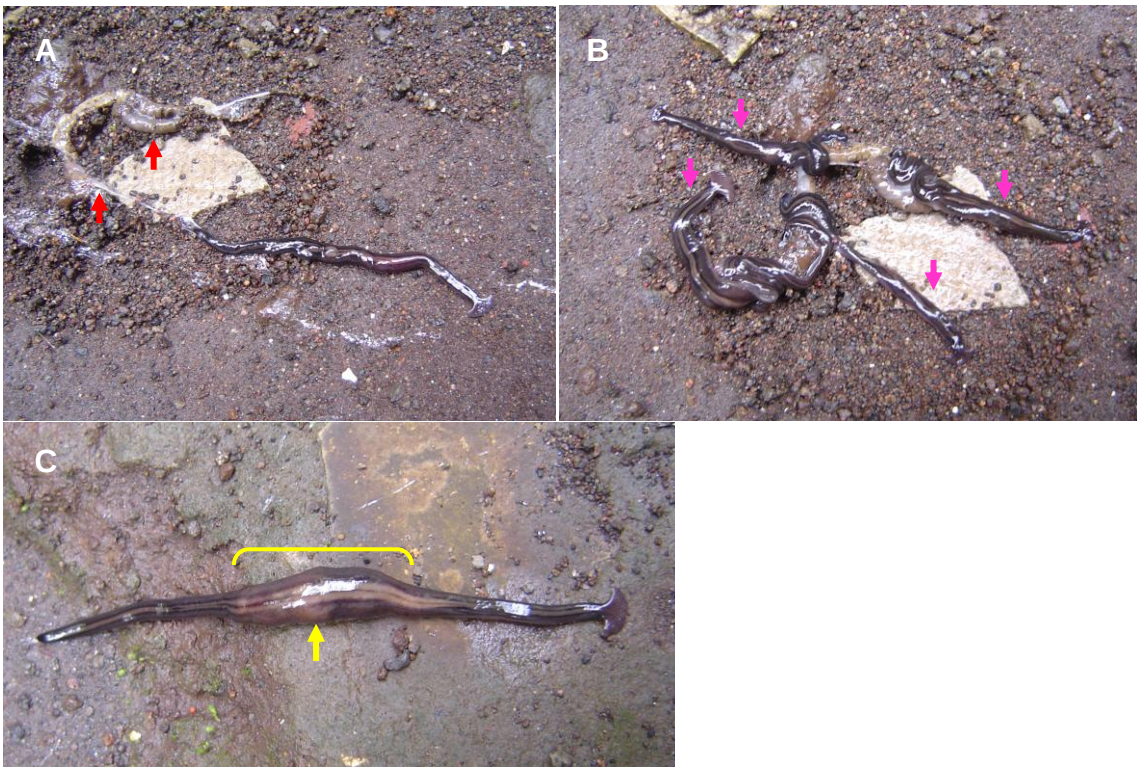

Fig. 4: Digesting the prey. A. After the party. Red arrows: remnants of the prey body. B. Four planarians sharing the food. C. Accumulation of food in the planarian body. Pink arrows: 4 eating planarians; yellow bracket: the area of food accumulation; yellow arrow: the food inside planarian body.

Summary

From this study we could summarize the preying behavior of land planarian living in Tidar Villa Estate Malang area as follow: first, land planarian searching the prey by detecting the trace left behind and follow it, once they found the trace of pray they will pursue until they catch it, they paralyze the captured prey, then cover the prey' with its own flattened body and externally digest with certain proteolytic enzyme, finally the softened body of the prey is absorbed using its oesophagus protruded from the ventral side of their body. Food is accumulated in their digestive tract.

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O-EC16

Study of Opisthobranchia Diversity in Bama Beach Baluran National Park

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Abstract. Bama beach is located in the eastern part of Baluran National Park which has complex terrestrial and water ecosystems. It has three different beach muddy zones in the south, sea grass zone in the middle, and rocky zone in the north. There are many kinds of invertebrate like echinoderms and gastropods. Gastropods are a group of organisms that have the highest diversity among Molluscs, it has been estimated that there are 1500 species in Indonesia. This research aims to determinate the level of diversity of gastropods especially Opisthobranchia in Bama Beach. Data obtained in June 2011 in the seagrass and rocky zone used sampling method. The data was analyzed using Shannon-Weaner diversity level index. It is found 5 species of Opisthobranchia. The result of data analysis shows that in the rocky and the sea grass zone have a little different diversity level of Opisthobranchia.

Keywords: Diversity, Opisthobranchia, Bama Beach, Baluran National Park, Shannon-Weaner diversity level index

INTRODUCTION

Indonesia has a very diverse marine living resources, one of them is marine invertebrates. Marine invertebrates are the dominant herbivore predators and determinant organism of the food pyramid system (Murniasih, 2005). There, one of them which has an important role is Opisthobranchia. It is one of mollusks which include in gastropod class. Its existence as one of the Indonesia's biological wealth and its role in the food chain, has put it as a species that should be preserved. One effort that can be done is creating a biodiversity database. Currently in Indonesia, there is no exact data about Opisthobranchia diversity and no research have done a lot. Therefore, research on Opisthobranchia is needed to make a better knowledge about these marine invertebrates.

Diversity is a combination of species richness and species evenness (Dharmawan, 2005), so diversity is a concept that describes the state of an ecosystem based on species found in a habitat with its distribution. Diversity is widely used to indicate the environmental conditions of an ecosystem. Odum (1993) stated that diversity is identical to the stability of an ecosystem, ecosystem diversity is relatively high if the ecosystem condition is stable.

Bell and Galzin (1984) mention in his research that there is a direct relationship between live coral cover and species diversity of benthic organisms. Alleged that the place

where the coral cover well, then the greater the number the better the Opisthobranchia and species diversity. Bama beach is a beach that has beautiful coral reefs, making it possible to obtain high diversity Opisthobranchia. Bama beach tourism is one of the objects located within the National Park area Baluran. Baluran National Parks Conservation of Natural Resources is an area which has an area of 25,000 ha with a land area of 23,937 ha and water area of 1063 ha.

MATERIALS AND METHODS

The experiment was conducted on 17-18 June 2011 in Bama beach, Baluran National Park, when the maximum low tide in the sea grass and and rocky zones. The observation method used in this study is the squares method using $1m^2$ quadrant size with 50 random plots each. The samples were taken its photograph within the plots as material identification. The identification is carried out using the morphological identification of some of the literature, including The Systematic and Phyllogeny of Phyllidiid Nudibranchs, Sea Slugs of the Red Sea, Sea Slug Forum Australian Museum, Sydney and from Nudi Pixel.

Opisthobranchia diversity is calculated using the Shannon-Weaner species diversity level index formula (Ludwig and Reynolds, 1998). Density was counted according to Krebs (1978).

RESULTS AND DISCUSSION

Opisthobranchia which is found on the Bama beach is Family Aplysiidae 1 species (3 individuals), Family Phyllidiidae 3 species (5 individuals), and Family Discodorididae 1 species (1 individual) (Yonow, 2008). The Opisthobranchias found in the sea grass and rocky zone is shown in Table 1. Family Phyllidiidae can be found in every stations approximately 4 species. Family Phyllidiidae is one of the Opisthobranchia species which are common in the tropics and in the Indo-Pacific region with abundant quantity during the day (Brunckhorst, 1993), the same as this research. Species found at each observation station (sea grass and rocky zones) is *Phyllidiella nigra*. It suggests that *P. nigra* suits with the Bama beach's waters conditions.

Table 1. Opisthobranchias found in sea grass and rocky zone

Zona	∑ individual	∑ sp	∑ sp (Family)		
			Aplysiidae	Phyllidida	Discodorididae
Sea grass	6	3	3	2	1
Rocky	3	3	0	3	0

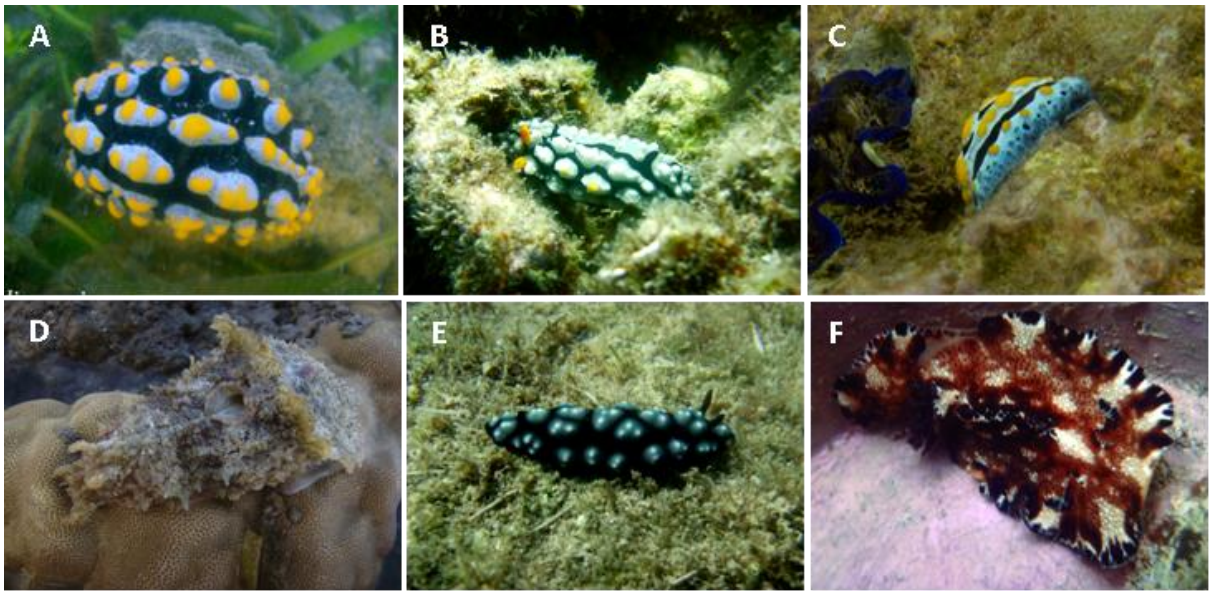

Fig. 1: Opisthobranchia found in Bama Beach. A. *Phyllidia varicosa*; B. *P. multifaria*; C. *P. coelestis*; D. *Dolabella auricularia*; E. *Phyllidiella nigra*; F. *Discodoris boholiensis*

Brower (1998) mention that species diversity is a measure of community stability (the ability of community structures to be unaffected by the disruption of its components). The stability of a community associated with the number and level of complexity of pathways of energy and nutrients (food web). The better level of complexity of food webs, the more stable communities and higher diversity it has. Thus, the H' value differences is affected by its substrate because the more variable the substrate, the greater the species diversity within it. This pattern was offered as one of the reasons why there are more species in a bigger area (more area covers a greater variety of habitat). The diversity value in sea grass and rocky zone is shown in Table 2.

Table 2. Shannon-Weaner diversity level index value

Zone	H'	E	R
Sea grass	1,097302228	0,998807531	0,548442467
Rocky	1,098436885	0,99984034	0,556276893

Margalef in Odum (1968) says that diversity is a parameter that can be used as clues to determine the trophic level of an ecosystem. Higher diversity means that the food chain will be longer and more interactions can be occurred in the ecosystem likes symbiotic relationship, such as mutualism, parasitism, and commensalism. Odum (1993) stated that diversity is identical to the stability of an ecosystem, which means if the ecosystem diversity is relatively high then the ecosystem condition is stable. Overall, the index of Opisthobranchia diversity (H') on both zone is ranged from 1.097302228 to 1.098436885.

Evenness index values indicate the extent of the dominant species (the activity) that can interfere with the existence of other types (Merman, 2004). The smaller of E value, the smaller its population uniformity, on the other hand, the greater the E value, then the population will show uniformity, which means that in the community may not be a dominant group of species. The E value in the sea grass zone is 0.998807531 and in the rocky zone is 0.99984034 which means that on both zone there is no a species dominance.

It was used to view the evenness of the distribution of individuals among species (Odum, 1993). Evenness index that ranges from 0.920619836 to 1 indicates that the habitat conditions at all study sites are heterogeneous, which means that the condition of biotic and abiotic factors and more varied. Evenness index values on both substrates indicates that Opisthobranchia at that location have nearly the same abiotic conditions tolerance limits and the availability of existing resources. It is the same Ahlfinger *et al.* (2008) that the existence of individuals of each species at a location fairly balanced if the evenness index (E) relatively close to 1.

Richness index in the rocky zone is 0.556276893 and in the sea grass zone is 0.548442467. It is known that richness index in the rocky is higher than in the sea grass, which means the Opisthobranchia which lives in the rocky substrate is more diverse, so it has an optimum environment condition. It is shown that there can be a longer food chain and a greater opportunities for interactions among its constituent species, so the environmental condition is steady.

Opisthobranchia density is the number of Nudibranchia individuals per unit area. It will give an overview on the condition of the population (Campbell, 2004). The substrate condition is one factor that affect the density. The highest density is *Dolabella auricularia* (0.239629) due to its nocturnal nature and the time sampling was at night. The Opisthobranchia diversity is shown in Table 3.

Table 3. Opisthobranchia's density

Zone	Taxa	D (ind/m2)	Di (%)
Sea grass	*Phyllidiella nigra*	0,25565	33,33333
	Phyllidia multifaria	0,239629	31,24431
	Phyllidia varicosa	0,271672	35,42235
∑		0,766951	
Rocky	*Phyllidiella nigra*	0,239629	33,33333
	Discodoris boholiensis	0,239629	33,33333
	Dolabella auricularia	0,239629	33,33333
∑		0,718886	

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ORAL - TOPIC 3

Systematic and Evolution (O-SE)

ICBS 2011

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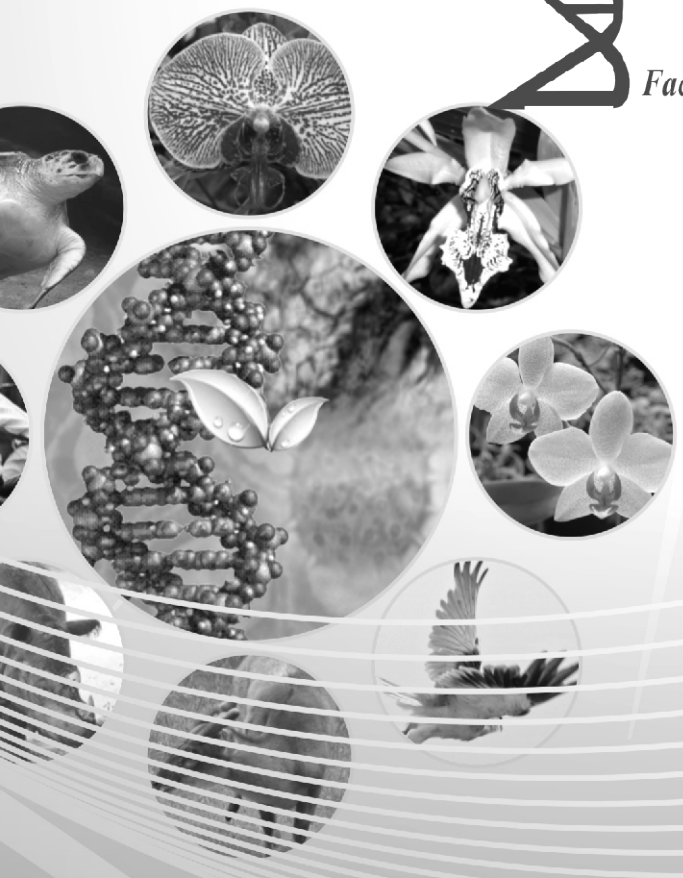

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

O-SE01

Diversity Orchid After 6 Years of Forest Logging at Malinau Research Forest (MRF)-CIFOR Seturan- Malinau Regency

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ABSTRACT

The aim from this research is to find out impact of the 6 years after logging to the various kinds of orchids at Malinau Research Forest (MRF-CIFOR) the village of Seturan – district of Long Loreh, the regency of Malinau.

Input data species of orchids using census method in the climax forest to the broadness of 6 hectares and the log over area to the broadness of 12 hectares. In the primary forest it could be found Orchids is consist 3324 individu from 43 species. In log over area being found 1649 individual from 38 species. Thus 6 years after logging bring about of degradation sum of individual 71.1% and species 18.1%.

The host tree in the climax forest to the amount of 696 trees are consisting of 179 species in 85 genera of 39 families, with 417 trees (59,9%) each of them has a diameter runs 36-67 cm, whereas in the log over area being found 610 trees consisting of 162 species in 101 genera of 42 families with 484 trees (79,9%) each of them has got a diameter runs from 20-51 cm.

Keywords: climax forest, log over area, microclimate, diversity

INTRODUCTION

Orchidaceae only a small group of plants, but it is a significant part of all plant species are found in tropical forests. Because it has a very importance role in characterizing the types of tropical forest, including nutrient recycling systems in various types of forest ecosystems (Mitchell, 1989).

The results Gandawidjaja (1997) showed in kalimantan known as Orchids land has recorded 2500-3000 orchid species (75% orchid Indonesia-Malaysia or or Malesia), or about 10% of all species of orchids in the world. The diversity of orchids in various types of trees, growth rates, and parts of trees that became the host for its dependence on microclimatic conditions of forest stands. That led to the existence of a number of colonies of orchidscan only be found in certain tree species or in certain parts of the tree, otherwise other colonies can be found in each type of tree and on every part of the tree. For that conducted the study with the objective to identify orchids and its host tree, in climax forest and in logged forest that is harvested with a conventional system (TPTI).

MATERIALS AND METHODS

A. Overview: Research Areas (Machfudh and Kartawinata. 2001)

1. Location

The experiment was conducted at the climax forest and logged-over forest at the Forest Research Station Malinau Research Forest (MRF) - Center for International Forestry Research (CIFOR), Seturan village-subdistrict Long Loreh in Malinau (180 km from the town of Malinau). Size total acreage of Forest Research Malinau (BRF-CIFOR) is approximately 321 000 hectares. The observation in 1997 to use the Landsat TM-5 showed a wet tropical forest in the area of Research Station Forest-CIFOR BRF Seturan consists of climax forest (97.84%), secondary forest (2.12%) and the open land (0.04%).

2. Topography

Topographic conditions of the area of Forest Research Station BRF - CIFOR Malinau Seturan-hilly, located at an altitude between 100-300 m above sea level, with slopes varying between 10% - 70%. While 40% of the total BRF area has slopes between 25-40% (including in Seturan), while areas with greater slope (steep to very steep) lots located on the west and southwest. Based on data obtained by using the Digital Elevation Model (DEM) from satellite Radarsat can be obtained information that the 84.24% area of BRF is hilly area with altitude of more than 300 m above sea level., 11.43% is an area with undulating topography, with little there is a flat area.

3. Climate

Climate data have been obtained from PT Inhutani II Unit Malinau show that the forest areas managed by the BRF-CIFOR and its surroundings are included in the precipitation type A on the basis of Schmidt and Fergusson (1951), with dry periods of less than 2 (two) months and wet months over 9 (nine) months, the average rainfall was recorded around 3790 annual mmyear^{-1}.

4. Hydrology

Topographic conditions are largely a local cause BRF-CIFOR area is passed by 3 (three) major rivers namely the Malinau River, which flows from east to west and then turned north; Tubu river, which crossed the mid-BRF area and flows northward and then then turned east to join the river Mentarang Mentarang river will join the Malinau River in the village of Cow Island and is the limit of BRF in the north, two rivers, will join with the river Sesayap. Also along the western boundary BRF Bahau river also flows from north to south direction, which would then meet with greater Kayan river. Based on the existence of these large rivers and the water flow pattern, the BRF area can be grouped into 3 (three) main water catchment area or water basin (DAS), the Malinau watershed (44.09%), DAS Tubu / Mentarang (36.04 %), and DAS Bahau (19.86%).

B. Permanent sample plots at the Forest Research Area BRF Seturan CIFOR Malinau (Machfudh, et al. 2001)

Dipterocarpaceae forest Lowland is a major extensive forest type contained in the BRF, very rich with trees that have a 35-40 m tall, dominated by trees with $\geq$ 10 cm a diameter tribes, particularly Meranti (*Shorea* sp.), Keruing (*Dipterocarpus* sp.) and Merawan (*Hopea* sp.). *Agathis borneensis*, are commonly found growing in forests with sandy soils in the BRF area, apart from that are commonly found in species of Fabaceae, especially *Koompassia excelsa*, or called "Bengeris" or “honey tree” by local residents.

Number of permanent sample plots in the plot (PSP) in the BRF-CIFOR totaling 24 plots, each measuring 100m x 100m (1 hectare), the whole is a mixed forest Dipterocarpeceae. PSP location is located approximately 30 km east of Forest Research station BRF-CIFOR.

The data have been obtained from 24 PSP, each with size 1 (one) hectare, prior to logging shows that the tree diameter (dbh) had an ≥ 20 cm average basal area 4.30 m^2/ha and density of 253 trees / ha. The trees from the Dipterocarpaceae family dominated the entire plot of the study, reaching 27% of tree density and 40% basal area, as well as the main component of the forest canopy. Other types that have a high density and basal area is *Shorea elliptica S. maxwelliana* and *S. parvifolia*, while Shorea is the largest tree with a diameter 199.6 cm

C. Diversity of orchids

Most orchids are epiphytes living at the canopy grows in colonies with *Licopodium Selliguea* sp. of the genera of ferns which are found-shaped pile substrate (moss). Overall the number of orchids are found in logged over area (LOA) of 12 hectares as 1492 individuals or as much as 124.3 individuals per hectare, which is included in 37 species from 18 genera.

Orchids which live singly or in the form of colonies generally found to grow and thrive in the tree canopy (97.6%) mainly on the branches that are large. Some other small (only three types, or 2.4%) were found living on the trunk free from branches and none of the orchid species are found living on the bole of the tree (ground level).

Table 1. 10 (ten) Orchids often found in the Canopy In Climax Forest (CF) and Log Over Area (LOA)

Species	Genera	Sum Of individu	
		CF	LOA
Bulbophyllum binnendijkii J.J.S.	*Bulbophyllum*	197	-
Bulbophyllum beccariu Rchb.f.	*Bulbophyllum*	165	102
Bulbophyllum gracillum Rolfe.	*Bulbophyllum*	143	-
Bulbophyllum lepidum (Bl.) J.J.S.	*Bulbophyllum*	132	-
Bromheadia finlaysiniana (Lindl.) Miq.	*Bromheadia*	117	-
Bulbophyllum vaginatum (Lindl.) Rchb.	*Bulbophyllum*	144	-
Cimbidium finlaysonium Lindl.	*Cymbidium*	110	-
Acriopsis javanica Reinw.	*Acriopsis*	108	102
Sarcanthus subulatus Rchb.f.	*Sarcanthus*	107	-
Bulbophyllum macranthum Lindl.	*Bulbophyllum*	-	98
Bulbophyllum purpurescens Ted. & B.	*Bulbophyllum*	-	89

These conditions correspond to the results of research from Partomihardja (1991) on the plot area of 6 ha in secondary forest Wanariset Sambodja-Kutai Kartanegara indicating that Orchidaceae is the type easy to find, rich in species, spread, and the most abundant. Something similar is also conveyed by Walter (1971), OOsting and Migenis (1993) that the presence and distribution of orchid generally abundant in the canopy, especially those that grow relatively flat at various canopy heights. In Table 1 are given 10 species of orchids orchids are often found in the canopy of trees and only three species that grow on the trunk free from branches.

In CF and LOA orchids found mostly in the form of colonies on the former branch or limb fractures were deep enough or the sidelines of the branches are large and filled with litter or organic ingredients as well mildew cracks in tree trunks. Dominant orchids found alive on a tree trunk with a large diameter and are not found living in other parts of the tree, because it did not like the shade in all parts of his life. Though often found to accumulate on one side of the rod opposite the sun. Where the stick on cracks or fissures are narrow tree trunks perakaraan system is much longer and extends over parts of the body, whereas if his life in the cracks or holes large enough fault branch and the (full litter) then the roots is almost invisible. It shows orchids although tolerant of direct sunlight but not resistant to

drought. The existence of orchid can be used as an indicator that shows the area is very humid environmental conditions and often foggy.

Table 2. The most Orchidaceae being found at the bark tress in the Climax Forest (CF) and Log Over Area (LOA)

Species	Genera	Sum Of individu	
		CF	LOA
Agrostophyllum Bl.	*Agrostophyllum*	67	-
Bulbophyllum gracillum Rolfe	*Bulbophyllum*	57	14
Bulbophyllum lepidum (Bl.) J.J.S.	*Bulbophyllum*	57	-
Bulbophyllum beccariu Rchb.f.	*Bulbophyllum*	33	-
Bulbophyllum vaginatum (Lindl.) Rchb.	*Bulbophyllum*	32	-
Bulbophyllum macranthum Lindl.	*Bulbophyllum*	23	-
Bulbophyllum purpurescens Ted.&B.	*Bulbophyllum*	17	-
Sarchantus subulatus Rchb.f.	*Sarcanthus*	-	14
Pholidota imbricata (Rchb.f.) Lindl.	*Phollidota*	-	9

Orchid on the bole of tree, in addition to type *Eria Javanica* (Bl.) Lindl. which is the tribe of Orchidaceae in the primary forest. This situation suggests that different types of orchids tolerant of sunlight, the humidity is not too high, this condition is ideally located on the canopy (Wolf, 1994).

Felling trees and looming large in diameter (emergent trees), which is often the host tree are many kinds of orchids, are potentially reduce the availability of local seeds and endemic orchids, including reduced vegetation growth and spread of orchids species, thereby reducing the presence and abundance or even extinct. The condition is caused by environmental conditions around the host tree supporting orchids growth has started to not fit as a result of a sudden and sharp changes and will take place in the long run. Since the penetration of sunlight on the forest floor logged greater than in primary forest floor, causing the tree dries faster (Sutton, 1983; Mitchell, 1989).

3. Tree diameter distributions Host On Primary and Forest Used Forest Felling

Stem diameter which generally indicates the age, seems closely related to the number of epiphytes especially orchids that attach to a host tree species. Regardless of species, genera and families, host trees with relatively large diameters tend to be more attached orchids, both in number of species and number of individuals. With large diameter trees over most of the bark has a condition favorable for the growth of orchids, because his skin is generally rough, cracks and a lot of indentations, holes and broken branches or scars rotting (Mitchell. 1989).

However, it does not mean that every large diameter trees that although of the same type will always be more attached orchids, not even found at all (walter, 1993) for example on the type *Koompassia excelsa* and *Agathis borneensis* (or in kind by the host tree but canopy is damaged, molt and nearly bald or already bald).

Observations on Table 3, show that in primary forest around 59.9% of the host tree has a trunk diameter of 36-67 cm, and 5.4% of all host trees or 38 host tree has a diameter of more than 84 cm, while 175 trees host or 25.1% of the host tree has a diameter between 20-35 cm. In logged over area shows the host tree or about 49.3% of all host trees have a diameter between 20-35 cm, 186 host tree or approximately 30.0% of the host tree has a diameter between 36-51 cm, 89 host trees or around 21.1% of all host tree has a diameter between 52-67 cm, 19 host tree or approximately 15.3% of the host tree has a diameter between 68-83 cm, while 18 host tree, or about 3.3% of the host tree has a diameter of between 84 - 131 cm. And when comparing the host tree diameter distribution between primary forests to forests logged, then the average diameter of the host tree in the primary

forest is greater than the average diameter of host trees in logged-over forests. At logged-over area are most at between 20-51 cm diameter class and have not found the host tree with a diameter of 132 cm, because the tree trees with diameters over 100 cm was cut out and left more because of poor quality trees ("growing", disability, branch of branch-free trunk is too short, or curved) or trees of the species harvested are prohibited.

Table 3. Tree Diameter Distribution of Host Tree on Climax Forest (CF) and Log Over Area (LOA)

No.	Class of Diameter (cm)	CF		LOA	
		Sum	%	Sum	%
1.	20 – 35	175	25.1	301	49.3
2.	36 – 51	234	33.6	183	30.0
3.	52 – 67	183	26.3	89	21.1
4.	68 – 83	66	9.5	19	15.3
5.	84 – 99	21	3.0	12	2.3
6.	100 – 115	9	1.4	4	0.7
7.	116 – 131	3	0.4	2	0.3
8.	132 – 147	3	0.4	-	-
9.	148 – 163	1	0.1	-	-
10.	164 – 179	1	0.1	-	-
	T o t a l	696	100.0	610	100.0

Tree of life orchids (host) often have special physical appearance. Most of the host plants have branches, branches or twigs that growth is relatively flat or sloping habitats encountered groups of orchids. Surface of the skin on the slippery wood trees and hard, for example *Legerstroemia lanceolata*, *L. duperreans, Kompassia exelsa* and others, rare live orchids that grow well, so that orchids often found in trees that have a rough skin, cracked and grooved so easy to save water, for example *Ehritia acuminata, Sonneratia caseolaris, Pithecellobium scalare , Calophyllum inophyllum* and others (Claudio, R. 1999; Partomihardja, 1984). Conversely though environmental conditions, especially climatic elements strongly support the presence of orchids was never found in plants pioneer (Essen, 1996).

CONCLUSION

The results of this study, several conclusions can be drawn as logging activities have caused degradation number of individuals and number of orchids species. Although logging activities have been implemented 6 years,felling trees and looming large in diameter (emergent trees), which is often the host tree are many kinds of orchids, are potentially reduce the availability of local seeds and endemic orchids, including reduced vegetation growth and spread of orchids species, thereby reducing the presence and abundance or even extinct.

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O-SE02

Acronychia spp : Species Diversity in Java and Its Potency

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not presented

O-SE03

THE DIVERSITY OF CRUSTOSE LICHENS IN THE FOREST OF TAHURA R. SOERYO, BATU, EAST JAVA

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Abstract. Lichen is an outstanding successful group of symbiotic organisms, which comprise the strand of algae partner (photobiont) and fungal partner (mycobiont). According to the morphological forms, there are seven growth-forms of lichen, however, there are five common growth-forms, i.e.: foliose, fruticose, crustose, squamulose, and leprose. A taxonomic study of the crustose lichens in the forest of TAHURA R. Soeryo had been conducted based on morphological, anatomical, and chemical characters. In this research involved a method of descriptive explorative and the aim of this research is to study the diversity of crustose lichens in the forest of TAHURA R. Soeryo. TAHURA R. Soeryo is a conservation forest located in Tulungrejo, Batu City, East Java. Its altitude is 1000-3000 m, the rainfall is about 2500-4500 mm per year, and the temperature is about 5°C-18°C. This research was conducted from September 2010 – Januari 2011. Twenty species of crustose lichen with one species unidentified and one specimen identified to the group of family are reported from the forest of TAHURA R. Soeryo. They are *Graphina anguina, Graphina columbina, Graphina ruiziana, Graphis galucescens, Graphis elegans, Graphis scripta, Phaeographis lyelli, Megalospora campylospora, M.* cf *sulphurata*, *Pertusaria amara, Pertusaria* sp., *P. corallina, Pachyphiale carneola, Pachyphiale* sp., *Phlyctis agelaea, Lepraria* sp., *Lecanora carpinea, Lecania cyrtella, Cyphellium inquinans* and one new record from Java, *M. kalbii*. The taxonomic, the current delimitation and description, altitudinal range, humidity, light intensity, temperature of the each species; and the identification key to its species of the TAHURA R. Soeryo's crustose lichen are presented in the article.

Keywords: diversity, crustose lichen, Forest of TAHURA R. Soeryo

INTRODUCTION

Lichens are also a plant, but a very special kind, for when we dissect and examine it under a microscope, we find that it is composed of two completely different organisms, microscopic green or blue-green algae and colorless fungal threads called hype. These two components grow together in a harmonious association referred to as symbiosis, or more simply a “living- together”. Lichen symbiosis, however, differs basically from all other kinds in that a new plant body, the thallus, is formed and this talus has no resemblance at all to either a fungus or alga growing alone (Hale, 1969). Lichens can be found from extreme low tide level on the sea-shore to the tops of high mountains, and from arctic to tropical regions (Dobson, 1992).

Growth form means the overall shape and configuration of the lichen talus. There are three major types: foliose, fruticose, and crustoce (Hale, 1974; Rout *et.al.*, 2010). A fourth type, the squamulose lichens, may also be recognized. The whole association grows at a rate ranging from one millimetre or less per year for crust (crustose) lichens up to a few centimetres a year for the most rapidly growing leafy (foliose) or shrubby (fruticose) lichens (Richardson, 1992). The distribution of lichens is governed by many interacting factor i.e. topography, substrate, light intensity, moisture, humidity (Termina *et.al*., 2009; Ramakantha *et.al*., 2003; Hayward *et.al*., 1975; Kenkel *et.al*., 1986) and altitude (Hayward *et.al*., 1975).

Tropical forest has a complex component either flora or fauna. One of the tropical forests in Indonesia is an Arjuno Lalijiwo forest which is more famous with Taman Hutan Raya R.Soeryo (TAHURA). Geographically**,** Tahura is located in Tulungrejo village, Bumiaji District, East Java. Topography in general has various configurations among superficial, hilly, mountain with 1000-3000 m high from the upper surface of sea shore. Its altitude is 1000-3000 m, the rainfall is about 2500-4500 mm per year, and the temperature is about 5^0C - 10^0C. Biotic potential of that area is flora condition dominated various kinds of plant. (Departemen Kehutanan, 2008). Deforestation is a major enviromental issue which may cause disappearance of many lichen species without being studied. Fifteen species of foliose lichen (Jannah *et.al.*, 2009) and eleven species of fruticose lichen (Jannah *et.al.*, 2010) is reported in this forest.

Lichens has a big diversity in this world, however, people pay very small attention in this subject. Lichens play a very important role in the ecosystem, as oxygen supplier, bioindicator of air pollution and air quality biomonitoring (Richardson, 1992; Negi, 2003; Rout *et.al.*, 2010). This rich diversity indicates good forest health. Lichens can also be very usefull,as medicine, antibiotic, antimutagenic, cosmetic (Nash, 1996; Negi, 2003) and pesticide (Dayan *et.al.,*2001).

Therefore, we had conducted a lichen taxonomical research in purpose to study the diversity of crustose lichens in TAHURA R. Soeryo, to identify the species of lichen in the TAHURA R. Soeryo, and to provide the identification key of its species, in which the study would support the conservation of lichen in situ in the ecosystem.

MATERIALS AND METHODS

This research is descriptive explorative which has purpose to study the diversity of crustose lichens in TAHURA R. Soeryo. This researches was conducted from September - January 2011, in which involved a method of descriptive explorative technique. The identification involved the method of determining the shape and colour of the thallus, the presence or absence of soredia, soralia, and isidia and the size and septation of the spores

within the fruit bodies and chemical tested. The chemical substances used are P (Phenylenediamin), K (Potassium hydroxide), and C (Calcium hypoclorite). The substances of thallus lichen are identified by their characteristic microcrystal formation in various reagents i.e. GAW (H_2O : glycerol: ethanol = 1:1:1), GE (acetic acid : glycerol = 1:3), An (aniline : glycerol : ethanol = 1:2:2), dan oT (o-toluidine : glycerol : ethanol = 1:2:2).

RESULTS AND DISCUSSION

The diversity of crustose lichen in the forest of TAHURA R. Soeryo, Batu, East Java consist of 9 families and 20 species of crustose lichen with one species unidentified and one specimen identified to the group of family. In this research the family Graphidaceae is the most common with 8 species being identified.

Identification Key

1. a. Without soralia.....6
   b. With white soralia 2
2. a. Soredia white (P+red, K-).....*Pertusaria amara*
   b. Soredia absent.....3
3. a. Without isidia (K+yellow, P+red, C+yellow).....*Unidentified*
   b. With white isidia.....4
4. a. Soredia absent (K-, P-. C-).....*Pertusaria corallina*
   b. Soredia green colour.....5
5. a. It has reddish yellow isidia (K+red, P+red, C+yellow).....*Lepraria* sp.
   b. It has green isidia (K+red, P+red, C-).....*Pertusaria* sp.
6. a. Apothecia with lirellate shape.....7
   b. Apothecia with disc shape.....15
7. a. Margin and disc of apothecia cant be differentiated.....*Lecania cyrtella*
   b. Margin and disc of apothecia can be differentiated.....8
8. a. Spore one septate with epispore warted (K+yellow).....*Megalospora campylospora*
   b. Spore one septate with epispore smooth.....9
9. a. Apothecia margin greyish white (K+ yellow,P+red) .....*Cyphellium inquinans*
   b. Apothecia margin reddish orange.....10
10. a. Spore muriform, ≥ 2 in each ascus (K+red, C+yellow).....*Phlyctis agelaea*
    b. Spore muriform, one in each ascus.....11
11. a. Apothecia green pruinose (P +reddish yellow).....*Megalospora kalbii*
    b. Apothecia epruinose.....12
12. a. Spora multiseptate, 2-40 in each ascus (K+yellow).....*Pachyphiale carneola*

b. Spora multiseptate, one in each ascus.....13
13.a. Apothecia with reddish brown disc (K-, C-, P-).....*Pachyphiale* sp.
b. Apothecia with dark brown disc 14
14.a. Apothecia lecideine (K+yellow, C-,P+red).....*Megalospora* cf. sulphurata
b. Apothecia lecanorine (K+yellow, P+ red, C+yellow) .....*Lecanora carpinea*
15.a. Margin and centre of lirellae apothecia cant be differentiated
(K+ kuning, C+kuning) .....*Graphidaceae*
b. Margin and centre of lirellae apothecia can be differentiated.....16
16.a. Lirellae innate (P+red, C+yellow).....*Graphina anguina*
b. Lirellae elevated 17
17.a. Apothecia with rarely branched (K+yellow) .....*Graphina ruiziana*
b. Apothechia with much branched.....18
18.a. Apothecia white pruinose (K+yellow).....*Graphina columbina*
b. Apothecia brown pruinose.....19
19.a. Spore multiseptate with thick-walled (K+yellow).....*Phaeographis lyelli*
b. Spore multiseptate with thin-walled.....20
20.a. Margins of lirellae with several furrows, often (K+red).....*Graphis elegans*
b. Margins of lirellae unfurrowed 21
21.a.Centre of lirellae with dull black (P+red, C+yellow) .....*Graphis scripta*
b. Centre of lirellae with pale brown (K+red,C+yellow) .....*Graphis glaucescens*

Taxonomic Descriptions

1. *Graphina anguina* (Mont.) Müll. Arg.

This lichen belongs to *Graphidaceae* family. **Thallus** grey, irregularly shaped, usually wrinkled and cracked; **chemistry**: 4-0-methylphysodic acid, roccellaric acid, and acetylportentol; **apothecia** lecideine, immersed, lirellate, innate, margins with gray, centre with black, much branched and serpentine; **spores** 2-8 in each ascus, colourless, muriform, 1,5-2 x 1-2 µm; **Chemical test** in medulla are C+yellow, K-, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1730 dpl, temperature is about 16°-19°C, humidity is about 83-98%, and light intensity is about 475-1700 lux.

2. *Graphina columbina* (Tuck.) Wirth et. Hale. Jr.

This lichen belongs to *Graphidaceae* family. **Thallus** greenish to brownish, irregularly shaped; **chemistry**: dissectic acid with atranorin, fumarprotocetraric acid, acetylportentol, barbatic acid, and retigeric acid; **apothecia** lecideine, lirellate, margins with pale green, centre with white and widely, white pruinose, much branched; **spores** 2-8 in each ascus,

colourless, muriform, 6-15 x 1-3 µm; **Chemical test** in medulla are C-, K+yellow, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1780 dpl, temperature is about 17,5-19°C, humidity is about 85-98%, and light intensity is about 600-1150 lux.

3. *Graphina ruiziana* (Fée) Müll. Arg.

This lichen belongs to *Graphidaceae* family. **Thallus** greenish grey or whitish, irregularly shaped, sometimes scarcely developed, it has orange soredia; **chemistry**: dissectic acid with atranorin, 4-0-methylphysodic acid, acetylportentol, and retigeric acid; **apothecia** lecideine, lirellate, margins with whitish grey, centre with black, elevated, and rarely branched (unbranched); **spores** >1 in each ascus, colourless, muriform, 6-12 x 1-2 µm; **Chemical test** in medulla are C-, K+yellow, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1780 dpl, temperature is about 17,5°C, humidity is about 98%, and light intensity is about 600 lux.

4. *Graphis galucescens* Wirth et. Hale. Jr.

This lichen belongs to *Graphidaceae* family. **Thallus** white to greenish grey, irregularly shaped; **chemistry**: fumarprotocetraric acid, bellidiflorin, nephroarctin, dan pseudo-norrangiformic acid; **apothecia** lecideine, lirellate, margins with greenish grey, centre with pale brown, spiral and much branched; **spores** 4-) 6 (8- in each ascus, colourless, multiseptate, 4-6 x 1 µm; **Chemical test** in medulla are C+yellow, K+red, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1780 dpl, temperature is about 17,5°C, humidity is about 98%, and light intensity is about 600 lux.

5. *Graphis elegans* (Sm.) Ach.

This lichen belongs to *Graphidaceae* family. **Thallus** greenish grey, irregularly shaped, smooth, rather thin, slightly, and wrinkled; **chemistry**: barbatic acid and acetylportentol; **apothecia** lecideine, lirellate, margins with black and several furrows, centre with black, spiral and carbonaceous; **spores** 6-13 in each ascus, colourless, multiseptate, 5 x 0,5-1 µm; **Chemical test** in medulla are C+yellow, K+red, P+red.

Habitat and ecology: In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1800 dpl, temperature is about 17,5°-19^{0}C, humidity is about 85-98%, and light intensity is about 600-1700 lux.

6. *Graphis scripta* (L.) Ach.

This lichen belongs to *Graphidaceae* family. **Thallus** grey, smooth or wrinkled, irregularly shaped; **chemistry**: 4-0-methylphysodic acid and acetylportentol; **apothecia** lecideine, lirellate, margins with gray and unfurrowed, centre with pale black, sometimes pruinose and carbonaceous; **spores** 11-15 in each ascus, colourless, multiseptate, 4-5 x 0,5-1 µm;

Chemical test in medulla are C+yellow, K-, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1730 dpl, temperature is about 16°-19^{0}C, humidity is about 85-98%, and light intensity is about 475-1150 lux.

7. *Phaeographis lyelli* (Sm.) Zahlbr.

This lichen belongs to *Graphidaceae* family. **Thallus** green, smooth, glossy, irregularly shaped; **chemistry**: thamnolic acid with decarboxythamnolic acid, acetylportentol, nephroarctin, and α-acetylsalazinic acid; **apothecia** lecanorine, lirellate, margins with green, centre with brown; **spores** > 3 in each ascus, colourless, multiseptate, 3-4 x 1 µm; **Chemical test** in medulla are C-, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18,5°-19^{0}C, humidity is about 83-85%, and light intensity is about 1050-1150 lux.

8. *Unidentified* Graphidaceae

This lichen belongs to *Graphidaceae* family. **Thallus** brwonish gray, smooth, irregularly shaped, it has yellowish white soredia; **chemistry**: 4-0-methylphysodic acid, acetylportentol, and pseudo-norrangiformic acid; **apothecia** lirellate, elevated, unbranched; **Chemical test** in medulla are C+yellow, K+yellow, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18°-19^{0}C, humidity is about 75-85%, and light intensity is about 1050-1700 lux.

Notes: This specimen identified to the group of family because spores in the apothecia was not found.

9. *Megalospora campylospora* (Stirt.) Sipman

This lichen belongs to *Megalosporaceae* family. **Thallus** greenish gray, irregularly shaped, rather thick, very rugulose with little cracks; **chemistry**: diffractaic acid and acetylportentol.; **apothecia** lecidine, scattered, sessile, orbicular or oblong and the large ones becoming lobed to reniform to irregularly shaped, margins with black, disc with black, up to 3 mm diam; **spores** 4-6 in each ascus, colourless, one septate with epispore warted, 10-25 x 20-28 µm; **Chemical test** in medulla are C+yellow, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1800 dpl, temperature is about 16°-18,5^{0}C, humidity is about 75-98%, and light intensity is about 45-1700 lux.

10. *Megalospora* cf. *sulphurata* Meyen

This lichen belongs to *Megalosporaceae* family. **Thallus** greenish gray, irregularly shaped, rather thin to rather thick, smooth to very rugulose, small cracks or with longitudinal, epruinose; **chemistry**: diffractaic acid, haemathamnolic acid, barbatic acid dan acetylportentol; **apothecia** lecidine, scattered, sessile, orbicular to oblong and the large ones

becoming lobed or irregularly shaped, margins with black, disc with brown to black, up to 3 mm diam; **Chemical test** in medulla are C-, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18,5°-19^{0}C, humidity is about 83-85%, and light intensity is about 1050-1150 lux.

Notes: This specimen identified to the group of *Megalospora* genus. This genus is characterized by olidroplet in the hymenium (Sipman, 1983). This specimen was not found spores, it may prove worthy of further identification. Spora type is an important aspect for identification, because it can be used as special characteristic of species and family.

11. *Megalospora kalbii* Sipman

This lichen belongs to *Megalosporaceae* family. **Thallus** grayish white, irregularly shaped, rather thin to rather thick, smooth to very rugulose, epruinose; **chemistry**: 4-0-methylphysodic acid and acetylportentol; **apothecia** lecidine, scattered, adnate, orbicular to oblong and the large ones becoming lobed or irregularly shaped, margins with black, disc with brown to black, dull green pruinose, up to 1 mm diam; **spores** one in each ascus, colourless, muriform, 10-15 x 20-50 µm**; Chemical test** in medulla are C-, K-, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18°-18,5^{0}C, humidity is about 75-83%, and light intensity is about 1050-1700 lux.

Notes: The avalaible specimen deviates from Sipman`s description (1983) by epruinose apothecia disc and brown to dark apothecia, but from Sipman`s description (1999) by white pruinose apothecia disc and pale brown to dull brown apothecia disc. As more collections become avalaible, it may prove worthy of further study and of a separate taxonomic status.

12. *Pertusaria amara* (Ach.) Nyl.

This lichen belongs to *Pertusariaceae* family. **Thallus** grayish white, irregularly shaped, thick and delimited, it has white soredia and soralia, very bitter taste of the soralia; **chemistry**: baeomycesic acid, 4-0-methylphysodic acid and acetylportentol; **Chemical test** in medulla are C-, K-, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18°-18,5^{0}C, humidity is about 83%, and light intensity is about 1050-11650 lux.

13. *Pertusaria corallina* (L.) Arn.

This lichen belongs to *Pertusariaceae* family. **Thallus** white to pale gray, irregularly shaped, thick, warted, it has white isidia; **chemistry**: fumarprotocetraric acid, pseudo-norrangiformic acid, hiascic acid, and acetylportentol; **Chemical test** in medulla are C-, K-, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from

1640 dpl, temperature is about 18,5°-19^{0}C, humidity is about 83-85%, and light intensity is about 1050-1150 lux.

Notes: The avalaible specimen deviates from Dobson`s (1992) by this specimen was found on rock.

14. *Pertusaria* sp.

This lichen belongs to *Pertusariaceae* family. **Thallus** gray, irregularly shaped, thick, warted, it has green soredia and isidia; **chemistry**: dissectic acid with atranorin, thamnolic acid with decarboxythamnolic acid, atranorin, barbatic acid, and acetylportentol; **Chemical test** in medulla are C-, K+red, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1780 dpl, temperature is about 17,5°-19^{0}C, humidity is about 85-98%, and light intensity is about 600-1150 lux.

15. *Pachyphiale carneola* (Ach.) Arnold

This lichen belongs to *Gyalectaceae* family. **Thallus** dull green, thin, irregularly shaped, smooth, epruinose; **chemistry**: 4-0-methylphysodic acid, baeomycesic acid, lichesterinic acid, retigeric acid, and acetylportentol; **apothecia** lecidine, scattered, sessile, orbicular to oblong and irregularly shaped, margins with reddish brown, disc with reddish brown, up to 2 mm diam; **spores** 2-40 in each ascus, multiseptate, fusiform shaped, colourless, 6-8 x 0,5 µm**; Chemical test** in medulla are C+yellow, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18°C, humidity is about 75-83%, and light intensity is about 1650-1700 lux.

16. *Pachyphiale* sp.

This lichen belongs to *Gyalectaceae* family. **Thallus** dull green, thin, irregularly shaped, smooth, it has reddish yellow soredia; **chemistry**: 4-0-methylphysodic acid, acetylportentol, lichesterinic acid, baeomycesic acid, and retigeric acid; **apothecia** lecidine, scattered, sessile, orbicular to oblong and irregularly shaped, margins with brownish black, disc with brown, up to 1,2 mm diam; **spores** one in each ascus, multiseptate, fusiform shaped, colourless, 5-6 x 0,5 µm**; Chemical test** in medulla are C-, K-, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640-1780 dpl, temperature is about 17,5°-18,5°C, humidity is about 75-98%, and light intensity is about 600-1700 lux.

17. *Lepraria* sp.

This lichen belongs to *Stereocaulaceae* family. **Thallus** grayish white, irregularly shaped, thin, pruinose, it has white soredia and reddish yellow isidia; **chemistry:** 4-0-methylphysodic acid, obtusatic acid, diffractaic acid, and acetylportentol; **Chemical test** in medulla are C+yellow, K+red, P+red. **Habitat and ecology:** In forest, this species was found on old and

cracked bark, at altitudes ranging from 1640-1730 dpl, temperature is about 16°-19^{0}C, humidity is about 85-98%, and light intensity is about 475-1150 lux.

18. *Phlyctis agelaea* (Ach.) Fw.

This lichen belongs to *Phlyctidaceae* family. **Thallus** gray, thin, cracked or warted, irregularly shaped; **chemistry**: fumarprotocetraric acid, obtusatic acid, acetylportentol, and bellidiflorin; **apothecia** lecanorine, scattered, sessile, orbicular to oblong and irregularly shaped, margins with reddish orange, disc with green, green pruinose, up to 1,5 mm diam; **spores** >2 in each ascus, muriform, colourless, 4-7x 1-2 µm**; Chemical test** in medulla are C+yellow, K+reddish yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18,5°-19^{0}C, humidity is about 83-85%, and light intensity is about 1050-1150 lux.

19. *Lecanora carpinea* (L.) Vain.

This lichen belongs to *Lecanoraceae* family. **Thallus** grayish green, thin, smooth or warted, irregularly shaped; **chemistry**: 4-0-methylphysodic acid, baeomycesic acid and acetylportentol; **apothecia** lecanorine, scattered, sessile or adnate, roundish to irregularly shaped, margins with brownish gray, disc with pale black, reddish green pruinose, up to 2 mm diam; **spores** 4-9 in each ascus, simple, colourless, 1x1 µm**; Chemical test** in medulla are C+yellow, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18^{0}C, humidity is about 75%, and light intensity is about 1700 lux.

20. *Lecania cyrtella* (Ach.) Th. Fr.

This lichen belongs to *Ramalinaceae* family. **Thallus** grayish green, thin, smooth, not strongly attached on subsrate, pruinose, irregularly shaped; **chemistry**: baeomycesic acid, roccellaric acid, pseudo-norrangiformic acid and acetylportentol; **apothecia** lecanorine, scattered, sessile, roundish to irregularly shaped, margins and disc with brown, thalline margin which usually becomes excluded as the apothecia mature and become convex, up to 2 mm diam; **Chemical test** in medulla are C-, K-, P-. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 18^{0}C, humidity is about 75%, and light intensity is about 1700 lux.

21. *Cyphellium inquinans* (Sm.) Trev.

This lichen belongs to *Caliceaceae* family. **Thallus** gray to reddish yellow, thin, warted, irregularly shaped, it has white and orange soredia; **chemistry**: norstictic acid, alectorialic acid and pseudo-norrangiformic acid; **apothecia** lecanorine**,** scattered, innate or sessile, roundish to irregularly shaped, margins with grayish white, disc with black, black pruinose, up to 1 mm diam; **spores** one septate, septate with black, brown, 1-3x 1 µm**; Chemical test** in medulla are C+yellow, K+yellow, P+red. **Habitat and ecology:** In forest, this species was

found on old and cracked bark, at altitudes ranging from 1640-1800 dpl, temperature is about 16°-19°C, humidity is about 75-98%, and light intensity is about 450-1650 lux.

22. *Unidentified*

Thallus grayish white, thin, warted, irregularly shaped; **chemistry**: fumarprotocetraric acid, dissectic acid with atranorin, roccellaric acid, pseudo-norrangiformic acid, and acetylportentol; **Chemical test** in medulla are C+yellow, K+yellow, P+red. **Habitat and ecology:** In forest, this species was found on old and cracked bark, at altitudes ranging from 1640 dpl, temperature is about 19°C, humidity is about 85%, and light intensity is about 1150 lux.

Notes: This specimen unidentified because not found reproductive organs in the thallus.

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O-SE04

ANALYSE OF MORPHOLOGICAL AND ANATOMICAL STRUCTURE AND GENETICS RELATION OF AMPHIBIOUS FISHES (GOBIIDAE) BY RAPD METHOD AT SUING BEACH

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ABSTRACT

Siung beach has a unique structure. This caused organism that lived on this beach has distinctive character. On the Siung beach was found several types of amphibious fishes. These fishes were belong to Family Gobiidae. These amphibious fishes has a similar morphological traits but has different integument paterns. Nowdays, the Morphological traits were less perceived as classification base for amphibious fishes, therefore genetics datas were needed for suporting the classification. In this study, the specimens were captured in Siung Beach, Wonosari, Gunung Kidul. Sample of fishes were identified, fifty one morphological characters were observed and measured. For acquiring data of the genetics polymorphisms, the sample of these fishes populations were observed by RAPD method. The morphological datas, macrograph photos of the specimens and RAPD profiles were analyzed descriptively and quantitatively by using algoritm software NTSys-pc (Numerical Taxonomy and Multivariate Analysis System for *Personal Computer*). The results revealed there were five species of common amphibious fishes found in Siung beach, they were: *Palutrus* sp., *Gobius paganellus*, *Acentrogobius* sp., *Istigobius* sp, and *Istigobius ornatus*. Quantitatively, *Palutrus* sp., *G. paganellus*, and *Acentrogobius* sp. were remain in same species because these three species shared morphological traits more than 85% similarity index, while *Istigobius* sp., *I. ornatus* were proven as close relative each other with coeficience similarity 74%. In other hand,these five species were genetically proven as different species. Their genetic has similarity index less than 70%.

Keywords: Gelodok, Gobiidae, morphology, RAPD

Introduction

Siung beach, Gunung Kidul regency, Yogyakarta (8°10'55"S 110°40'58"E) has a unique structure. There is two diferrent structure in this beach. Coral zone in the west area and volcan rock in the east area. This caused organism that lived on this beach has distinctive character. On the Siung beach was found several types of amphibious fishes. These fishes were belong to Family Gobiidae. The gobioid fishes (Teleostei: Gobiidae) comprise the most speciose of all the teleost suborders, with over 2500 nominal species arranged in at least 300 genera (Birdsong *et al.*, 1988; Miller, 1993).

The Gobiidae is the largest family of marine fish in the world (Larson dan Murdy, 2001). Gobioid which live in intertidal zone in Siung beach have high variety of spesies Because of their small size and cryptic behavior, few Gobioid species have been reported or recorded based on their morphological traits. Nowdays, the Morphological traits were less perceived as classification base for amphibious fishes, therefore genetics datas were needed for suporting the classification. The objective of study diversity of gobioid in Siung beach is to improve the data base of the diversity of Indonesian gobioid fish.

Material and Methods

In this study, the specimens were captured in Siung Beach, Wonosari, Gunung Kidul ($8^{o}10'55''S$ $110^{o}40'58''E$) by *purposive sampling method* (Patton, 1990). Sample of fishes were identified (Larson dan Murdy, 2001). Fifty one morphological characters were observed and measured as the morphological datas. For acquiring data of the genetics polymorphisms, the sample of these fishes populations were observed by RAPD method. A random primers OPA-01, OPA-02, OPA-06, OPA-08, OPA-09, OPA-11, OPA-12, OPA-15, OPA-16, dan OPA-20 was used to determine genetic differences among the fishes.

Tabel 1. Komposisi Reaksi PCR

Composition PCR	Konsentration	Volume used
Go Taq Green Master Mix 2 X	1x	12,5 µl
Primer RAPD µ10 M	0,6 mM	1,5 µl
DNA *template*	10 pg - 1 µg	1 µl
dH2O	-	10 µl
Volume total		25 µl

The amplification was done in a Touchdown Thermal Cycler (Hybaid, England). PCR cycling procedures were as follows; 45 cycles of 94°C for 0,5 min, 55°C for 1 min and 72°C for 1,5 min. A final step of extension was applied at 72°C for 5 min (Ambak *et al.*, 2006). The amplicon of RAPD profils was the quantitatif data of genetic characterisation.

The morphological datas, macrograph photos of the specimens and RAPD profiles were analyzed descriptively and quantitatively by using algoritm software NTSys-pc (Numerical Taxonomy and Multivariate Analysis System for *Personal Computer*).

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Result and Discussion

An example of the electrophoretic profiles generated by RAPD analysis using OPA-01, OPA-02, OPA-06 primer of (I1*) Istigobius ornatus, (A) Acentrogobius sp., (B) Gobius paganellus.,(P) Palutrus sp., (I2) Istigobius sp.*

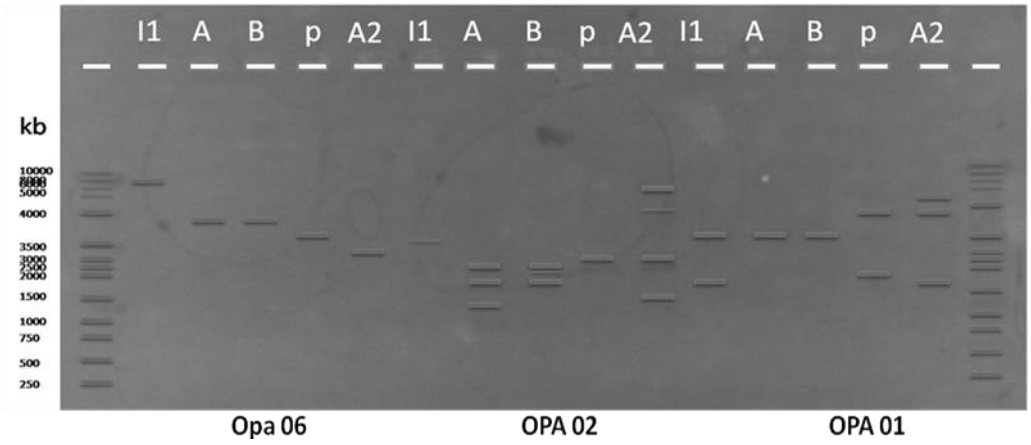

Figure 1. RAPD analysis of (I1*) Istigobius ornatus, (A) Acentrogobius sp., (B) Gobius paganellus., (P) Palutrus sp., (I2) Istigobius sp.* by primer OPA-01, OPA-02, OPA-06.

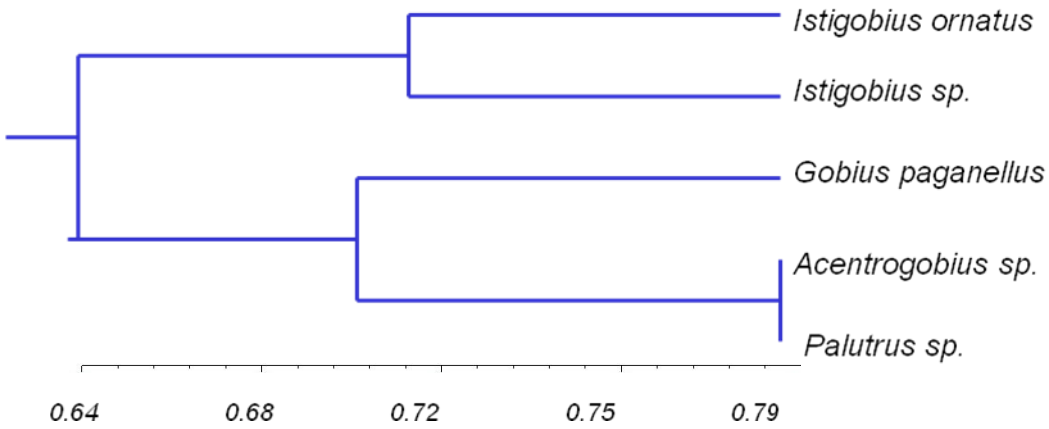

Figure 2. Fenogram Analysis similarity by *Jaccard (J)* Coefisien dan clustering UPGMA of the Fishes

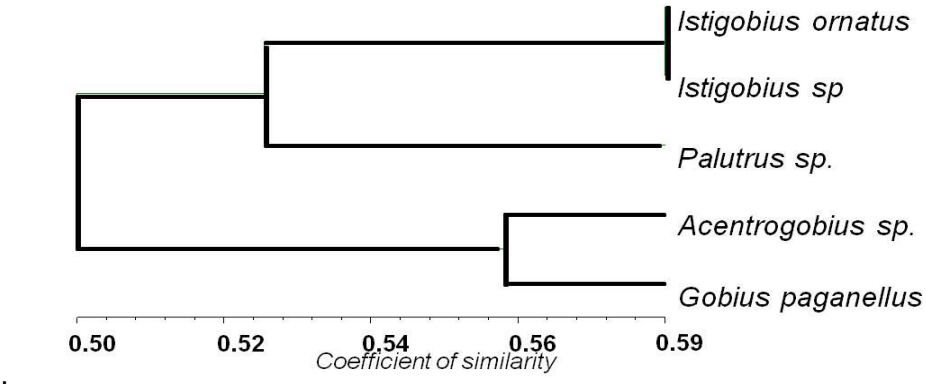

Figure 3. Dendogram Analysis similarity by *Simple Matching (SM)* Coefisien and clustering UPGMA of genetic karacter

The results revealed of the study, there were five species of common amphibious fishes found in Siung beach, they were: *Istigobius* sp.(Whitley, 1932), *Istigobius ornatus* (Rüppell, 1830), *Acentrogobius caninus* (Valenciennes, 1837), *Gobius paganellus Indonesian name is Bathygobius padangensis* (Bleeker, 1878), dan *Palutrus* sp. (Smith,

1959). Genus Istigobius have the same k*ey to genera:* 1b-1b-8b-11b-16b-21b-22b-23b-24b-25b-30a-31b-32a (Istigobius) (Larson dan Murdy, 2001). *Istigobius* sp. have characteristics **D_1 VI; D_2 I, 10; A I,8; C 20; P 18**, while *Istigobius ornatus* are **D_1 VI; D_2 I, 10; A 1,9; C 18; P 20**. *Istigobius ornatus* **D_1 VI; D_2 I, 10; A 1,9; C 18; P 20**.

Genus Acentrogobius k*ey to genera* : 1b-2b-8b-11b-16b-21b-22b-23b-24b-25b-30b-34b-35b-39b-43b-44b-48b-49a-55a-56b(Acentrogobius). The formula of fin are **D_1 VI; D2 I,7; A I,8; C 14; P 20.** Gobius Paganellus in Indonesia *well known as Bathygobius padangensis* (Bleeker, 1878). This Genus have k*ey to genera* is 1b-1b-8b-11b-16b-21b-22b-23b-24b-25b-30a-31a (Bathygobius) and have the fins formula **D_1 VI; D_2 I, 9; A I,8; C 24; P 20.** (Larson dan Murdy, 2001). And The last Genus that Identified is *Palutrus* sp.. *Palutrus* sp. formula fins are **D1 VI; D2 I, 10; A I,8; C 16; P 20**. *Key to genera* 1b-1b-8b-11b-16b-21b-22b-23b-24b-25b-30a-31b-32b-33b (Palutrus).

Based on Jaccard and Simple Macthing Coefisien, Quantitatively, *Palutrus* sp., *G. paganellus*, and *Acentrogobius* sp. were remain in same species because these three species shared morphological traits more than 85% similarity index according FAO key of Identification (Larson dan Murdy, 2001). *Istigobius* sp., *I. ornatus* were proven as close relative each other with coeficience similarity 74%. In other hand,these five species were genetically proven as different species. Their genetic has similarity index less than 70%.

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O-SE05

IDENTIFICATION OF POECILIIDAE FISHES FROM SARI LAKE, PURWODADI, PASURUAN REGENCY THROUGH MORPHOLOGICAL CHARACTERISTICS AND *DNA BARCODE CYTOCHROME-C OXIDASE SUB UNIT I (COI)*

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ABSTRACT

Poeciliidae family consists of small live-bearer fishes with sexual dimorphic. This fishes are widely distributed across Indonesian archipelago, but biological studies on this fish are very limited. This research is aimed to identify the species of Peciliidae fishes which lives in Sari Lake Pasuruan based on morphological characteristics and *DNA Barcoding*. Morphological parameters including morphometric, meristric, anatomic, pigmentation characteristics and gonopodium structure as well, while *DNA barcode* using *cytochrome-c oxidase I (COI)* gene. Based on morphological characteristics, we find three species i.e: *Xiphophorus hellerii*, *Poecilia mexicana*, and *Poecilia reticulata*. *Neighbor joining*, *minimum evolution, maximum parsimony,* and *maximum likelihood* analysis with bootstraps 1000 result shows that four samples are closely related to genus Poecilia of Poeciliidae family. Genetic distance and sequence divergence analysis was calculated using Kimura-2 parameter distance model. It shows that monophyly and intraspesific variation are ranged 0.55 % between sample 3 and *Poecilia mexicana* MX573, so we were able to identify the third sample as *Poecilia mexicana*. Other three samples are members of genus Poecilia, yet, we are unable to identify up to the species level. Further research is needed to determine their exact taxonomic position.

Keywords: identification, Poeciliidae family, morphological characteristics, *DNA Barcode*, *Cytochrome-c Oxidase Sub Unit I (COI)*

INTRODUCTION

Fresh water fishes includes the order of Cyprinodontiformes, among them is Poeciliidae family. The Poeciliidae family consists of fishes which are widely distributed throughout the world. The fertilization of this family is internal. Transfer of sperm into female reproduction tract is accomplished by a gonopodium, a generally highly modified anal fin. Members of this family can be used as an object or a model for biological studies, such as ecology, embryology, morphology, physiology, and behavioral study [1)]. Some members of the Poeciliidae family have the advantage of being able to control the spread of malaria by eliminating population of Anopheles larvae [2).] However, the systematic, diversity, and phylogenetic relationship between genus is still lacking of study [1).]

In our previous study, Poeciliidae family which lives in Sari Lake, locally named as Gatul fish, morphologically belongs to genus Xiphophorus. Furthermore, based on *16S mitochondrial ribosomal RNA* gene sequence, they are closely related to *Xiphophorus*

hellerii (XHU80047) [3]. This result still leaves a room for questions concerning the taxonomical position of this fish. Currently, *DNA Barcoding* gives an alternative to better identifications of unknown species in animal [4, 5, 6]

DNA Barcoding is a sequence of a short standardized region of DNA [5, 6]. *Barcoding* markers which are commonly used are *cytochrome-c oxidase* I (COI). As a *barcoding* marker, *COI* is a relatively conserved gene within fishes, and most of the above species low levels of intraspecies variation. *COI* have two important advantages, i.e.: (1) the universal primers for this gene are very robust, enabling recovery of its 5' end from representatives of most, if not all, animal phyla, (2) *COI* appears to possess a greater range of phylogenetic signal than any mitochondrial genes[7, 8]. It has 648 bp in length [9].

In this paper, we report our latest work. We identify Poeciliidae fishes living in Sari Lake Purwodadi based on morphological characteristics and *DNA Barcode* using *COI* gene. The morphological characters including morphometric, meristric, anatomic, pigmentation, and gonopodium structure had been analyzed.

MATERIAL AND METHODS

Observation of Morphological Characteristics

Morphological observations were done on 50 samples male and female from each type we found. Morphological characteristics observed were morphometric, meristric, anatomic, pigmentation, and gonopodium structure as well. We did very careful observation on this structure is one of important key to identify in this genus and species level.

DNA Barcoding analysis

DNA Isolation

Total cellular DNA was extracted from the pectoral fin of each sample using DNA Isolation Kit (*(NucleoSpin® Tissue, Macherey-Nagel, Germany*) with several modification. The DNA concentration and purity were assessed by spectrophotometry using *Nanodrop 2000*.

Amplification of *COI* gene region

The amplification of the *COI* gene region from mitochondrial genome was performed using universal primers [4,5,6] *COI Barcoding Forward* 5'-GGTCAACAAATCATAAAGATATTG-3' and *COI Barcoding Reverse* 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'. The amplified DNA was check in 1 % agarose gel electrophoresis. PCR products were then sequenced using the Big Dye Terminator ABI 3130 XL in Eijkman Gene Institution, Jakarta.

Data Analysis

Barcode COI sequence analysis was done using a number of software which is: DNA Baser to form *consensus sequence*; BLAST to determine the conformity of target gene with the *Query*; and Clustal-X to form *multiple alignments*. Aligned sequences were analyzed by the *maximum parsimony* (MP; *Close Neighbor Interchange on random tree* with use *Random addition sequence* added 10 repetition), *maximum likelihood* (ML; setting the *Hasegawa Kishino Yano* model with a gamma distribution of substitution rate among different nucleotide sites), *neighbor joining* (NJ) dan *minimum evolution* (ME) using the Kimura 2 parameter. MP, ML, NJ, and ME analyses were performed using *MEGA5*. Genetic distance and sequence divergence analysis was calculated using Kimura-2 parameter distance model. The internal stability of the inferred methods was measured by bootstrap using 1000 replications.

RESULTS AND DISCUSSION

Morphological Characterisics

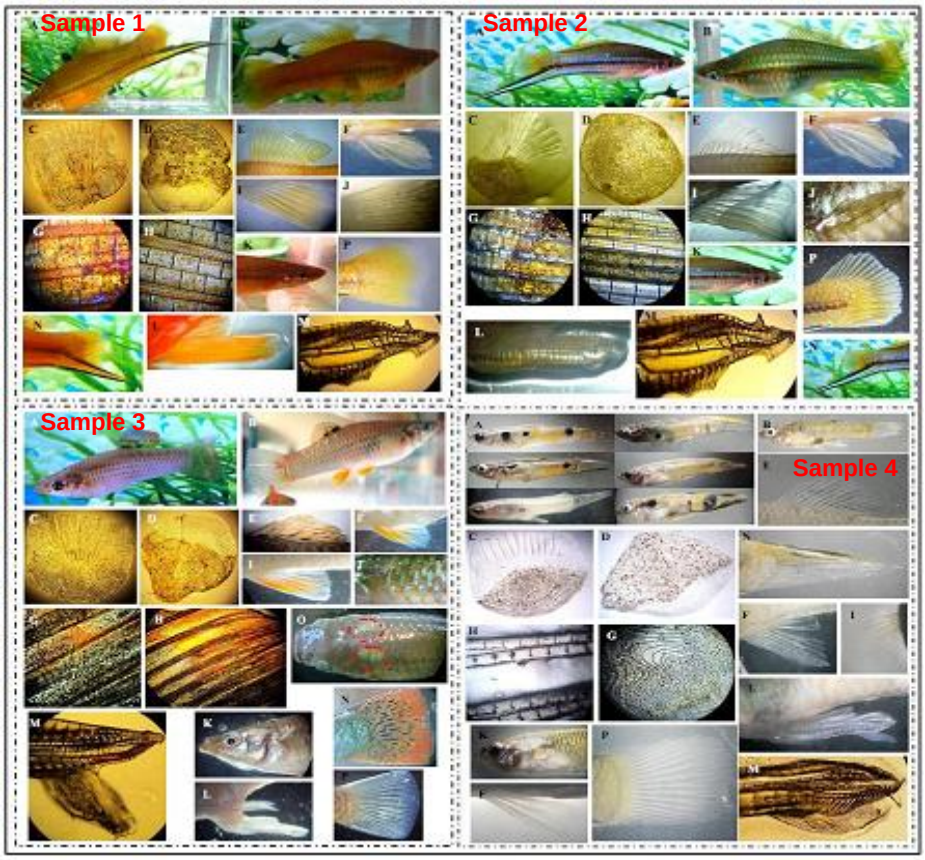

Fig.1. Morphological characteristics. A) male, B) female, C) abdomen scale, D) head scale, E) dorsal fin, F) ventral fin, G) pigmentation of abdomen, H) pigmentation of fin, I) anal fin (female), J) pectoral fin, K) type of mouth, L) complete gonopodium (male), M) structure of gonopodium, N) male caudal fin, O) smear of head, P) female caudal fin

A specific characteristic of the Poeciliidae family is its gonopodium structure which is a modification of 3rd, 4th, and 5th rays of the male's anal fin. Sample 1 and 2 (Fig 2.A & B), possess large claw on the terminus of ray 5 (orange arrow), hook is small crescent-moon shaped on 3rd ray (green arrow), and lacking of *gonopodium palp* (blue arrow). A compared sample 1 and 2, samples 3 and 4 possesses a *gonopodium palp (blue arrow)*. Sample 3 (Fig 2.C) possesses *distal platform* with 11 *retrosse serrae* (purple arrow), membranous hook (red arrow), and membranous tissue on the 3rd ray around distal tip is well developed (light green arrow), whilst sample 4 (Fig 2.D) has a long-pointed comb-like spines on ray 3rd (white arrow), and has a small hook on the tip of 5p ray (pink arrow).

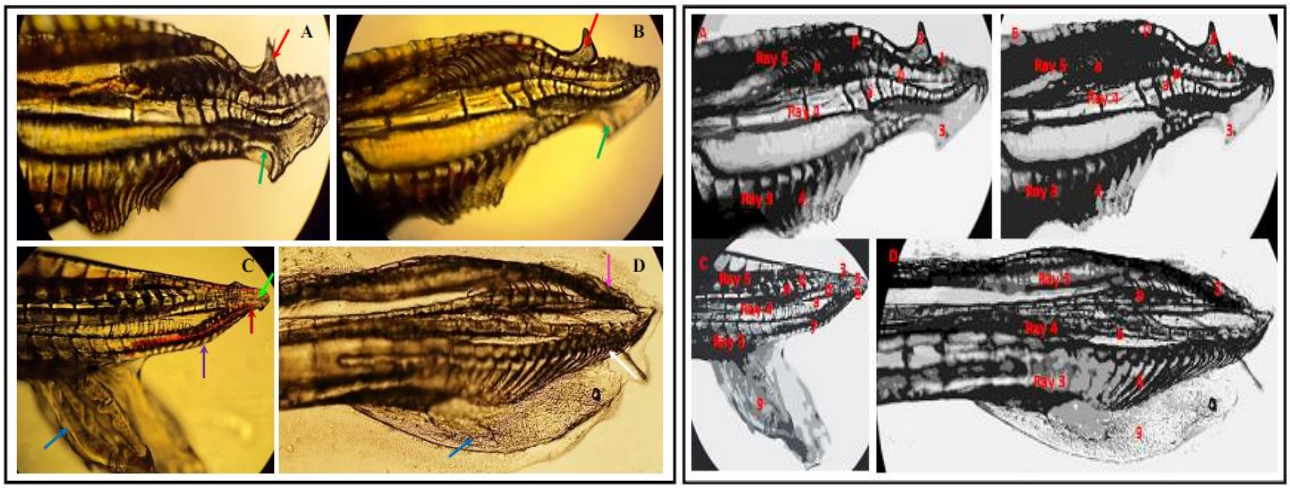

Fig 2. Gonopodium structure and Sketch. A) Sample 1, B)Sample 2, C) Sample 3, dan D) Sample 4. Description: 1: *distal serrae*, 2: claw, 3: hook, 4: comb, 5: membranous tissue, 6: *blade*, 7: *retrose serrae*, 8: membranous hook, 9: *gonopodium palp*, 10: *cephalic ramus*, 11: *distal platform*

Identification Key

1. a. Membranous hook and membranous tissue on the 3rd……..*Poecilia mexicana*
   b. Do not have membranous hook and membranous tissue on the 3rd ray……..2
2. a. Possess large claw on the terminus of ray 5th, hook is small crescent-moon shaped on 3rd ray, and lacking of *gonopodium palp*……..……..*Xiphophorus hellerii*
   b. Do not have claw on the tip of the 5a ray segment, have a small hook on the tip of the 5p ray and has *gonopodium palp*.....*Poecilia reticulata*

Based on those morphological characters, we propose to classify these samples in the hierarcy as follow: Kingdom Animalia, Phylum Chordata, Class Actinopterygii, Ordo Cyprinodontiformes, Family Poeciliidae, sub family Poeciliinae [1,10]. Sample 1 belongs to genus Xiphophorus, species *Xiphophorus hellerii* Heckel; sample 2 belongs to genus Xiphophorus, species *Xiphophorus hellerii* Heckel; sample 3 belongs to genus Poecilia, species *Poecilia mexicana* Steindachner; and sample 4 belongs to genus Poecilia, species *Poecilia reticulata* Peter.

Genetic Analysis Using *DNA Barcoding*

We successfully amplified 605 bp *COI* fragments from all samples. Gene sequence from each sample shows a high conformity (87-99%) with *COI* gene of *Poecilia mexicana* MX573 (EU751930.1). It can be concluded that the fragments we amplified belongs to *COI* gene. The sequences were then aligned using CLUSTAL-X program. There are transition and transversion in some bases. The alignments used to construct phylogenetic trees.

Pair wise sequence divergence sample 1 and *Poecilia mexicana* MX 573 has 16.13 %, sample 1 and Xiphophorus group has 23.57 %, sample 1 and *Poecilia reticulata* group has 5.51 % (black arrow); sample 2 and *Poecilia mexicana* MX 573 has 16.54 %, sample 2 and Xiphophorus group has 24.52 %, sample 1 and *Poecilia reticulata* group has 5.51 %; sample 3 and *Poecilia mexicana* MX 573 has 0.55 %, sample 3 and Xiphophorus group has between 19.74 % to 22.76 % (SD=1.51%), sample 3 and *Poecilia reticulata* group has 16.99 %; and sample 4 and *Poecilia mexicana* MX 573 has 16.13 %, sample 4 and Xiphophorus group has 23.57 %, sample 4 and *Poecilia reticulata* group has 5.51 % divergence sequence.

Table 1. Levels of nucleotide divergence within and between four samples with *data base.* The estimates were based on Kimura-2 Parameters

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. G.sexadata ECO-CH-P5474D																							
2. G.yucatana ECO-CH-P5474A	0.1310																						
3. P.reticulata LBP-29428	0.2287	0.2404																					
4. P.reticulata LBP-29431	0.2287	0.2404	0.0000																				
5. P.reticulata LBP-294290	0.2287	0.2404	0.0000	0.0000																			
6. P.reticulata LBP-29430	0.2287	0.2404	0.0000	0.0000	0.0000																		
7. Jenis 1	0.2224	0.2398	0.0551	0.0551	0.0551	0.0551																	
8. Jenis 4	0.2224	0.2398	0.0551	0.0551	0.0551	0.0551	0.0000																
9. Jenis 2	0.2304	0.2446	0.0655	0.0655	0.0655	0.0655	0.0110	0.0110															
10. P.mexicana MX011	0.2087	0.2230	0.1668	0.1668	0.1668	0.1668	0.1583	0.1583	0.1625														
11. P.mexicana MX015	0.2087	0.2230	0.1668	0.1668	0.1668	0.1668	0.1583	0.1583	0.1625	0.0000													
12. P.mexicana ECO-CH-P5475A	0.2087	0.2230	0.1668	0.1668	0.1668	0.1668	0.1583	0.1583	0.1625	0.0000	0.0000												
13. P.mexicana MX594	0.2087	0.2230	0.1668	0.1668	0.1668	0.1668	0.1583	0.1583	0.1625	0.0000	0.0000	0.0000											
14. P.mexicana ECO-CH-P5475B	0.2119	0.2264	0.1699	0.1699	0.1699	0.1699	0.1613	0.1613	0.1654	0.0055	0.0055	0.0055	0.0055										
15. P.mexicana ECO-CH-P5475C	0.2119	0.2264	0.1699	0.1699	0.1699	0.1699	0.1613	0.1613	0.1654	0.0055	0.0055	0.0055	0.0055	0.0000									
16. P.mexicana ECO-CH-P5475D	0.2119	0.2264	0.1699	0.1699	0.1699	0.1699	0.1613	0.1613	0.1654	0.0055	0.0055	0.0055	0.0055	0.0000	0.0000								
17. P.mexicana MX573	0.2087	0.2230	0.1699	0.1699	0.1699	0.1699	0.1613	0.1613	0.1654	0.0018	0.0018	0.0018	0.0018	0.0036	0.0036	0.0036							
18. Jenis 3	0.2023	0.2162	0.1699	0.1699	0.1699	0.1699	0.1613	0.1613	0.1654	0.0073	0.0073	0.0073	0.0073	0.0092	0.0092	0.0092	0.0055						
19. H.bimaculata MX568	0.2133	0.2379	0.2068	0.2068	0.2068	0.2068	0.1898	0.1898	0.1919	0.1725	0.1725	0.1725	0.1725	0.1787	0.1787	0.1787	0.1756	0.1725					
20. H.bimaculata MX571	0.2167	0.2343	0.2103	0.2103	0.2103	0.2103	0.1930	0.1930	0.1951	0.1756	0.1756	0.1756	0.1756	0.1818	0.1818	0.1818	0.1787	0.1756	0.0018				
21. X.helleri MX562	0.2381	0.2172	0.2017	0.2017	0.2017	0.2017	0.2357	0.2357	0.2452	0.2130	0.2130	0.2130	0.2130	0.2200	0.2200	0.2200	0.2165	0.2200	0.1731	0.1763			
22. X.helleri MX566	0.2381	0.2172	0.2017	0.2017	0.2017	0.2017	0.2357	0.2357	0.2452	0.2130	0.2130	0.2130	0.2130	0.2200	0.2200	0.2200	0.2165	0.2200	0.1731	0.1763	0.0000		
23. X.helleri MX564	0.2381	0.2172	0.2017	0.2017	0.2017	0.2017	0.2357	0.2357	0.2452	0.2130	0.2130	0.2130	0.2130	0.2200	0.2200	0.2200	0.2165	0.2200	0.1731	0.1763	0.0000	0.0000	
24. X.alvarenzi MX623	0.2269	0.2068	0.2250	0.2250	0.2250	0.2250	0.2535	0.2535	0.2634	0.2228	0.2228	0.2228	0.2228	0.2228	0.2228	0.2228	0.2193	0.2228	0.1955	0.1989	0.0205	0.0205	0.02
25. X.maculatus ECO-CH-P5545B	0.2265	0.2127	0.2072	0.2072	0.2072	0.2072	0.2285	0.2285	0.2365	0.1898	0.1898	0.1898	0.1898	0.1898	0.1898	0.1898	0.1866	0.1898	0.1668	0.1699	0.0683	0.0683	0.06
26. X.maculatus MX628	0.2230	0.2094	0.2038	0.2038	0.2038	0.2038	0.2250	0.2250	0.2329	0.1866	0.1866	0.1866	0.1866	0.1866	0.1866	0.1866	0.1834	0.1866	0.1637	0.1668	0.0660	0.0660	0.06
27. P.catemaco ECO-CH-P5482D	0.2610	0.2554	0.2825	0.2825	0.2825	0.2825	0.2736	0.2736	0.2798	0.2633	0.2633	0.2633	0.2633	0.2672	0.2672	0.2672	0.2633	0.2595	0.2386	0.2350	0.2438	0.2438	0.24
28. P.catemaco ECO-CH-P5482C	0.2610	0.2554	0.2866	0.2866	0.2866	0.2866	0.2776	0.2776	0.2839	0.2672	0.2672	0.2672	0.2672	0.2711	0.2711	0.2711	0.2672	0.2633	0.2423	0.2386	0.2475	0.2475	0.24

Calculation of genetic distance and sequence divergence using Kimura-2 parameter distance model showed that sample 3 is intraspecies (sequence divergence < 3 %) in *Poecilia mexicana*. Sample 1, 2, and 4 have sequence divergence greater than 3 % [4)]. It means that those samples in different species from gene reference (intraspecies) [4)].

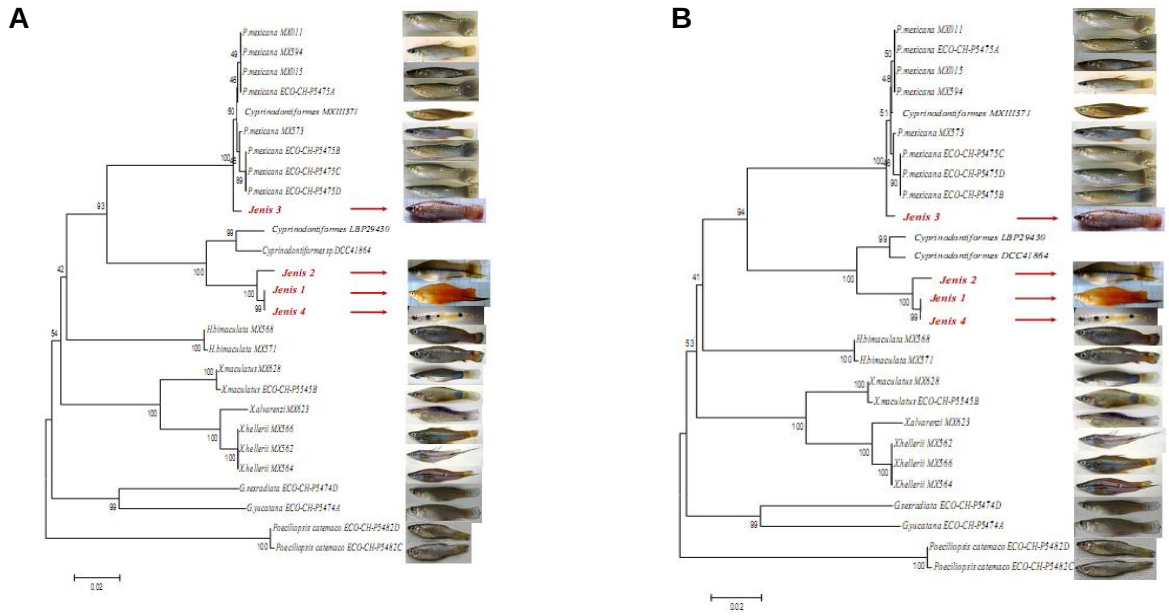

Fig. 3. Phylogenetic trees built with the *Neighbor Joining* (A) and *Minimum Evolution* (B) methods based on sequenced COI gene, *Consistency index* (CI) = 0,589977, *Retention Index (*RI) = 0,842244. Numbers at nodes represent percentage recovery in bootstrap analysis (1000 replicates)

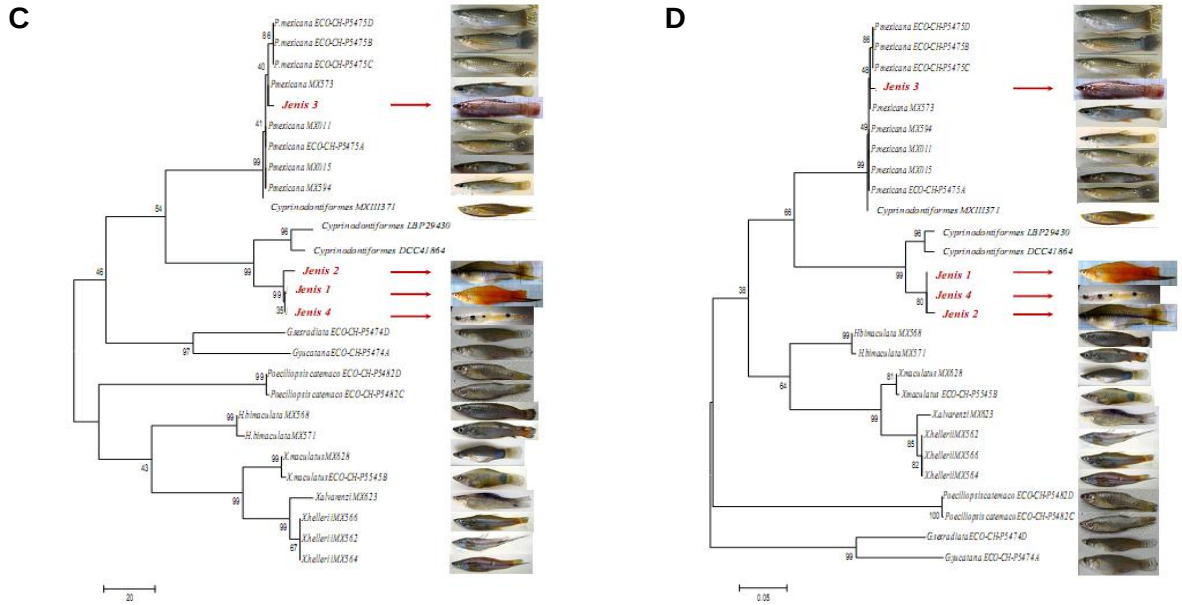

Fig 4. Phylogenetic trees built with the *Maximum Parsimony* (C) and *Maximum Likelihood* (D) method based on COI gene fragments. The ML analysis using HKY model. The estimated parameters for likelihood analysis: -Ln L = -2514,29.

Topology tree of these samples were constructed using *Maximum Parsimony, Maximum Likelihood, Neighbor Joining and Minimum Evolution* (Fig. 3 & Fig.4) gave the same results, yet different only in bootstraps support value. Sample 3 is closely related with *Poecilia mexicana* group, and stand in different group with Xiphophorus, Heterandria, Gambusia, and Poeciliopsis genus; whilst the construction using *Maximum Likelihood* showed that sample 3 is in one clade (monofiletic) with *Poecilia mexicana* MX573 followed

by bootstraps value 48. The taxonomic position of sample 1, 2, and 4 showed the same topology tree based on those four methods. Three samples (sample 1, 2, and 4) are closely related to Poecilia and stand in different group with Xiphophorus. It showed that the four samples are closely related to genus Poecilia.

Based on morphology and *DNA barcode*, sample 3 and 4 could be classified into genus Poecilia. There is a controversial result in this study. Taxonomic position of sample 1 and 2 is closely related to Poecilia based on *DNA barcode* instead of Xiphophorus, whilst morphological characteristics showed the resemblance to *Xiphophorus hellerii*. This difference may be caused by incompleteness of the *COI* barcode sequence obtained, especially for 5' end. Members of Poeciliidae family originally endemic in Central & South America[1, 2)] which were introduced to Indonesian archipelago a long time ago. Adaptation towards the Indonesian water environment might have caused a different phenotypic expression on certain gene. Further research is needed to determine their exact taxonomic position for these samples.

CONCLUSSION

Based on morphological characteristics, we found three species; those are *Xiphophorus hellerii* Heckel; *Poecilia mexicana* Steindachner, and *Poecilia reticulata* Peter. *DNA barcode* using *COI* gene showed sample 3 is *Poecilia mexicana*, while sample 1, 2, and 4 belongs to genus Poecilia. Construction of phylogenetic trees based on *barcode COI* gene with *Neighbor Joining*, *Minimum Evolution*, *Maximum Parsimony* and *Maximum Likelihood* showed that the four samples were closely related to genus Poecilia rather that other genus from Poeciliidae family.

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O-SE06

Molecular evolution and taxonomy of the Northern Australian and New Guinean Species of Freshwater Crayfish of the Genus *Cherax* Erichson (Decapoda: Parastacidae)

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Abstract

Systematic information on freshwater crayfish of the Genus *Cherax* from northern group (Papua-Indonesia, northern Australia and Papua New Guinea) is still limited. This genus represent the only group of crayfish in the region. The principal aim of this project is to place these poorly known species of the Genus *Cherax* into a taxonomic and evolutionary framework using molecular genetic data. This will not only greatly extend out understanding of the biodiversity of this group but also provide a basis for comparison with the better studied species of southern and eastern Australia the biogeography of the region. We use DNA nucleotide data from mitochondrial genes (16S, 12S and COI) and nuclear gene (GAPDH) to study the taxonomy and evolution of northern Australian and southern New Guinean species of *Cherax*. We also investigated the relationship of these species to *Cherax* species from eastern and southern Australia using GenBank and unpublished sequences. Preliminary results from 16S rDNA sequence data set show that all northern Australian and New Guinean crayfish form a monophyletic group with the exception of *C. wasselli* from North Queensland. Freshwater crayfish species from the Wissel Lakes, a biodiversity "hotspot" for *Cherax,* formed a monophyletic group supporting a single evolutionary origin for this morphologically diverse assemblage. The phylogenetic analysis supports the presence of cryptic species in other parts of Papua and that New Guinean and Northern Australian species are interrelated consistent with the geological history of the region.

Keywords: *Cherax*, systematic, DNA, Northern Australia, Papua – Indonesia

O-SE07

The Identification and Isolation of Microalgae Planktonic from Selorejo Dam in Kabupaten Malang, East Java

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Abstract: Selorejo Dam is a kind of freshwater dam located in Kabupaten Malang. Its water has verdure color that indicates the existence of microalgae. Microalgae has many advantages in food, industry or medicinal, such as *Chlorella* as a single cell (PST). The utilization of microalgae can be done through some stages, first identification and isolation of microalgae. This study aims for knowing microalgae species that are successfully identified and isolated. It is expected can give information to the society concerning microalgae potential and microalgae species that are successfully identified and isolated from Selorejo Dam. Microalgae that is utilized in this study is planktonic microalgae that is taken from plankton net of 200 mesh in Selorejo Dam, Kabupaten Malang. The media used to isolate is Walne. The method used to isolate microalgae is scratch isolation. This study is descriptive explorative study. The data concerning microalgae that are found and isolated are analyzed descriptively and are made as identification key. *Planktonic microalgae that are successfully identified consist of 3 divisions (Chlorophyta, Chrysophyta dan Cyanophyta), 15 families, 23 genera and 24 species that are different. Planktonic microalgae that are successfully isolated consist of 3 divisions, 8 families, 9 genera and 10 species that are different.*

Keywords: Identification, Isolation, Microalgae Planktonic, Selorejo Dam

INTRODUCTION

Dam is an example of artificial freshwater made its way to stem a particular river (Apridayanti, 2008). Dam waters have natural resources that are very high of microalgae. Selorejo dam in Kabupaten Malang, has an area of approximately 640 hectares. River water is dammed at the dam containing organic and inorganic materials that can fertilize the waters of the dam (Apridayanti, 2008). In the waters there are bodies of living organisms, one of them is the microalgae, kind of micro-organisms that float in water. Water in Selorejo dam have a greenish tint which indicates microalgae. Microalgae in the dam has a role as a natural food of fish that live in it.

According Freaky (2010) there are so many advantages of microalgae, good advantages to other organisms, ecosystems, and humans. It contain many nutrients that a lot of microalgae are used as food supplements and medicines. One of the microalgae that has many advantages is Chlorella as a source of highly nutritious food supplements or used for PST (single cell protein).

Utilization of microalgae requires several stages. The first steps are the identification and isolation of microalgae, and then as the second step do the breeding (cultivation) for 7-

10 days and after that can be done the harvesting (Freaky, 2010). Microalgae from dam waters identified in advance to determine the species that have the potential to be exploited. This research of identification and isolation of microalgae is an early stage prior to the utilization of microalgae in the Selorejo dam. The purpose of this research is to determine which species of planktonic microalgae that has been identified and isolated from Selorejo dam Kabupaten Malang that can be utilize.

MATERIALS AND METHODS

Tools used in this study were; petri dish, autoclave, stoves, scales, light microscope, micrometer, pipette, test tubes, cotton, paper, aluminum foil, glass beaker, bucket, net plankton, bunsen, micropipette, flakon, sprayer, needle inoculation , glass objects, glass lid and erlenmeyer flask. Materials used in this study were water samples taken in the Selorejo dam and formalin (5%) as a preservative of samples to be identified. Medium for culturing microalgae is Walne medium. The materials to make the media are NaNO3, H3BO3, Na2EDTA, NaH2PO4.6H2O, FeCl3.6H2O, MnCl2.4H2O, ZnCL2, CoCl2.6H2O, (NH4) 6Mo7O24.4H2O, CuSO4.5H2O, vitamins B1 and B12, and aquades (Isnansetyo & Kurniastuty, 1995:107-108).

The study began with the sampling done using plankton nets. Sampling was carried out 2 times for identification and for isolation. Sampling for identification is done by using plankton net that has been sprayed by sprayer and added 3 drops of formalin (5%), while samples for isolation without the addition of formalin.

Samples were taken from the dam and then observed by light microscopy and the record of the characteristics of microalgae are obtained. Identify the characteristics of microalgae obtained clarified by using the book The Freshwater Algae of the United States written by Gilbert M. Smith (1950), the book How To Know The Freshwater Algae essay GW Prescott (1978) and the book Freshwater Algae Identification and Use as Bioindicators written by Edward G. Bellinger & David C. Sigee (year 2010).

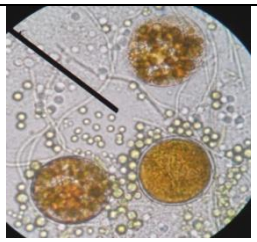
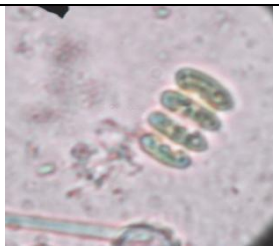
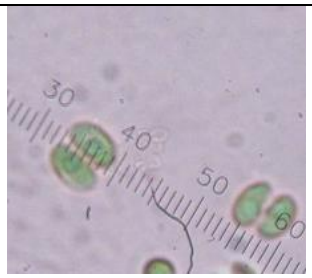
Isolation phase begins with the manufacture of medium walne and inoculation microalgae by the following ways: 1) drop 1 ml of sample water on the medium plate and flattening it 2) observe the grown microalgae in the petri dish, 3) culturing microalgae that observed at medium oblique to purposes of pure cultures by scraping microalgae using aseptic inoculation needle, 4) perform subcultures formed repeatedly until pure isolated.

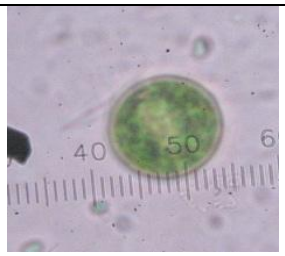
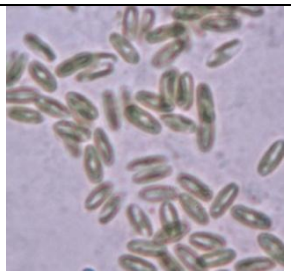
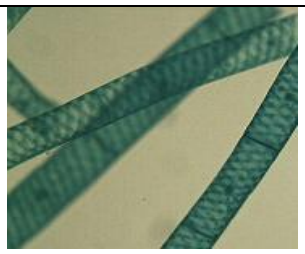
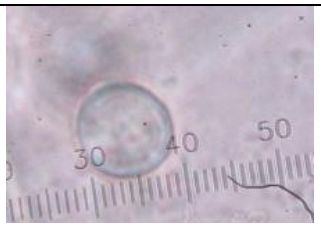
RESULTS AND DISCUSSION

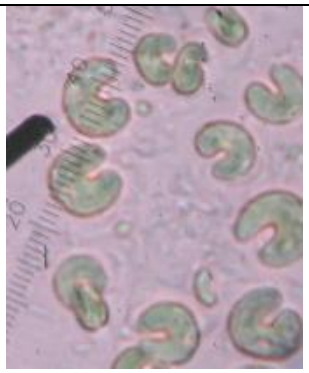
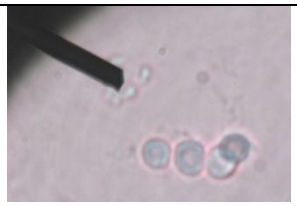
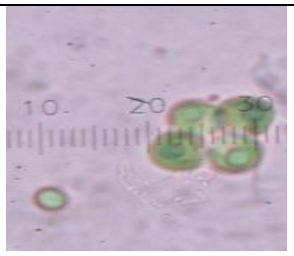
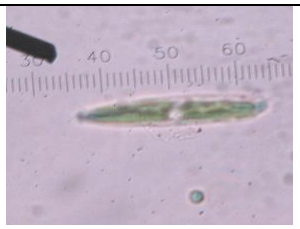
Based on the research has been conducted, the obtained microalgae have a variety of colors, shapes and sizes. Identification of the type based on some of the major characters

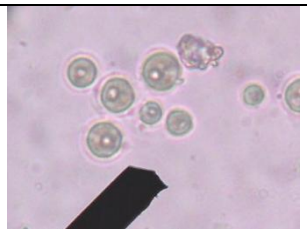
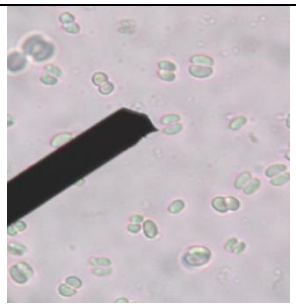
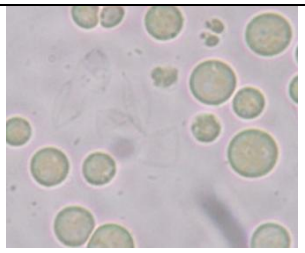
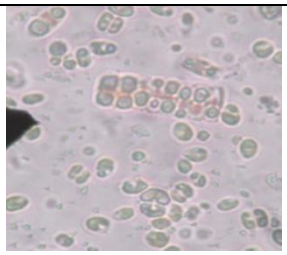
in microalgae. Planktonic microalgae found in Selorejo dam and identified as many as 24 species.

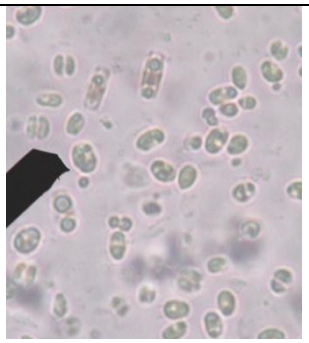
Table 1. Species and Characteristics of Planktonic Microalgae Identified in Selorejo dam Kabupaten Malang

No	Nama spesies	Characteristic	Photo
1	*Botrydiopsis arhiza*	a solitary cell, golden brown color, round shape, covered with gelatin, do not have a flagellum, have many nuclei (multinucleate), has 2 chromatophore, the cell size of 51 µm	
2	*Navicula sp.*	Solitary cells, brown, fusiform shape, symmetrical, not flagell, has 2 chromatophore, cell size 22 x 5 µm	
3	*Scenedesmus bijuga*	colony cells senobium, consisting of four cells composed of linear and not curved, green color, oval shape, covered with gelatin, do not have a spine, have chloroplasts with a pirenoid, uninukleat, cell size 20 x 10 µm.	
4	*Palmella miniata*	Colony cells, consisting of two cells, green color, oval shape, covered with gelatin, colony aggregate palmeloid, chloroplast shape bowl with 1 pirenoid, cell size of 8 x 5 µm.	
5	*Scenedesmus quadricauda*	Colony senobium cells, consisting of 4 cells are composed of linear, green color, oval shape, covered with gelatin, has 4 spines in each end, has a chloroplast with a pirenoid, uninukleat, cell size 21 x 12 µm.	

6	*Chlorococcum sp.*	Solitary cells, green, round shape, not flagel, has a chloroplast with a cell edge pirenoid, chloroplasts bowl shape, cell size 50-10 µm	
7	*Selenastrum gracile*	Colony cells, green, crescent cell shape with pointed tip, not covered with gelatin, chloroplasts with a pirenoid cell, the cell size of 12 x 2 µm.	
8	*Dacthylothece confluens*	Colony, dark green, oval shape, not flagel, clothed gelatin, chloroplasts 1 in the parietal, cell size 7 x 3 µm.	
9	*Spirogyra sp.*	Colony cells, filament shape, color green, spiral chloroplasts, there are several pirenoid, cell size 15 µm	
10	*Gyrosigma sp.*	Solitary cells, brownish, fusiform shape asymmetric, a two chromatophore with some pirenoid, cell size 37 x 5 µm	
11	*Pluto caldarius*	solitary cell, the color blue, round cell, not covered with gelatin, do not have a flagellum, the cell size of 8-12 µm	

12	*Kirchneriella obese*	Colony cells, green, curved in a crescent shape and blunt tip, covered with gelatin, has a chloroplast with a pirenoid, cell size 10 x 2 µm.	
13	*Closterium sp.*	Solitary cells, green, elongated cell shape with an enlarged middle section, not covered with gelatin, has two chloroplasts, cell size 15 x 3 µm	
14	*Gleocapsa magma*	Colony aggregate cells, round cell shape, bluish green color, not flagel, clothed gelatin, cell size 11 x 4 µm	
15	*Pseudotetraspora gainii*	Colony cells, round shape, green color, not berflagel, not covered with gelatin, has a chloroplast with a pirenoid, cell size 12 µm	
16	*Netrium digitus*	Solitary cell, fusiform shape, color green, covered with gelatin, chloroplasts have 4, each cell has a pirenoid, cell size 20 x 7 µm	
17	*Ourococcus bicaudatus*	Solitary cell, green, fusiform shape, not flagell, has a chloroplast with a pirenoid, cell size 15 x 5 µm	

18	*Westella botryoides*	Colony, round cell shape, the color is green, not flagell, clothed gelatin, does not have pirenoid, chloroplasts in the form of bowls or fulfill the parietal cells, cell size 11 µm	
19	*Chroococcus sp.*	Colony cell, bluish green color, not berflagel, clothed gelatin, oval shape cells, each colony consists of two cells, cell size 10 µm	
20	*Chlorella sp.*	Solitary cells, green color, round shape, chloroplast bowl shape, has no pirenoid, cell size 10 µm	
21	*Terpsinoe Americana*	Colony cells, brownish color, with a quadrangular form septa, not berflagel, cell size 16 x 7 µm	
22	*Borzia trilocularis*	Colony cells, bluish color, oval shape, not berflagel, colonies consisting of 3 cells, cell size 18 x 8 µm.	
23	*Cymbella cistula*	fusiform cells form asymmetric, brownish, solitary cells, no berflagel, has a chromatophore, clothed gelatin, cell size 43 x 15 µm	

24	*Nannochloris bacillaris*	solitary cell, an oval, green, not covered with gelatin, chloroplasts near the poles, does not have pirenoid, cell size of 8 x 2.5 µm	

Identification key to 24 species

1. a. cell eukaryotic……..……2
   b. cell prokaryotic……21
2. a. green ……....3
   b. golden brown ……...17
3. a. solitary cell ……....4
   b. colony cell …..……..9
4. a. non-fusiform shaped cells…….. 5
   b. fusiform shaped cells ……..7
5. a. round cells ……...……...6
   b. cylindrical cells ... ……..……*Nannochloris bacillaris*
6. a. has pirenoid ……*Chlorococcum sp.*
   b. without pirenoid .....*Chlorella sp.*
7. a. chloroplasts only 1 .. ……*Ourococcus bicaudatus*
   b. chloroplasts more than 1 ……8
8. a. has two chloroplasts at cell .... ……*Closterium sp.*
   b. has 4 on his cell chloroplasts ……*Netrium digitus*
9. a. colony filaments ……*Spirogyra* sp.
   b. colony non-filament…… 10
10. a. colony senobium ……...11
    b. colony aggregate ……..12
11. a. there are 4 spina ……. ……*Scenedesmus quadricauda*
    b. there are no spina ……*Scenedesmus bijuga*
12. a. forms of sickle cell ……...13
    b. spherical cell shape ……14
13. a. has a gelatinous sheath ..... *Kirchneriella obesa*
    b. has not gelatin sheaths ……*Selenastrum gracile*

14. a. chloroplast form of bowl ……15
    b. chloroplasts do not form of bowl ……16
15. a. boundaries of individual cells clearly …….. *Westella botryoides*
    b. Individual cell boundaries are not clearly ..... *Palmella miniata*
16. a. colony consists of 4 cells ... .. ……..*Pseudotetraspora gainii*
    b. colony consists of 2 cells .....……..*Dacthylothece confluens*
17. a. globose-shaped cells ... ……*Botrydiopsis arhiza*
    b. non globose-shaped cells ……..18
18. a. cell shape quadrangular ……*Terpsinoe americana*
    b. spindle cell shape ……19
19. a. are symmetrical ..... …….....*Navicula* sp.
    b. asymmetrical …….20
20. a. have a chromatophore ..... *Cymbella cistula*
    b. has 2 chromatophore ..... *Gyrosigma* sp.
21. a. multicellular…….22
    b. unicellular ..... *Pluto caldarius*
22. a. irregular shape …….. *Gleocapsa magma*
    b. subglobose to elliptical shape ……23
23. a. each colony consists of two cells ... . *Chroococcus* sp.
    b. each colony consisting of 3-8 cells ……..*Borzia trilocularis*

The second stage after the identification of microalgae is isolation. Isolation is an activity to obtain isolated microalgae. Isolation of microalgae done using medium plate and medium tilt. There are 10 species that can grow on medium plate Walne is *Pluto caldarius*, *Chlorococcum* sp., *Chlorella* sp., *Nannochloris bacillaris*, *Navicula* sp., *Scenedesmus quadricauda*, *Scenedesmus bijuga*, *Westella botryoides*, *Dacthylothece confluens* and *Chroococcus* sp. Not all species of microalgae that are identified can be isolated. This is because tolerance and nutritional needs and environmental conditions needed microalgae different between species microalgae.

Andersen (2005:84) explains that diatoms require silica, euglenoid requires ammonia and some genera of microalgae requires selenium. The second stage in the isolation of microalgae is to remove contaminants. The last stage is necessary to subculture sustainable growth of microalgae. It is also necessary to prevent the death of species that are isolated if the culture medium to lose some elements or organic components.

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O-SE08

Study of Diversity and Association between Gastropods and Macroalgae in Pasir Panjang Coast, Sempu Island

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ABSTRACT

Sempu Island is a natural reservation area located southward of Java Island which has a complex terrestrial and aquatic ecosystem. Some aquatic areas on Sempu Island directly face to high wave Indian Ocean. It makes that areas develop a specific ecosystem which is adapted to that extreme condition. Gastropod is a group of Mollusca with highest diversity which has adapted to that condition. Each species of Gastropods in each habitat is known to be distributed according to the resource patterns in the environment, including the existence of macroalgae. In the type of aquatic area some macroalgae appear periodically. This condition builds a dynamic ecosystem. Our research aims to determine the diversity ofliving gastropods in Pasir Panjang coast Sempu Island and its asssociation with macroalgae. Data was obtained from six different habitats along Pasir Panjang coast, include: rocky plates with algae, rocky plates (without algae), rocks, cliffs, dead reefs, and corals. The diversity index of gastropod was analyzed by using ANOVA continued with LSD. To find out the association between macroalgae and gastropod, coefficient of association was calculated qualitatively. Totally 26 species of gastropods were found in these 6 areas. Rock plates with algae habitat has highest diversity of Gastropod, and highest coverage of macroalgae. On the other hand, Cliff area has the lowest diversity of gastropods and this area was not covered by macroalgae. There are 8 positive associations, 37 negative association, and 15 no association between gastropods and macroalgae in rock plates with algae area.

Keywords: diversity of gastropod, association, macroalgae

INTRODUCTION

Sempu Island is a natural reservation area located southward of Java Island. The area of Sempu Island is 877 hectares (1), but it has a complex terrestrial and aquatic ecosystem. Some aquatic areas on Sempu Island directly face to high wave Indian Ocean. It makes that areas develop a specific ecosystem which is adapted to this extreme condition. Several groups of animals have been adapted to this condition, such as echinoderms and gastropods.

Gastropod is a group of Mollusca with highest diversity. It has been estimated around 1500 species in Indonesian Archipelago. Gastropods have been well adapted in to this extreme condition, especially high waves with its ability to attach to rocks or other substrates which protect them from high wave and against predator (2). They exhibit a wide range of

shell shape, many of which are adjusted to provide some defense from high waves and against predator. Gastropods play important roles in recycling materials from in particular ecosystem. Quantitative studies have shown that some activities of the gastropod are imperative to smoothen the ecosystem dynamics(3).

Qualitative composition and availability of provided food and the nutritional needs of the gastropod affectits food selection (4). Each species of gastropods in each habitat is known to be distributed according to the biological, physical, and chemical factors, including the appearance of macroalgae. Some macroalgae appear periodically depend on the season in aquatic area build a dynamic ecosystem (5). Macroalgae have important roles either directly or indirectly to the life of some marine animal, including gastropods, such as for nursery, food and as spawning ground (6). As primary producers in aquatic ecosystems, it plays important rolesin matter cycle and energy flow. It also plays important roles in growth, reproductive capacities, and character of the population of aquatic organism (7). Based on those characteristics of gastropod, our research aims to determine the diversity of Gastropod in Pasir Panjang coast, Sempu Island and its association with macroalgae in the area with highest diversity.

MATERIALS AND METHODS

Collection and Identification of gastropod and macroalgae

This research had been done in Pasir Panjang Coast, Sempu Island, covering around 410 meters along the coast during the lowest tide on May 26th 2011. Samples were takenfrom six different habitats those are: rock plates with algae, rock plates without algae, rocky zones, cliffs, dead reefs, and corals (Fig. 1). Sampling was done using 1x1m^2 quadrat with four replications for each area.

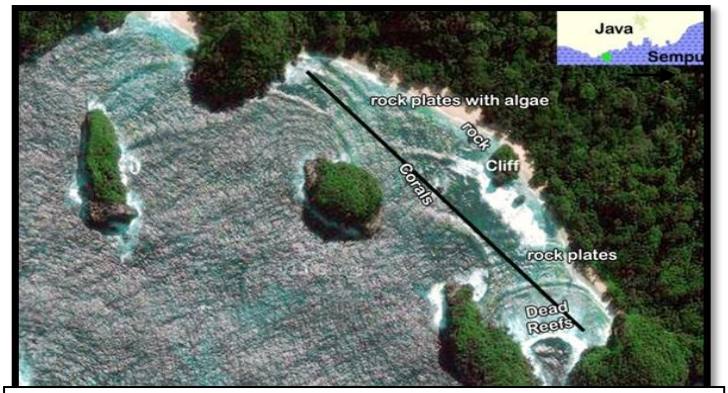

Fig. 1: Research Location. Black line depicts the target

Identification of gastropodswas done based on morphological characteristics according to Oliver (8), Powel (9), www.gastropods.com, and nudipixel.com for Sacoglosa.

Macroalgae samples were taken from each type of habitat and then dried for further identification based on morphological characteristic (www.iptek.net.id).

Data analysis

The data was analyzed using H' Shannon-Wienner to get the diversity of gastropod.Then it was analyzed by using ANOVA continued with LSD to compare the diversity index of gastropod from each habitat. To find out the association between macroalgae and gastropod, coefficient of association (V_{ab}) was calculated qualitatively according to Michael (10). The number of presence of species insert in the association table (Table 1) then continue to calculate the coefficient of association formula (V_{ab})

Table 1 Association Table

	Gastropod species	
Macroalgae species	Present	Absent
Present	a	b
Absent	c	b

$$Vab = \frac{ad - bc}{\sqrt{(a+b)(a+c)(c+d)(b+d)}}$$

RESULTS AND DISCUSSION

From six different habitats, we found 26 species of gastropods (Fig.2) with 19 species belong to subclass Prosobranchia, whereas 7 others belong to Opistobranchia. Among them *Conus striatus*, *Conus ebaus, Morula* spp1.,*Conus carnifonicus, Pirenidae, Conus* spp1., *Conus* spp2., *Conus lividus., Strigaltella litterata, Strigatella paupercula, Strigatella* spp. belong to order Neogastropoda; *Cyprea moneta, Cyprea caputserpentis*, *Littorina* spp, Litorinidae 1, Littorinidae 2, Littorinidae 3 belong to order Neotaeniglossa; *Pattela* sp., *Patella longicosta*, *Patella caerecula, Acmaea* sp belong to order Pattelogastropoda; *Turbo* sp. belongs to order Archaeogastropoda; *Nerita lineata* belong to order Neritopsina; and *Plakobranchus ocelatus* belongs to Sacoglosa.

Eight different species of macroalgae was found (Fig. 3). Most of them belong to Chlorophyta division, they are *Valonia* sp., *Halimeda* sp.,*Chaetomorpha* sp., *Cladopohora* sp. There also found *Amphiroa* sp., *Halimenia* sp, and *Galaxaura* sp which belong to Rodhopyta division, and *Dictyota* sp. which is belong to Phaeophyta division.

The diversity index of gastropod from six different habitats is significantly different; in more detail Rock plate with algae is different for other 5 habitats (table 1). Our data shows that rock plate with algae has the highest diversity level compared to others. Its diversity index categorizedas medium level diversity (1≤H'≤3).The development and growth of

gastropods are influenced by the environment where they live. Thus, our result show that Rock Plate with algae has the highest carrying capacity compared to those other five (11)

Table 1 Result of LSD

Habitat	Score	Notation
Rock plate with algae	1,383	a
Rock plate	0,75575	b
Rocky	0,7065	b
Coral	0,67825	b
Dead Reef	0,5095	b
Cliff	0,41725	b

Fig. 2 Gastropod in six variation of habitat

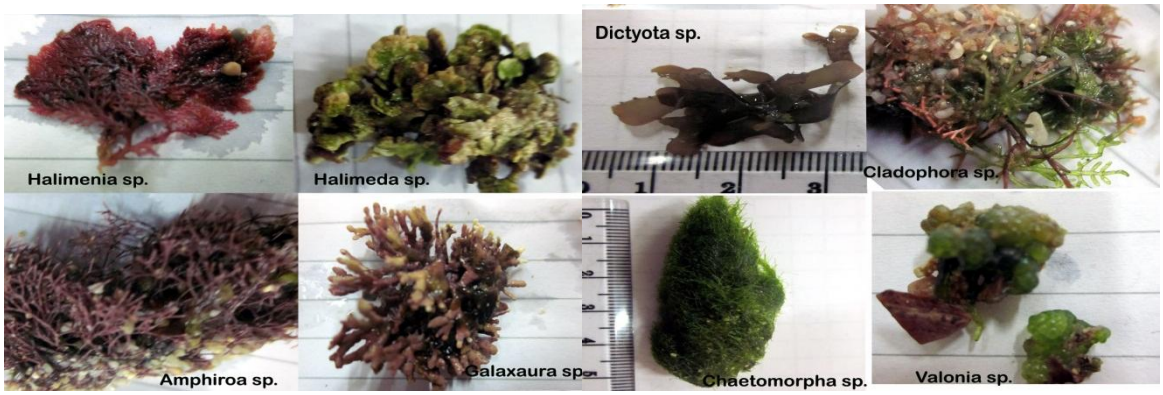

Fig. 3 Macroalgae in six variation of habitat

Rock plates with algae have the largest coverage of macroalgae compared to other habitats. In contrast with rock plates with algae, Cliff area which was not found macroalgae has the lowest diversity (Fig. 3). This condition supports the assumption that the life of gastropod affected by the availability of Plant (11). Quantitative study showed that macroalgae gave a great contribution for gastropod biomass than other food resources (5)

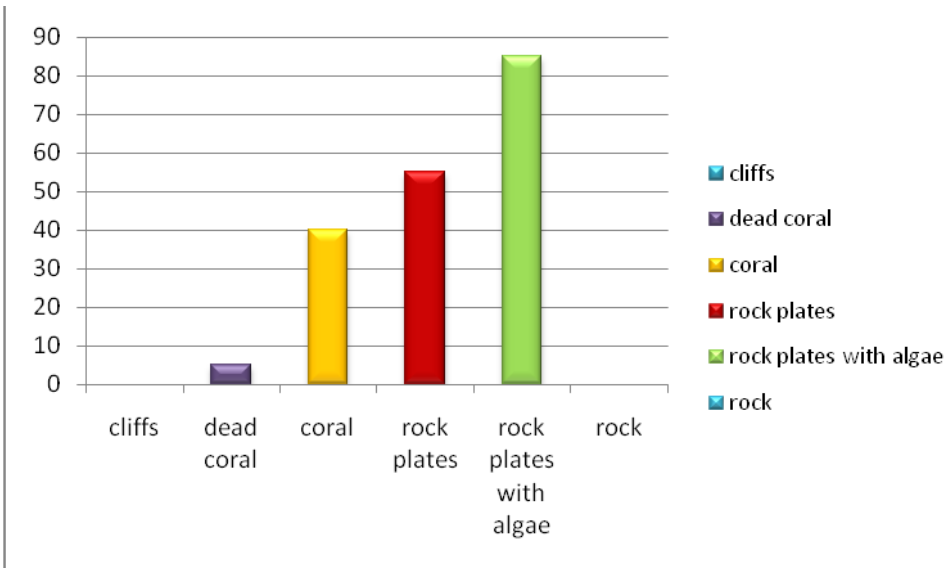

Fig. 3 Coverage of Macroalgae

In this paper we focus our analysis on the association between gastropod and macroalgae found in rock plate with algae area, since this area shows the highest diversity. From the association coefficient between Gastropod and Macroalgae in rock plate with algae (Table 3), we found three kinds of association, those are: negative, positive and no association. Some gastropods which have positive association with one or more macroalgae, they are, *Tronchus intextus, Conus striatus, Conus ebaus, Cabestana sp,* and *Strigatella* sp. The others gastropods which have negative association with all kind macroalgae in that area are *Littorina* sp, *Plakobranchus ocellatus, Conus californicus,*

Pirenidae, *Mitra* spp, *Haminoea* sp, and *Marginella gabrielle*. While *Cyprea* moneta and *Morula* sp. have both negative and no association with macroalgae in that area.

Positive association between gastropod and macroalgae might occur because some macroalgae have to induce settlement and metamorphosis of invertebrate's marine larvae (12). On the other hand, some carnivore gastropods such as Strigatella and Conus eat the others small mollusc including herbivore gastropods (13, 14). Negative association between some gastropods for example Littorina sp., Cyprea, and Haminoea sp. and macroalgae might occur because macroalgae have been the main source of food for them (15,16,17). It means that gastropod only take advantage from the macroalgae but not the vice versa. *Morulla* sp., *Cabestana* sp.,and *Conus striatus* are carnivore gastropods, but they are not have association with macroalgae in rock plates with algae area. That might occur because both of them are predator for another kind of animals which are not directly having association with macroalgae (14, 17, 18).

Table 3 Coefficient of Association between Gastropod and Macroalgae

	Gatropod Species	Coefficient of Association between Gastropod and Macroalgae							
		Amphiroa sp		Halimenia sp.		Cladophora sp.		Dictyota sp.	
1	*Trochus intextus*	0.57	+	-0.577	-	0.578	+	0.578	+
2	*Conus striatus*	-0.41	-	0	0	0	0	1	+
3	*Conus ebaus*	0	0	-0.577	-	0.578	+	1	+
4	*Morula sp*	-0.41	-	-0.316	-	0	0	0	0
5	*Cabestana sp.*	0.5	+	-0.577	-	0	0	0	0
6	*Strigatella spp1*	0.5	+	-0.333	-	0	0	0	0
7	*Cyprea moneta*	0	0	-0.577	-	0.167	0	-0.333	-
8	*Littorina sp.*	-1	-	-0.577	-	-0.578	-	-0.577	-
10	*Plakobrancus ocellatus*	-1	-	-0.577	-	-0.578	-	-0.333	-
11	*Conus carnifonicus*	-1	-	-0.577	-	0.578	-	-0.333	-
12	*Pirenidae*	-1	-	-0.577	-	0.578	-	-0.333	-
13	*Mitra (Strigatella) spp.*	-1	-	-0.577	-	-1	-	-0.333	-
14	*Haminoea sp*	-0.57	-	-0.577	-	-1	-	-0.333	-
15	*Marginella gabrielle*	-0.57	-	-0.577	-	-1	-	-0.333	-

From this study, we conclude that the diversity of gastropod varies depend on the presence of macroalgae, the highest diversity is found in rock plates with algae area. Three kindsof associations between gastropod and macroalgae were found there. The variation of these associations might occur because there are both herbivore and carnivore gastropods found. We leave the association between gastropods and macroalgae in other areas for further study.

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O-SE09

Study of *Pekarangan* Biodiversity in The Upper Stream Of Kalibekasi Watershed Bogor District, Indonesia

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not presented

O-SE10

Detection of Methanotrophic Bacteria in Borneo-Peatland Soil

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ABSTRACT

Borneo is the third Indonesian peat possession island. Peat potential for absorber and carbon storage. When environmental condition is disturbed, carbon deposits in the form of CO_2 and CH_4 are release from the peat. Methane is an important global warming gas. It is 25-26 times more reactive than carbon dioxide. That gas decreased by methanotroph activity. Methanotrophs oxidize methane to formaldehyde use methanol by using unique monooxygenase include methane monooxigenase (sMOO and pMMO), and methanol dehydrogenase. The aims of this study were to obtained methanotrophic bacteria on decreasing methane emission and to identify their metabolic by gene cluster. The research was carried out, including isolation and selection; measuring methane emission, and identification methanotroph from peat based on molecular methods. The results showed that the peat consisted of 12 isolate methanotroph. Those bacteria consumed methane up to 80% within 6h. Not all selected isolates were partially identified. Methanotrophic bacteria belong to type I (M-1), II (M-2 and M-3), and X (M-4). Methanol dehydrogenase were found in three strain of methanotrof used mox*F* gene that proved to be gram-negative methylotroph specific MDH.

Keywords: methane, peat, Borneo, methanotroph, type X, methylotroph

Introduction

Borneo is the third Indonesian peat possession island that have wide peat region approximately 5.7 million hectare **(1)**. The peat was made from residual accumulation of tropical vegetation. It is a unique ecosystem where have carbon management directly or indirectly affect environmental balances. The biggest carbon is deposit under surface soil and accumulated for million years. Wide of peatland in the world just 3 %, but have 550 gT deposit of carbon **(17)**. When environment condition is disturbed, carbon deposits in the form of carbon dioxide (CO_2) and methane gas (CH_4) release from the peat. The atmospheric concentration of methane increasing for many decades **(11)**. Methane emission from wetland and peatland ecosystem in the world is 100-231 gT year^{-1} **(16)**, whereas Kalimantan Tengah, Borneo-Indonesia is 0.014 Ton hectare^{-1} year^{-1} **(15)**. Methane is potential gas to contribute long-term global warming in the world. It is 25-26 times more reactive than carbon dioxide **(3, 16)** and very dangerous for global climate change.

Methane emission from peat is effect of methanogens that production of methane. In the ecosystem, methane emission will naturally reduce by methanotroph. The oxidation of methane in the soil provide major sink for greenhouse gas in atmosphere **(11)**. Methanotroph is an important group of methane oxidizing microorganism that use methane as sole sources

of carbon and energy **(8, 10, 14)**. These bacteria are widespread in nature **(19)** such as wetlands, fresh and marine waters, lakes, sediments **(28)**, and acidic peatland (**6**). It can thrive as free-living bacteria or symbioses with mosses. Methanotrophs are classified into 2 major group include methanotrophic bacteria and anaerobic archaea **(14)**. Arcoding to previous studies about methane emission reduction, there are three group of methanotrophic bacteria involved **(27),** that is methanotroph type I, II, and X base on multiple criteria **(11, 23)**. Type I mostly belonged to *gammaproteobacteria* are indicated by particulate methane mono oxygenase enzyme (pMMO) in their cell wall and use RuMP pathway for formaldehyde assimilation; type II having soluble methane monooxygenase (sMMO) in their cytoplasma and use serin pathway for formaldehyde assimilation; and type X which has both sMMO and pMMO **(22)**. Methane monooxygenase enzyme is important for catalyze methane oxidation to methanol **(13, 23)**. Type I nclude *Methylomicrobium*, *Methylobacter*, and *Methylomonas* **(5, 18)**, *Methylocaldium, Methylothermus* **(4)**, *Methilospaera* **(5)**; type II include *Methylocystis* and *Methylosinus;* type X include *Methylococcus capsulatus.* The sMMO consist of protein A, B, and C. Protein A is made up of two copies each of subunit α, β, γ which are docded for mmoX, mmoY, and mmoZ genes. The pMMO consist of subunit 47, 27, and 25 kDa **(25**).

Understanding of microbial ecology in peatland soil is very limited. Introduction of molecular technique enable to explain microbial processed in the peat. It is very important because it has shown us that methane oxidation is biological process for reducing methane emission in environment. Convert methane to methanol by methane monooxygenase and transform methanol to formaldehyde encoded by methanol dehidrogenase enzyme is so amazing. The aims of the study to obtained determine methanotophic bacteria on decreasing methane in peatland soil and to identify their metabolic by gene cluster. The research carried out, including isolation charcterization; measuring methane emission, and identification methanotroph from peat based on molecular method by using real-time PCR to study the abundance of methanotrophs in Borneo-Peatland soils.

Materials and Methods

Soil sampling

The sampling site is located at tropical rainforest of Kalampangan, Kalimantan Tengah, Indonesia, several years after fire. Peats were collected from water flooded area and surface soil in the depth less than 1 m at the end of January 2010 from twenty different random location within peatland soils. The peat samples were put into one sterile serum bottles capped with butyl rubber stopper. It can represent Kalampangan for general condition and increasing probability to get microbial diversity and microbial superior. Then samples were

kept cool in ice box during transport to the microbial ecology laboratory. Peat samples were stored at -20°C until use.

Extraction of total DNA

Extraction of DNA from 0.5 g peat was performed using Ultra Clean ™ Soil DNA Extraction Kit (Mo Bio Laboratories, Carlsbad, CA, USA) according to manufacturer's instruction. The DNA yield was approximately 20 µl for final volume and stored at -20°C. The DNAs were diluted by 10-fold to inhibit effect of humic substance via PCR method. Then use for template. Methanotrophic bacteria in the peats was analyzed based on the pmo*A*, mmo*B*, and mox*F* gene copy numbers using real-time PCR (Takara thermal cycler, Shiga). Specific primer pairs from Table 1 were used to amplify the metanotrophic pmo*A*, mmo*B*, and mox*F* gene fragments. Amplification was performed by using Takara thermal cycler in 0.2 ml PCR tube. Real-time PCR assay was performed with protocol of Otsuka *et a.l* **(26)**. The 25 µl reaction mixture consisted of 5.5 µl nuclease free water (ddH_2O), 1 µl template (peat's DNA), 3 µl forward primer, 3 µl reserve primer, and 12.5 µl Go Taq® Green Master 2X. The PCR programs were as follow: an initial denaturation for 5 min at 94°C follow by 30 cycles (94°C for 30 s, annealing for 30 s at 55°C, and ending with extention step at 72°C for 1 min). Then, additional 1 cycle (72°C for 7 min, colling down for 5 min at 4°C, and incubation at 16°C). Then, PCR tube take from PCR thermal cycler. The PCR products were cofirmed by Agarose gel 1% (1 gr agarose in 100 ml TAE Buffer 1 X) with electrophoresis machine (Mupid-EXU Sub Marine Electrophoresis System, Seraing) and were stained by ethidium bromide. Finaly, the band was performed by UV Transiluminator and was printed by Printgraph.

Table 1. Primer for detection of functional of methanotroph.

Gene	Primer Design	Sequens (5'-3')	Reference
pmo*A*	f A189b	GGNGACTGGGACTTCTGG	Otsuka *et al.,* 2008
	r A682	GAASGCNGAGAAGAASGC	
pmo*B*	f 77	AGTTCTTCGCCGAGGAGAACCA	McDonal *et al.,* 1994
	r 369	TGCCCAGGGTGTAGGCGCGGCCGA	
mox*F*	f 1003	GCGGCACCAACTGGGGCTGGT	McDonal *et al.,* 1994
	r 1561	GGGCAGCATGAAGGGCTCCC	

Isolation of methanotrophic bacteria

The peats were diluted on 10^{-2} until10^{-6}. The enrichment of 2 ml serial dilution was performed in double side arms tube and 30 ml modification nitrate mineral salt (NMS) liquid medium by Higgins *et al.* () at a starting pH of 7.2. The medium includes 1.0 g/l $NaNO_3$; 0.25 g/l NH_4Cl; 0.26 g/l KH_2PO_4; 0.74 g/l $K_2HPO_4 \cdot 3H_2O$; 1.0 g/l $MgSO_4 \cdot 7H_2O$, 0.2 g/l $CaCl_2$; 0.004 g/l FeS SO4·$7H_2O$; 0.01 g EDTA; 10 ml/l trace element; and 100 ml/ sterile soil extract. The

headspace of double side arm tube injected an methane-air mixture at 6:4 ratio for twice per day until 1 month. Enrichment cultures were incubated at room temperate with shaking (180 rpm) for 1 month. The mix culture from the last positive dilution was used for isolation of a pure culture from the single colonies that appeared on NMS solid medium at pH 7.2 during prolonged incubation of the plates under an methane-air atmosphere. Single colonies were transferred into a NMS liquid medium and the methanotroph candidate cultures showing growth with methane were selected. The purity of the selected clones was checked by repeate passage onto a ANMS agar medium and by absence of growth on rich organic media. The candidate methanotrophs were characterized.

Methane consumption assay in batch culture

Batch culture was performed at pH 7.2 using NMS liquid medium as indicated above. Four methanotroph candidates were transferred into double side arm with suspension density of bacterial cells are 1.6-1.8 optical units at 600 nm in 9 ml NMS liquid medium. The headspace of double side arm tube injected an methane-air mixture at 6 ml : 6 ml used by syringe. Cultures were incubated at room temperate with shaking (180 rpm) every 2 hour. Methane consumption culture was detected by Gas Chromatography (SHIMADZU PORAPAK Q GC 14 B, Kyoto) with Frame Ion Detector (FID) at 140^{o}C, column at 70^{o}C, and injector at 100^{o}C for 2, 4, 6 hour. The data was analyzed by Microsoft Excel **(11, 13)**.

Methanotroph gene analysis

Total DNA extraction, purification, and gen analysis based on the pmo*A*, mmo*B*, and mox*F* gene copy numbers using real-time PCR (Takara thermal cycler, Shiga) as indicated above **(26)**. The DNA band was performed by UV Transiluminator after run by electrophoresis.

Functional gene analysis

Functional gene analysis was needed for detection other potential abilities from methanotroph in soil. The potential gene includes denitrification (nirK1F-nirK3R), nitrification (NitA-NitB), nitrogen fixation (nifHf-nifHr), and phosphate solubilizing ability (F_{ow}-R_{ow}).

Result and Discussion

Detection of functional gene responsible for methane biotransformation in peats

Detection pmo*A*, pmo*B*, and mox*F* gene in the peat use for predicate methanotroph before direct isolation. From figure 1, all three group gene responsible for methane transformation were observed in peat indicating that peat are important source of gene responsible for oxidation of methane into methanol which could be done by type I methanotrohp belonged to gammaproteobacteria and use pMMO for transform methane to methanol coded by pmo*A,*

type II belonged the alphaproteobacteria and use sMMO and code by pmo*B*, and type X which has both pMMO and sMMO genes. The mox*F* gene which play role for synthesis of methanol dehydrogenase (MDH) that transform methanol to formaldehyde was commonly observed in peat (figure 1). Soluble methane monooxygenase coded by pmo*B* show many band caused by annealing at 55^{o}C is not optimum. Yuwono (2006) reported that temperature optimum for annealing mmoB gene is 59^{o}C. PCR product for mmoB gene is 290 bp **(23)**.

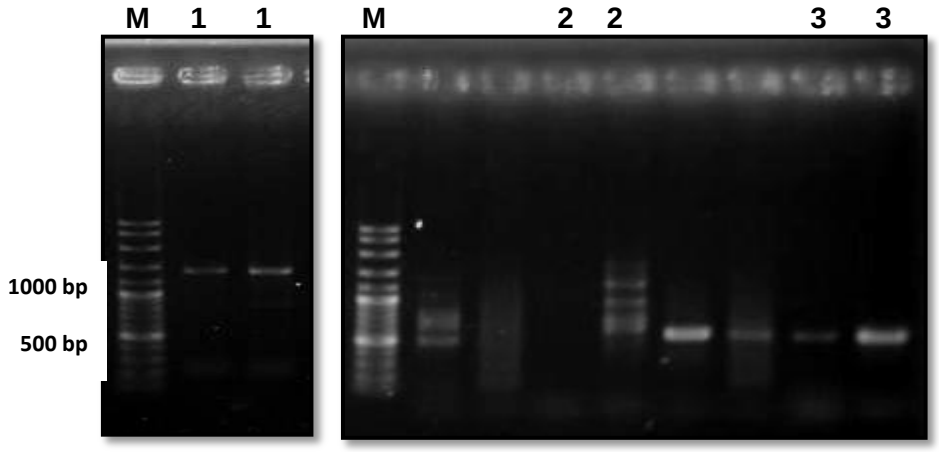


Fig 1. Polymerase chain reaction amplification product of pmo*A* (1), mmo*B* (2), dan mox*F* gene (3) in peats.

Morphology

Three isolates were a non-motile, Gram-negative, opaque, round colonies that formed pink pigmented when grown on NMS agar or liquid medium. They were similar to pink pigmented facultative methylotophic (PPFM) bacteria that can use all variety carbon compound **(7, 20, 21)** such as C_1 like methanol and methylamine **(20)** and other C like sugarcane **(22)**. Pink pigmented also formed in nutrient agar, but not strength like in NMS medium because color pigment depend on environmental nutrient. Lo and Lee **(21)** reported that different intensity of chromogene or color expression of facultative methanotrphic in AMS and NA caused by nutrient and carbon compound. Pink color indicate carotene pigment for protect from sunlight, ion, UV radiation, and use for extreme adaptation **(21, 29)**. In phosphate medium, all isolate were grown and performed of transparent zone. They have wide spectrum for dissolve of phosphate.

The isolates were spread growth in liquid medium. It show that methanotrop bacteria is facultative aerobic. Methane oxidation use oxygen for terminal acceptor electron. Methanotroph combine oxygen and methane to form methanol and formaldehyde **(11)**. Base on table 1, all isolates from peat were showed Gram-negative because it role important for methane binding. Never found that methanotroph bacteria is Positive-gram **(18, 30).**

Table 2. Morphological of the strain methanotroph

Characteristics	Strain M-1	Strain M-2	Strain M-3	Strain M-4
Morphology Cell	coccus	coccus	rod	coccus
Morphology Colony	circular	circular	circular	circular
Elevation	flat	flat	flat	flat
Surface layer	rough	rough	rough	smooth
Inner Structure	opaque	opaque	opaque	translucent
Edge	lobate	entire	entire	entire
Extracellular pigment	absent	absent	absent	absent
Gram reaction	negative	negative	negative	negative
Size cell	1-2 µm	0.3-1 µm	1.5-3 µm	0.2-0.9 µm
Colony color (NMS)	pink	red	red	white-pink
Motility	negative	negative	negative	negative
Exospore	absent	absent	absent	absent
pH	5-8	5-8	5-8	5-8
Temperature				
Growth range	25-35	25-35	25-35	25-35
Optimal	30	30	30	30
Growth in				
Liquid medium	Spread	Spread	Spread	Spread
Nutrient agar	+, pink	+, pink	+, pink	+, white
Phosphate agar	+, zone	+, zone	+, zone	+, zone
Poly-β–hydroxybutirat	absent	present	absent	absent

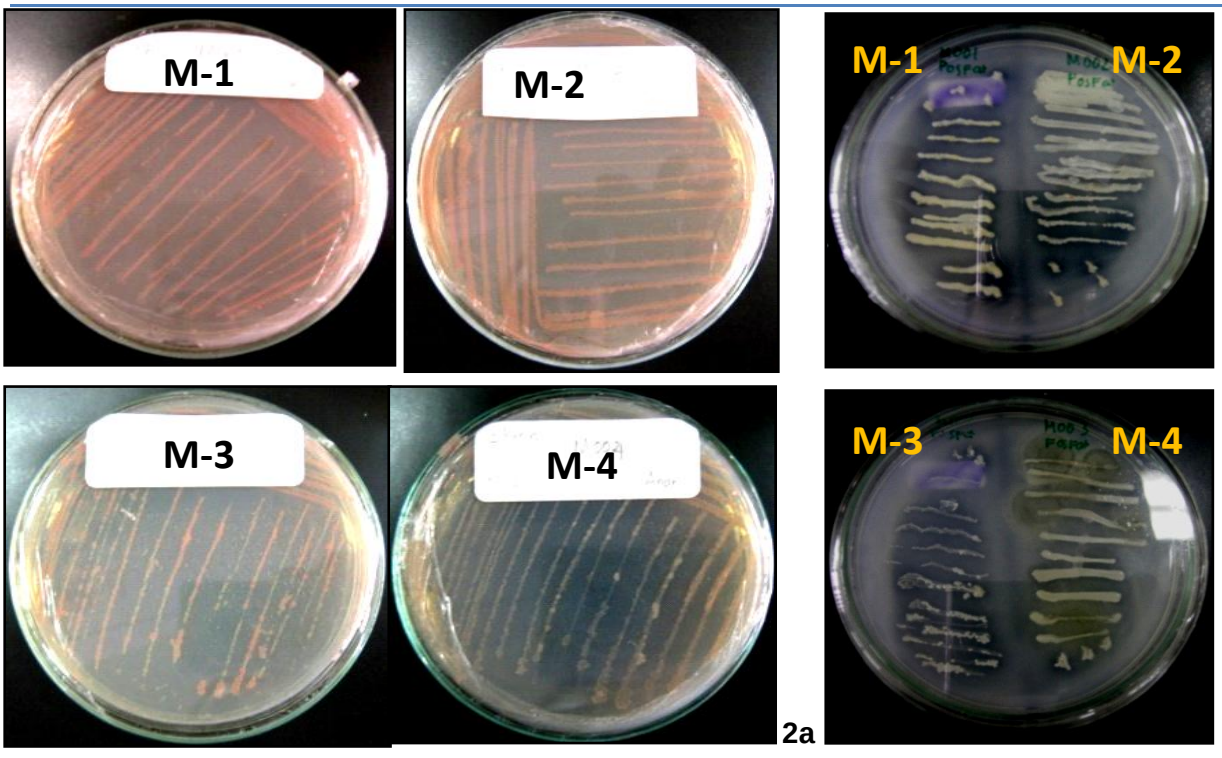


Fig 2b. Strain methanotroph in (a) modification nitrat mineral salt agar (NMS), all strain were showed pink-pigmented; (b) phosphate medium agar (PSB), all strains were showed transparant zone

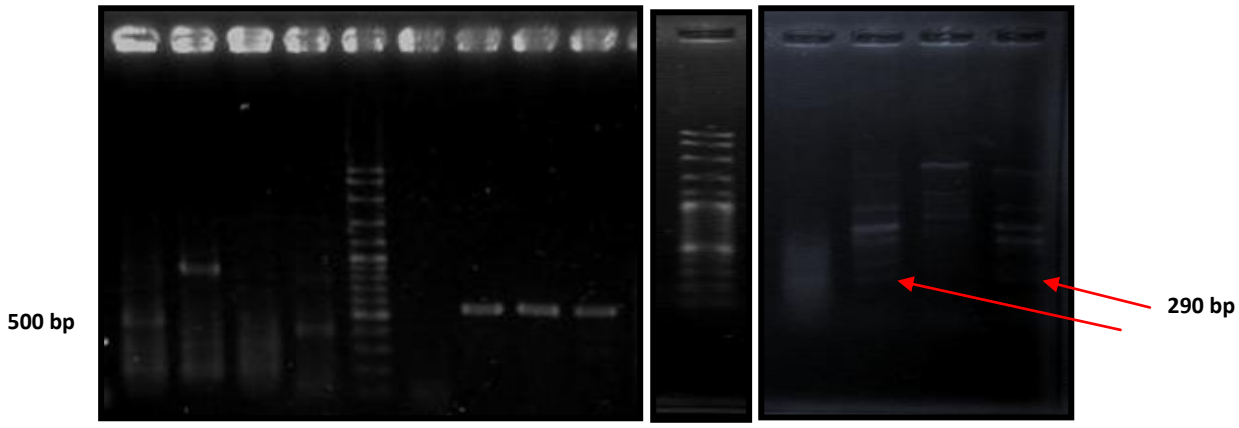


Fig 3. Polymerase chain reaction amplification product of pmo*A*, mmo*B*, and mox*F* gene in pure culture (4 strain from left are pmo*A*, mmo*B*, and mox*F*)

Methanotrophic bacteria with functional gene diversity that responsible for methane biotransformation are illustrated in Figure 2. Particulate methane monooxygenase (pmo*A* gene) were founded in strain M-1 and M-4. Particulate gene in M-2 strain was showed in 900 bp, it mean pmoA primer is not specific binding. PCR product for pmo*A* gene approximately 500 bp. Methanol dehydrogenase (mox*F* gene) were found in strain M-2, M-3, and M-4 (Figure 2) with PCR product is 550 bp **(23)**. All three strain can transform methanol to formaldehyde. MDH was commonly observed in large quantities than pmo*A* and pmo*B*. Its support of Henckel *et al.* **(13)**. The primers for mox*F* gene was design and proved to be gram-negative methylotroph specific MDH **(23)**. Soluble methane monooxygenase (pmo*B* gene) were founded in M-2 and M-4 with PCR product approximately 290 bp. Product PCR was performed with multiple band maybe caused by annealing temperature or primer is nonspecific binding, so gene sequence that same amplificated by PCR. Yuwono (2006) reported that PCR product of mmoB gene optimal at 59^{o}C and was formed single band. We were founded methanotroph type X from strain M-4 which have particulate and soluble form **(22, 23, 24)**.

Methane consumption assay in batch culture

Methane emission was much lower in ANMS medium (M-1, M-3, M-4, and M-5). All strain were absorbtion +/- 80% for 6 h. Methanotroph use methane monooxygenase to convert methane to methanol and will convert again by metahe dehydeogenase (MDH) to form formaldehyde.

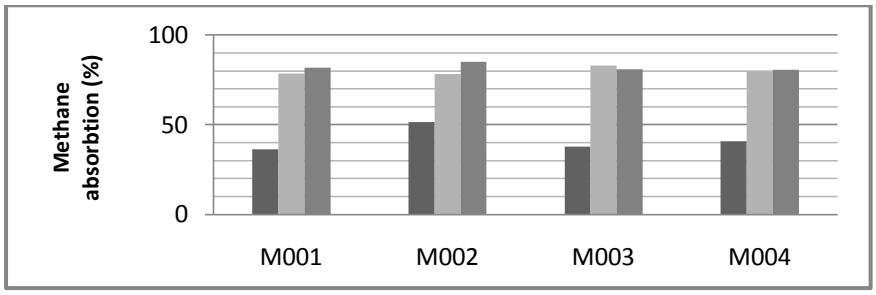
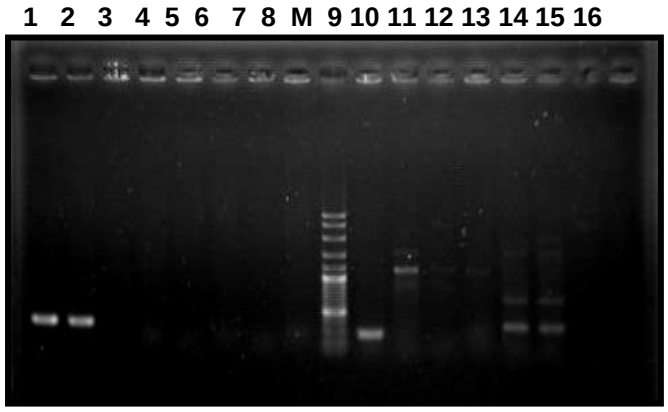


Fig 4. Methane absorbtion from strain methanotrop after 2 h, 4h, and, 6 h. From left to right is M-1, M-2, M-3, M-4. Methane consumption for 6 h by all strain are +/- 80%.

Detection of functional pure culture



1. PCR product of nirK gene M-1
2. PCR product of nirK gene M-2
3. PCR product of nirK gene M-3
4. PCR product of nirK gene M-4
5. PCR product of Nit gene M-1
6. PCR product of Nit gene M-2
7. PCR product of Nit gene M-3
8. PCR product of Nit gene M-4
9. PCR product of ow gene M-1
10. PCR product of ow gene M-2
11. PCR product of ow gene M-3
12. PCR product of ow gene M-4
13. PCR product of nifH gene M-1
14. PCR product of nifH gene M-2
15. PCR product of nifH gene M-3
16. PCR product of nifH gene M-4

Fig 5. Polymerase chain reaction amplification product of nirK, Nit, ow, and nifH gene in pure culture.

Strain M-1 and M-2 presented nir*K*, ow, and nif*H* genes. They have ability to expression denitrification process, dissolve of phosphate, and nitrogen fixation in soil. Those bacteria is potential for bioremediation agent. Strain M-3 and M-4 just can solve phosphate. Nitrogen fixation ability just found in methanotroph type 2 and 1 from genera Methylococcus **(2)**. Dissolve phosphate from all strain were showed in phosphate medium with wide transparent zone (fig. 2b).

Conclusion

In this study, we presented first data on molecular diversity and morphology characteristic of methanotrophic bacteria in Kalampangan, Borneo peatland soil, Indonesia. All three group gene responsible for methane transformation were observed in peat indicating that peat are important source of gene responsible for oxidation of methane. MDH also were found in three strain methanotrof used mox*F* gene that proved to be gram-negative methylotroph specific. All the strain present pink-pigmented in NMS medium, it show that they have ability to use all carbon compound like PPFM. The data show those bacteria consumed methane up to 80% within 6h. This indicates a high level adaptation to high methane present of methanotroph communities in Borneo peatland soil. All the result reported in this study are an initial investigation biological and physical mechanism of methanotrophic bacteria that contributes to further understanding of methane emissiin in Borneo peatland soil.

Acknowledgments

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O-SE11

Parasitization Intensity of Parasitoid (*Psyttalia makii* Sonan) to the Fruit fly (*Bactrocera carambolae* Drew & Hancock) on Red Chili (*Capsicum annuum* L.) Farm in Sleman and Bantul, Yogyakarta

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ABSTRACT

Fruit fly, *Bactrocera carambolae* is a pest on chili fruit. The fruits is very importance commodity in this country. *Psyttalia makii* Sonan is a soliter opiinae parasitoid that attack egg and 1st instar larvae of tephritid fruit fly The objectives of this research was to study parasitization of *Psyttalia makii* to the fruit fly, *B. carambolae* at Chili (*Capsicum annuum* L) farm in Sleman and Bantul. The research was conducted from May to September 2005. Fruit samples were bought to the Entomology Laboratory of Biology Faculty Gadjah Mada University. Adult fruit flies and parasitoids emerged from pupae were held for seven days for the identification. Parasitism intensity were calculated as the total number of enclosed parasitoids by the total number of fruit flies pupae. The average parasitization intensity of *P. makii* to immature stage of *B. carambolae* was 25,4% in Bantul and 33,23% in Sleman. It indicated that fruit and host (fruit fly) abundance may have influenced parasitoids abundance and its parasitization intensity.

Keywords: parasitization, *Psyttalia makii*, *Bactrocera carambolae*, red chili

Introduction

Carambolae fruit fly, *Bactrocera carambolae* Drew and Handcock are major pest on chili fruit (*Capsicum annuum* L.) in Indonesia. Early attempts to control of these pest resulted in using insecticide and male attractant, methyl eugenol[1)].

Soesilohadi et al. (2005) reported that *Biosteres vandenboschi* Fullaway, *Psyttalia makii* Sonan attack immature stage of *B. carambolae*, a chili fruit fly in wide area of chili farm in Sleman and Bantul [2)]

Psyttalia makii Sonan is a soliter opiinae parasitoid that attack egg and 1st instar of tephritid fruit fly[3,4)]. It is an endoparasitoid that oviposits primary in the fruit fly larvae and completes a holometabolous type of metamorphosis inside the host. According to Vargas et al. (1993), adult parasitoid emerge from the host puparium 18-20 day after oviposition, usually 2 days longer than eclosion of adult flies[5)]. Its ability to parasitize the early stage of the host and outcompete the immatures of other fruit fly parasitoids when multiparasitism occurs [6)].

This study was conducted to determine Quantitative effect on the relative abundance of Psyttalid fruit fly parasitoids based on chili fruit collected from chili farm in Sleman and Bantul.

Materials and Methods

Chili Fruit fly survey. Chili fruit were collected from host plant at the chili farm in Sleman and Bantul, Yogyakarta Special Province during May to September 2005. Just tree fruits were included in collection. Chili fruits were collected to obtain chili fruit fly infestation and associated parasitoid data. Variety of the Chili that planted in Sleman was cipanas. Chili fruits were panted In Bantul have more bigger than those in Sleman.

Fruit processing. Fruit were placed on cylindrical fiberglass containers (dia 20 cm and h 30 cm) containing 1,5 cm sand and held for 2 to 4 weeks (depending on rate of fruit decomposition). Sand from each the container was sifted weekly and larvae and pupae were placed in small containers. Fruit were held in containers in room maintained at 25 ± 2 ^{o}C and 60 ± 10 RH. Number of pupa and chili fruit fly adults and Psyttalid parasitoids that emerged were recorded. Percentage parasitism was calculated on basis of live parasitoids recovered from total pupae. Fruit fly and Parasitoid were identified to species with the taxonomic key [3,4,7].

Data Analysis. Degree of association between chili fruit fly, *B. carambolae* and parasitoid (*P. makii*) abundance was based on formula :

$$\% \text{ parasitization} = \frac{\sum \text{ adults parasitoid}}{\sum \text{ total fruit fly puparium}} \times 100\ \%$$

Result and Discussion

The average intensity of *P makii* parasitizing immature stage of fruit fly in Bantul and Sleman were 25,24 - 33,23% (Table 1) In Sleman, the chili host plant were planted in the farm by mulsa plastic to covering the ground. In Bantul the chili were planted in farm without mulsa plastic. The mulsa protecting the plant from diseases transmission, pest development, included fruit fly and minimize weed growth[1]. In Sleman, mulsa plastic did not influence parasitization intensity of *P. makii*. That was indicated by the parasitization level of *P. makii* in Sleman was higher than those in Bantul (Table 1). In Bantul, there were poly culture system in chili farm but in Sleman, chili were planted as mono culture.

Table 1. Total puparium, adult fruit fly (*B. carambolae*) emerged and patrasitization of *P. makii* parasitoid that sampled from chili farm in Sleman and Bantul

Sampling Location	Total pupae	% emerged adult of		
		ff	*P.m*	ff + *Pm*
Sleman	3221	49,22	33,23	82,45
Bantul	2557	50,88	25,24	76,12

ff: fruit fly; *P.m: Pyittalia makii*

Parasitization *P.makii* to immature stage of *B. carambolae* in Sleman was more higher than those in Bantul. Fruit fly has around 40 species host plant [8,9,]. Soesilohadi (1995) reported that parasitization level of *Opius* sp. on chili fruit fly was 50% in Sleman [10]. In the poly culture farm, there were more than one species fruit host that

were attacked by fruit fly. Therefore the fruit fly population dynamic in a host plant was influenced by planting pattern. On the Figure 1 and 2 indicated that the abundance of adult parasitoid was influenced by chili fruits. That is why parasitization levels of *P. makii* to immature stage of *B. carambolae* in Sleman was higher than those in Bantul. Fruit type affected parasitization rates by *B. arisanus* but the reason why are not fully understood [6]. According to Vargas et al. (1993), field parasitization rates of fruit vary with fruit varieties [5].

There were positive correlation between number of adult parasitoid and chili fruit number both in Sleman and in Bantul chili farm (Figure 1 and 2), indicated that parasitoid abundance influenced by availability of host fruit.

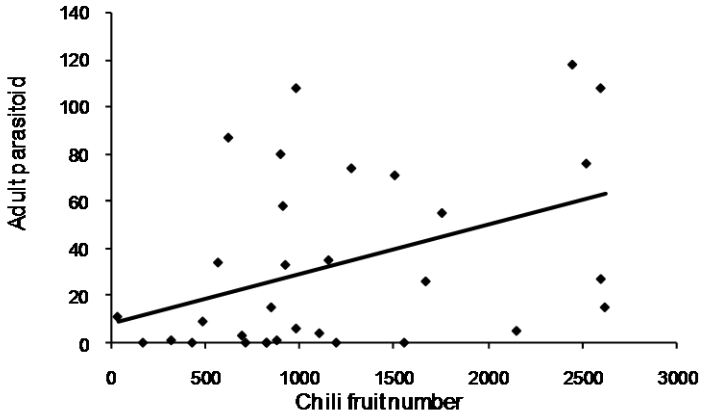

Figure 1. Regression between adult parasitoid and chili fruit number at Chili farm, Sleman
Y= 0,02x + 7,99, R^2=0,1700

Psytalid parasitoid, the most abundant and widely distributed psytalid parasitoid in Sleman (500 m asl) and Bantul (50 m asl), but the parasitization *P makii* to chili fruit fly in Sleman was higher than those in Bantul. For example, number of oriental fruit fly infesting fruit were 10-fold higher at 550 m that at 1,100 m [5]. What did it indicate that in Sleman with 500 m abs elevation as the optimum elevation for parasitoid, *P. makii* to parasitizing immature stage of fruit fly ? To answered the question there are still need supporting data.

Positive correlation between number of puparium and adult parasitoids emerged (*P. makii*) in Sleman and Bantul indicated that increased adult parasitoids emerged was influenced by increased number of puparium (Figure 3 and 4).

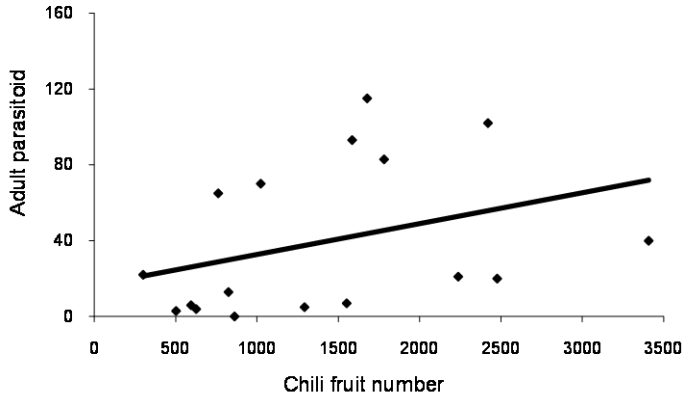

Figure 2. Regression between adult parasitoid and chili fruit number at Chili farm, Bantul Y= 0,02x + 16,46; R2 = 0,1225

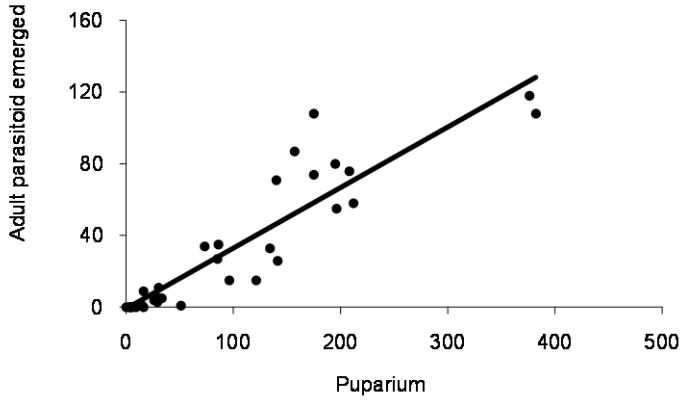

Figure 3. Regression between Adult parasitoid emerged and Puparium number at Chili farm in Sleman, Y = -0,89 + 0,34X, R^2 = 0,8301

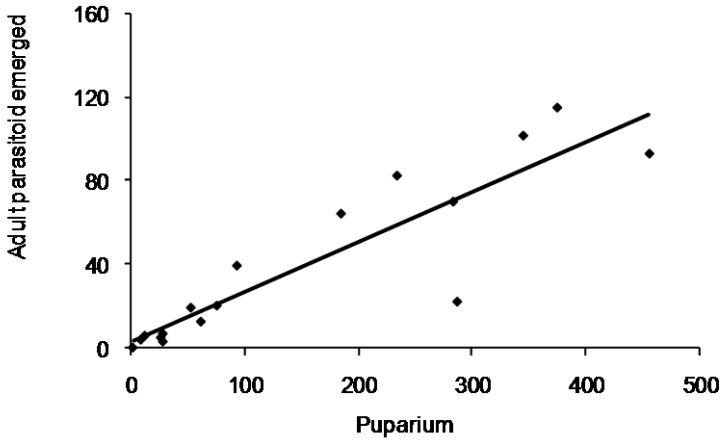

Figure 4. Regression beetween Adult parasitoid emerged and Puparium number at Chili farm, Bantul, Y= 0,2382 x + 3,5323, R^2=0,8090

Conclusion

1. P. makii reduced between 25,25 (in Bantul) – 33,23% (in Sleman) of chili fruit fly population.

2. Pattern of planting in chili farm did not influence parasitism degree

Acknowledgment

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O-SE12

SKELETAL ORGANIZATION OF CAUDAL FIN IN *Andamia reyi* (Perciformes, Blenniidae)

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ABSTRACT

Caudal fin skeleton modification is common in the Teleostei. Structural and functional adaptations cause changes in external morphology and provide an important consequence for the movement of fish. *Andamia reyi* is a fish that spends most of his life in the terrestrial. Behavior *Andamia reyi* require caudal fin structure capable of supporting the movement on land. In addition, research on the structure of the caudal fin skeleton in *Andamia reyi* not been done. This study aims to determine the structure of the caudal fin skeleton *Andamia reyi* as the adaptation of terrestrial life. Staining results of Alcian blue-Alizarin red's show that *Andamia reyi* has 12 principal rays and 6 procurrent rays. Caudal fin is divided into 6 segmented rays in each lobe and 6 unsegmented rays, 3 on the upper lobe and 3 on the lower lobe. Three procurrent rays in the upper lobe contained on uroneural while the third part of the lower lobe contained on hypural. Principle contained in hypural rays. Hypural clearly separated into two parts, the superior and inferior. The formula of caudal fin rays is 3+6+0+0+6+3 =18.

Keywords : caudal fin skeleton, *Andamia reyi*

Introduction

Caudal fin evolution has become a textbook case of structural and functional modification in vertebrate, and is used to illustrate how changes in external morphology have occurred and had important functional consequences. Within the Teleostei, modifications of the caudal skeleton are common and include fusion of the hypural bones and reduction and fusion of epurals and uroneurals. In most teleost fishes the hypurals have expanded and are oriented posteriorly, supporting fin rays both above and below the precaudal vertebral axis. In modern teleosts, reduction and fusion of many skeletal elements of the caudal fin can be observed (Lauder, 1989; Gosline, 1961).

Andamia reyi, rockskiper, active movements such as skipping, skimming the surface of water, or swimming when disturbed by waves, by the rise of the tide, or by human beings have been referred to above. In this movement the whole of the tail takes part, and enables the fish to move either from side to side or forward. Forward progression may be effected by the flexure of the tail either on one side only, or on both the side alternately, but

this flexure does not involve any zig-zag movement on the part of the fish. The new positions reached in this kind of progression are nearly always in a straight line in front of them (Rao and Hora, 1938).

Behavior *Andamia reyi* require caudal fin structure capable of supporting the movement on land. In addition, research on the structure of the caudal fin skeleton in *Andamia reyi* not been done. This study aims to determine the structure of the caudal fin skeleton *Andamia reyi* as the adaptation of terrestrial life.

Material and method

Alizarin and alcian blue staining is very useful method for staining of skeletal and cartilageous tissues. Female *Andamia reyi* fixed in alcohol 96%. Skin, eyes, thoracic and abdominal viscera removed from sample. The sample fixed in aseton for three days. Stain minimum of 24 hour in the following solution at 37° C : 1 volume 0,3 % filtered alcian blue in 70 % alcohol, 1 volume 0,1 % filtered alizarin red in 95 % alcohol, 1 volume glacial acetic acid, 17 volume 70 % alcohol. After stain, wash in 3 changed of destilled water. Place in 1 % KOH for 24-72 hour until the skeleton is visible through the soft tissues. Transfered to equal parts of pure glycerol and 1 % KOH (1:4, 1:1, 4:1) until clear. Sample stored in pure glycerol. Anatomical terminology used according to Lauder (1989) and Gosline (1997).

Result and discussion

Staining results of Alcian blue-Alizarin red's show that *Andamia reyi* has 12 principal rays and 6 procurrent rays. Caudal fin is divided into 6 segmented rays in each lobe and 6 unsegmented rays, 3 on the upper lobe and 3 on the lower lobe. Three procurrent rays in the upper lobe contained on uroneural while the third part of the lower lobe contained on hypural. Principle contained in hypural rays. The formula of caudal fin rays is 3+6+0+0+6+3 =18 (Fig. 1).

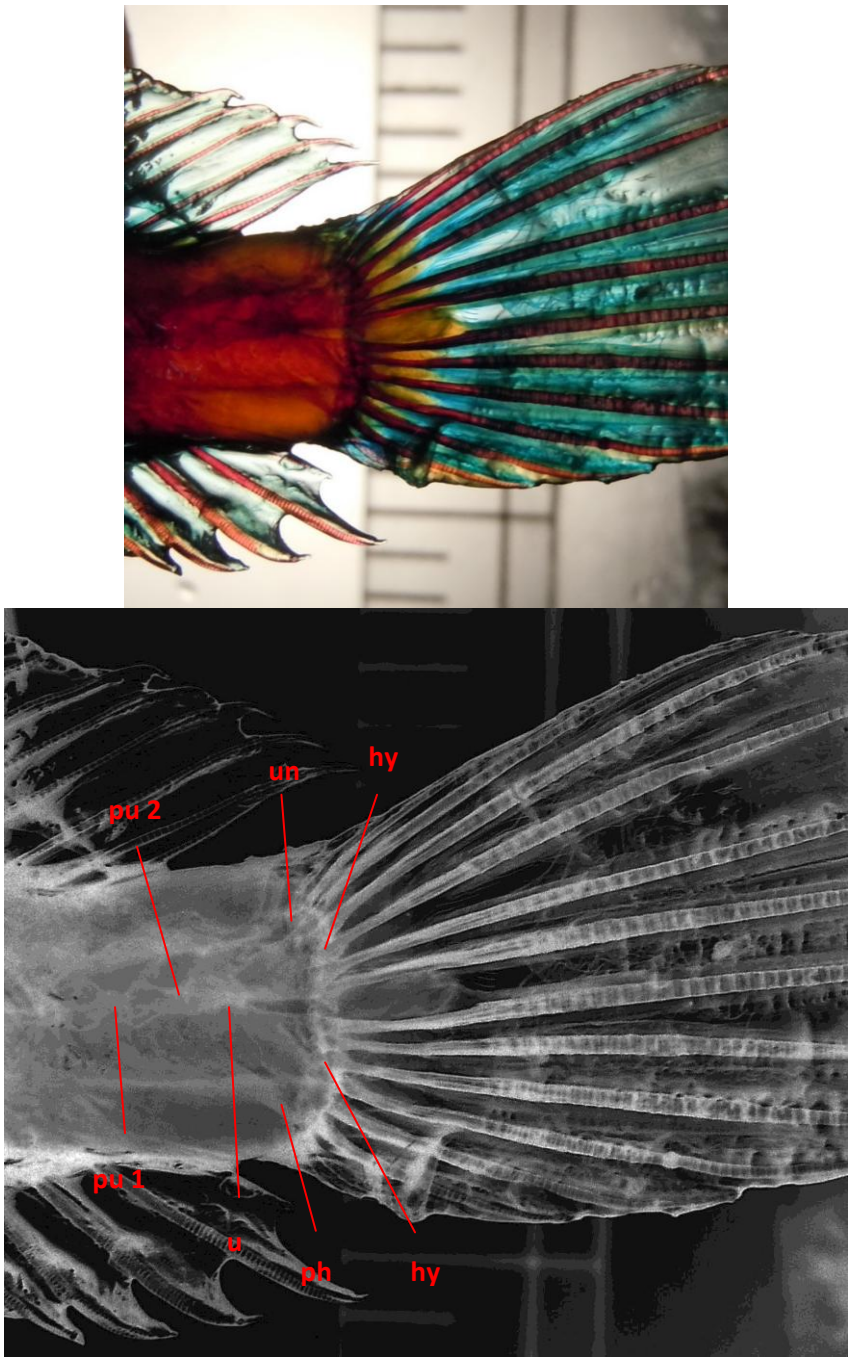

Fig 1. Caudal skeleton of female *Andamia reyi*. hy-hypural; ph-paryhypural; pu-preural centrum; u-ural; un-uroneural;

Hypural clearly separated into two parts, the superior and inferior. Tail can be moved freely toward the superior and inferior. Hypural have a system "lock and keys" (Fig. 2). This system causes the bone does not slip when the tail is used as a pedestal for the jump. In addition, this system reinforces the interradialis causing caudal rays can be widened so that the caudal rays can be used as a footstool. This system led to *Andamia reyi* able to jump to the left or right side. System "lock and key" allows *Andamia reyi* to determine the direction

and strength of the leap. The inferior caudal rays can be bent so as to facilitate the determination of the direction of the leap. In addition, the system supports the ability *Andamia reyi* to attach to rocks.

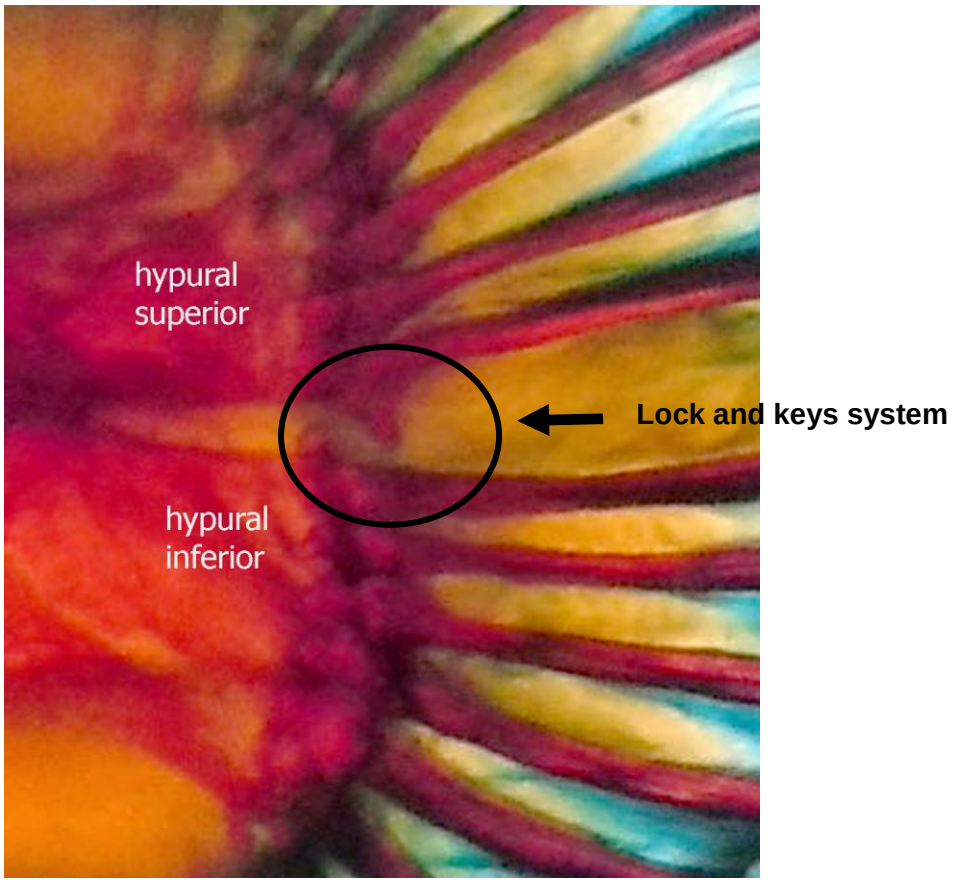

Fig. 2. “Lock and keys system in hypural’s *Andamia reyi*.

In many teleosts caudal rays are attached also to paryhypural and to the hemal spine of the preural 2 (Bartolino, 2005). But, in *Andamia reyi* paryhypural only supports procurrent rays rays while the principle is supported by hypurals. This is probably due to caudal on *Andamia reyi* used as a means of locomotion on land that requires a strong supporter of principle rays. Paryhypural size smaller than hypurals.

On the axis there are only pairs of caudal fin preural (PU 1 and PU 2) and the urals in the posterior directly associated with hypural. In addition, there is one uroneural that supports procurrent rays. In many lower teleosts there is only one to three uroneural located above the caudal axis (Gosline, 1997). This shows that Andamia reyi possibility is lower teleosts.

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O-SE13

Biodiversity of Intertidal Fish in Intertidal Zone of Drini Beach, Gunung Kidul, Yogyakarta

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Abstract

Intertidal zone is an area which is transition between sea and land. High tide and low tide happen periodically twice in intertidal zone of Drini, this make Intertidal zone has unique characteristics making it attractive to study. Meiofauna in this area is also unique, especially the types of fish. This research aims to determine the diversity of fish species in the intertidal zone of Drini Beach. The research was conducted in March 2011. The method used is free sampling at the afternoon and evening when the tide is low. The results show that there are 11 families and 12 species of fish. The families are *Ephipipidae, Chaetodontidae, Siganidae, Atherinidae, Mugilidae, Terapontidae, Apogonidae, Gobiidae, Scorpaenidae, Tetraodontidae,*and *Muraenidae.* The species are *Platax orbicularis, Chaetodon auriga*, *Siganus canaliculatus*, *Atherinomorus* sp., *Valamugil engeli, Terapon sp., Apogon angustatus, Istigobius ornatus, Scorpaenopsis* sp., *Arothron* spp., and *Echidna* sp. Most of them are reef fish, this is indicate that the coral reef ecosystem condition in Drini is still in good condition. The big wave in Drini may take them into the intertidal zone. There is typical fish in intertidal zone, *Gobiidae*. This family usually live in the intertidal zone and known as rock skipper. This result shows that the intertidal zone of Drini has high diversity of fish and still possible there are many kinds of fish in the intertidal zone Drini. Periodically research need to be done for better data.

Keywords: Intertidal Fish, Intertidal Zone of Drini Beach

INTRODUCTION

Indonesia located in the tropical zone and consist of more than 1000 islands. Indonesia also has the longest beach line in the world. This is make Indonesia has very high biodiversity. Intertidal zone is an area which is transition between sea and land. This area high tide and low tide happen periodically. This situation makes Intertidal zone has very unique meiofauna, different from sea, freshwater and terrestrial creatures.

Drini located in Gunung Kidul, Yogyakarta, Indonesia. Drini has very unique characteristics. High tide and low tide happen periodically twice in intertidal zone of Drini. There is a river estuary in Drini. Drini also has seagrass bed bed (*Thalasia hemprichii*) beside seaweed bed. Seagrass systems are important habitat for early life stages of many commercially important species that seek protection from predators [1]. This seagrass can

promote sediment deposition and also avoid resuspension of sediments and suspended particulate matter, which in have highly contribute to stabilize the coast [2].

Meiofauna play important role in Intertidal zone. Contributes in food chain and ecological trophic meiofaunaa has unique characteristics. It has to adapt the extreme condition in intertidal zone when the low tide is come, the temperature increase rapidly. Absent of water also happen periodically. Meiofauna has special ability to adapt with this condition. one of the meiofauna is fish. Fish in this area still need water, but it can live with only small water. Special physical character in Intertidal zone of Drini makes this area interesting to know. And there are no data base about intertidal fish in Intertidal zone of Drini. This research aims to determine the diversity of fish species in the intertidal zone of Drini Beach.

MATERIAL AND METHOD

The research was conducted on March 2011 in the Intertidal zone of Drini, Gunung Kidul, Yogyakarta, Indonesia. Sample taken 4 times in a month during Materials and tools used in this research are camera, millimeter block paper, alcohol 70%, net, identification book “FAO fisheries” and “Reef Fish Identification: Tropical Pacific Fishes” and plastic box. Method use in this research is free sampling at the afternoon and evening when the tide is low along intertidal zone of Drini.

Figure. 1. Location of the study site at coastal areas of Drini, Yogyakarta, Indonesia

RESULT AND DISCUSSION

The results show that there are 11 families and 12 species of fish. The families are *Ephipipidae, Chaetodontidae, Siganidae, Atherinidae, Mugilidae, Terapontidae, Apogonidae,*

*Gobiidae, Scorpaenidae, Tetraodontidae,*and *Muraenidae.* The species are *Platax orbicularis, Chaetodon auriga*, *Siganus canaliculatus*, *Atherinomorus* sp., *Valamugil engeli, Terapon sp., Apogon angustatus, Istigobius ornatus, Scorpaenopsis* sp., *Arothron* spp., and *Echidna* sp.

The first reef fish in this area is *Platax orbicularis*. This fish is belong to family *Ephipipidae*. *P. orbicularis* usually live in shallow protected coastal waters to deep, somewhat silty habitats, often with deep shipwrecks. Juveniles usually live inner sheltered lagoons while adults move out to open waters over sandy areas of deep lagoons [3]. In Intertidal zone of Drini, we found the juvenile of *P. orbicularis*. The juvenile has unique characteristics, has dark brown line in through eye. They occasionally mimicking like dead leaves. The Intertidal zone of Drini's condition has large seagrass bed, large algae bed with rocky substrate is ideal for juvenile fish to schooling.

The next reef fish is Chaetodon auriga**.** This fish is belong to family Chaetodontidae, the keystone species of coral reef ecosystem. C. auriga has very unique character, it has black spot in the yellow dorsal fin and has black line trough eye vertically. This fish live in coral, seagrass bed and seaweed bed. C. auriga found gathering in juvenile phase in Intertidal zone of Drini. the seagrass and seaweed bed in Drini make this area ideal for searching food. The present of this fish can indicate that coral reef ecosystem in Drini area is still in good condition.

Siganidae usually live in coral reef ecosystem the depth is 1-30 m and oceanodromous. This family especially *Siganus canaliculatus* can also live in the Inhabits inshore, algae reefs, estuaries and in large lagoons with Algae-rubble habitats, mainly common on rocky substrates. This species seems to tolerate more turbid waters, occurring within the vicinity of river mouths especially around seagrass beds just like Intertidal zone of Drini beach condition. Juveniles form very large schools in shallow bays and coral reef flats; school size reduces with size, with adults occurring in groups of 20 individuals or so [3]. *S. canaliculatus* are herbivorous, feeds on benthic algae and to some extent on seagrass. *S. canaliculatus* can consumed as food but have to be careful because have poisonous dorsal spines.

Atherinomorus sp. found form schools in large count. This fish is belong to family *Atherinidae*. This small fish has silver color, silvery midlateral band and Fins mostly uniform yellowish, pectorals with dusky patch at the base. This fish are pelagic neritic non migratory and found always swim around in Intertidal zone of Drini. This fish feeds on a variety of

planktonic crustaceans, seaweed and seagrass [4]. Local fisherman usually use this fish as bait fish.

Valamugil engeli found form schools in large count. This fish is belong to family *Mugilidae*. This fish inhabit coastal waters but enters estuaries and rivers where they feed on microalgae, filamentous algae, forams, diatoms, and detritus associated with sand and mud [5]. *V. engeli* has Dorsal and upper lobe of caudal fin with dark-blue tip. Anal, pelvic, and pectoral fins yellow. Pectorals also with dark blue spot dorsally at origin. Local fisherman usually use this fish as commercial fish.

Terapontidae form school in small count. This family are typically catadromous fish. Juveniles commonly found in sandy intertidal areas, often in tidal pools. Spawn in the sea and juveniles migrate into fresh water, eggs are guarded and fanned by the male parent. Drini has small estuaries so that this fish can be found easily. Beside, Drini also has seagrass and seaweed bed that can provide food [6].

Apogon angustatus is typically reef fish. This fish is belong to family Apogonidae. *A. angustatus* found in juvenile phase. This fish has specific characteristic, it has 5 horizontal stripes (brassy to dark brown in color), dark spot at base of middle caudal fin rays, fin rays light red. This fish is omnivore, it eat seaweed, benthic inverts and small crustacean. Intertidal zone of Drini with seagrass and seaweed bed provide much food for *A. angustatus* so it easily found in this area.

Istigobius ornatus is amphibious fish that usually live in intertidal zone. This fish is belong to family *Gobiidae*. This fish has specific characteristic, it has fluorescent scales in the both side of the body. *I. ornatus* is carnivore that eat eel, small crustacean, and small fish. This fish has special ability to go out of the water. Rocky substrate in Intertidal zone of Drini became ideal habitat to search food or to hide from the predators, so *I. ornatus* became easy to found almost in all Intertidal area.

Scorpaenopsis sp. is very poisonous benthic fish. This fish is belong to family *Scorpaenidae*. It has poisonous dorsal and pectoral fins. *Scorpaenopsis* sp. usually live in coral reef area, and intertidal zone with rocky or sandy substrate. It can camouflage well, so we have to be careful if swimming or walking in Intertidal zone of Drini. *Scorpaenopsis* sp. is a carnivore. It eat small fish and small crustacean. The characteristic of Drini became ideal for this fish. The fish usually found in seagrass bed and well camouflage.

Arothron spp. or puffer fish are belong to family *Tetraodontidae.* This family has unique characteristics; body covered with prickles and can double its size when feel threatened. *Arothron* spp. live in the lagoon, coral reef, seagrass and seaweed bed. The juvenile usually live in estuaries, and coastal bays. In the sampling location only the juvenile phase found. We can found easily in the sampling location, especially at night [7].

Last species found in Drini is *Echidna* sp.. This fish belong to famliy *Muraenidae. Echidna* sp. found between rocks and corals of intertidal reef flats. It's always hideaway in the rock and very difficult to catch. *Echidna* sp. has special characteristic, it has black and white pattern in alongside the body. *Echidna* sp. is nocturnal fish and also carnivore. Feed mainly on crustaceans [8]. In the Intertidal zone of Drini this fish is very easy to found, the abundance is high.

The condition of Intertidal zone of Drini with the seagrass and seaweed bed are still in good condition. Drini still became place for juvenile fish to grow. Most of the fish are reef fish, this is indicate that the coral reef ecosystem condition in Drini is still in good condition. This result shows that the intertidal zone of Drini has high diversity of fish and still possible there are many kinds of fish in the intertidal zone Drini. Periodically research need to be done for better data.

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O-SE14

Tadpoles in Southern Slopes of Mount Merapi after Eruption 2010: Diversity and Threat to Amphibians

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ABSTRACT

In 2010 Mount Merapi had erupted with pyroclastic flows heading towards southern area. This eruption caused a huge damage to Merapi's ecosystem, included the forest and water bodies. Anuran is one order of Amphibian which depends on water for complete of their life cycle, most tadpoles are usually aquatic. Study of Anuran and its tadpoles are urgently required since they are also used as bio-indicator. Tadpoles might used to recognize the species because it was easier to find in their habitats than the adult. This research was carried out from May-July 2011 in Plawangan and Turgo Hills which located in southern slopes of Mount Merapi. Tadpoles were taken from water bodies captured by fish-net, preserved in ethanol 96% and identified in Laboratory of Animal Taxonomy Faculty of Biology Universitas Gadjah Mada using Iskandar (1998), Manthey and Grossmann (1997). Based on this research five species of Anuran tadpoles from four different families were recorded. The highest frequency appeared is *Limnonectes kuhlii* and the lowest frequency is *Megophrys montana.* Tadpoles of endemic species in Java (*Limnonectes kuhlii, Megophrys montana* and *Rhacophorus* margaritifer) has been found. It indicated that they may survive after eruption. Potential threats to tadpoles are drought of waterfalls and stream, human waste products also construction of water reservoir which may disrupt breeding sites and the life cycle of amphibians. Water management and ecology-based water education are needed to local communities to conserve amphibians in Mount Merapi area.

Keywords: tadpoles, Mount Merapi, eruption 2010

INTRODUCTION

The southern slopes of Mount Merapi are a part of Mount Merapi National Park. This region become water reservoir area, buffer system of life also springs to life in the surrounding communities and ecosystems in Sleman, Boyolali, Klaten and Magelang Regency. Plawangan-Turgo Nature Reserves including to the southern slopes of Mount Merapi. Plawangan Hill and Turgo Hill has a hilly topography with an altitude 900 - 1700 meters above sea level and has a submontane forest type (Binarwan, 2008).

Anuran is one order of Amphibian which depends on water for complete their life cycle, since the tadpoles usually aquatic. Anuran plays an important role in an ecosystem food chain, either as prey or predators, and used as bio-indicators. Tadpoles play a key role

in determining the abundance of algae in a pond or water bodies, and mediates the flow of nutrients from aquatic habitats to terrestrial habitats. Anuran becomes a major food source for several species of birds, mammals, and other large predators (Pierce, 1985).

According to Gregoire (2005), Anuran larvae might used to recognize the species because it easier to find in their habitats than adult. Research on early life stages of Anuran in Indonesia is still very little known, especially in Java (Inger *cit.* Iskandar, 1998). In 2006, Eprilurahman have studied the morphological characters and habitats of the southern slopes of Mount Merapi tadpoles. However, monitoring the types of Anuran tadpoles in southern slopes of Mount Merapi needs to be done continuously, especially after the eruption in 2010.

This research was aimed to determine the type of Anuran tadpoles in the southern slopes of Mount Merapi after the eruption in 2010. The results are the Order Anuran tadpoles descriptions based on morphological characters and habitat. The research is expected to be used as a guide and information about the diversity of Order Anuran members in the southern slopes of Mount Merapi after the eruption. This also can be used as a conservation effort, either Anuran tadpoles or adults, and their natural habitats.

MATERIALS AND METHODS

Inventory of Anuran tadpoles were carried out during the months of May to July 2011. Sampling was conducted at Plawangan Hill which includes Telaga Putri, Telaga Muncar and Petak Pitu, Turgo Hill which includes Siraman Lanang, Siraman Wedhok and Siraman Candi, and Kali Kuning. Quantitative samplings of Amphibian larvae method were used to get the data of Anuran tadpoles (Heyer *et al.*, 1994). Tadpoles were caught using fishing net, then photographed their distinguishing characteristics following their natural habitats. Tadpoles were preserved using ethanol 96% and then identified with D.T. Iskandar (1998) also Manthey and Grossman (1997). Nomenclature in this research follows Iskandar (2006). Environmental parameters are taken include water temperature, air temperature, and pH of the water. The results of tadpoles types based on morphological characters and habitat were analysed descriptively.

RESULT AND DISCUSSION

Based on this research, five species of Anuran tadpoles from four different families were recorded. Three of them are endemic to Java. The type and location of encounter Anuran tadpoles are presented in Table 1.

Table 1. Diversity Anuran tadpoles southern slopes of Mount Merapi

No.	Family	Species	TP	TM	PP	SL	SW	SC	KK
1.	Dicroglossidae	*Limnonectes kuhlii**	-	-	-	√	√	√	-
		Limnonectes microdiscus	-	-	-	√	√	-	-
2.	Megophryidae	*Megophrys montana**	-	√	-	√	-	-	-
3.	Ranidae	*Hydrophylax chalconotus*	√	-	√	-	-	-	√
4.	Rhacophoridae	*Rhacophorus margaritifer**	-	√	-	-	√	-	-

Description: TP = Telaga Putri, TM = Telaga Muncar, PP = Petak Pitu, SL = Siraman Lanang, SW = Siraman Wedhok, SC = Siraman Candi, KK = Kali Kuning, √ = common, − = not found, * = endemic to Java

On the Plawangan Hill found three types of tadpoles, i.e *Megophrys montana* and *Rhacophorus margaritifer* in Telaga Muncar and *Hydrophylax chalconotus* in Telaga Putri and Petak Pitu. *M. montana* found in streamy calm water bodies that flows from Telaga Muncar. Around Telaga Muncar contained logs and fallen trees. *R. margaritifer* was found in Telaga Muncar which currently forms a small pool of water. Landslides and fallen trees caused by tectonic and thermal cloud were situated surrounding Telaga Muncar area. While *H. chalconotus* found in a small river that flows in Petak Pitu and Telaga Putri. Water bodies in Petak Pitu were filled with sand and stone, from small to medium sized stones. Riparian vegetation were seen in the area and dominated by bamboo plants and grass. Telaga Putri area were still covered with grass, shrubs and herbaceous plants, while Telaga Putri filled with sand material.

On the Turgo Hill found four types of tadpoles, i.e *M. montana* in Siraman Lanang, *L. kuhlii* in Siraman Lanang, Siraman Wedhok, and Siraman Candi, *L. microdiscus* in Siraman Lanang and Siraman Wedhok, *R. margaritifer* in Siraman Wedhok. Siraman Lanang flanked by cliffs. *M. montana*, *L. kuhlii* and *L. microdiscus* were found in small stream which flowing calm and on the flow of water dammed by the Turgo citizens. *L. kuhlii* and *L. microdiscus* were found in small streams, while *R. margaritifer* found in pools formed by water flow of Siraman Wedhok. Vegetation in Siraman Lanang and Siraman Wedhok which are often found is *Caliandra* sp., ferns, epiphytes, grasses and herbaceous plants. Water sources and water bodies on Turgo Hill are still in good condition after the eruption of Mount Merapi, so Anuran can survive and proliferate at this location.

On the Kali Kuning we only found *H. chalconotus* tadpoles which found in temporary pools and ponds formed by the flow of small rivers. Most of the Kali Kuning region was damaged affected by hot clouds of volcanic ash and rivers filled with volcanic material, ranging from sand to large stones.

Tadpoles which are most common to be found, namely *L. kuhlii* encountered at three research sites in Turgo Hill and *H. chalconotus* encountered on the Plawangan Hill, the Turgo Hill and Kali Kuning. While the tadpoles are rarely found is *M. montana*, which is only found in Telaga Muncar and Siraman Lanang. *R. margaritifer* only found in two locations, namely Telaga Muncar and Siraman Wedhok, but the number of individuals encounter higher than *M. montana*.

Based on research conducted Eprilurahman (2006), there are eight types of tadpoles on the southern slopes of Mount Merapi, which is *M. montana*, *Leptobrachium haseltii*, *Rana hosii* (*Odorrana hosii*), *R. chalconota* (*H. chalconotus*), *L. microdiscus*, *L. kuhlii*, *R. reinwardtii* and *R. javanus* (*R.margaritifer*). Three species which are not found in the research in 2011 was *L. haseltii*, *R. hosii* and *R. reinwardtii*. On the research carried out by Eprilurahman (2006), *L. haseltii* can be found on water bodies in Petak Pitu, *R. hosii* can be found on water bodies in Petak Pitu and Telaga Muncar, while *R. reinwardtii* can be found in the pool on the top of Plawangan Hill. Research in 2011 did not cover all types of tadpoles because there are still many unknown breeding sites.

Anuran tadpoles descriptions encountered in three study sites on the southern slope of Mount Merapi are as follows:

1. *Limnonectes kuhlii* Tschudi, 1838

   Tadpoles are benthic, found in the river that flowing quietly. Characteristic of tadpoles is rhomboid-shaped body, body and tail muscles are light brown colour, tail muscles with dark brown horizontal bands, there are a pair of white nodule in the ventral part of the body, tail fin has a low arch with a tapered tip. Oral apparatus located at anteroventral, nares near the snout, vent tube in dexter, eyes position on the dorsal, spiracles sinister. Labial tooth row formula: I+5-5/1-1+II.

2. *Limnonectes microdiscus* Böttger, 1892

   Tadpoles are benthic, found in the river that flowing quietly. Characteristic of tadpoles is oval and flattened body, body and tail muscles are light brown colour, tail muscles and fins with black patch, tail fin has a low arch with a tapered tip. Oral apparatus located at anteroventral, nares near the snout, vent tube in dexter, eyes position on the dorsal, spiracles sinister. Labial tooth row formula: I+1-1/1-1+II.

3. *Megophrys montana* Kuhl & Van Hasselt, 1822

   Tadpoles are neustonic, found in the river that flowing quietly. Characteristic of tadpoles is longitudinally and flattened body shape, body and tail muscles are black, tail fin has a low arch with a tapered tip. Oral apparatus widened into lateral with bi-triangular shapes, nares near the snout. Vent tube in medial, eye position on the lateral, spiracles sinistral.

4. *Hydrophylax chalconotus* Schlegel, 1837

Tadpoles are benthic, found in temporary pools and ponds formed by the flow of small rivers. Characteristic of tadpoles is oval and flattened body, body and tail muscles are dark brown, there is a pair of white nodule in the ventral part of the body, tail fin has a low arch with a tapered tip, oral apparatus located at anteroventral, nares near the eye. Vent tube in dexter, eyes position on the dorsal, spiracles sinistral. Labial tooth row formula: I +3-3/1-1+ III.

5. *Rhacophorus margaritifer* Schlegel, 1837
   Tadpoles are benthic, found in pools formed by water flow. Characteristics of tadpoles are oval and flattened body shape, body and tail muscles are light brown, ⅔ tail muscle and the posterior part of caudal fin with dark spots, tail fin has a low arch with a tapered tip. Oral apparatus located at anteroventral, nares near the snout. Vent tube in dexter, eyes position on the dorsal, spiracles sinistral. Labial tooth row formula: I +5-5/III.

Measurement of environmental parameters included water temperature, air temperature and pH of water conducted during the study are presented in Table 2.

Table 2. Environmental parameters on the southern slopes of Mount Merapi

No.	Location	Water temperature (°C)	Air temperature (°C)	pH of water
1.	Telaga Putri	21	19-24	8.3
2.	Telaga Muncar	19-20	19.5-20	8.6-9.2
3.	Petak Pitu	21	20.5-22	7.9-8.1
4.	Siraman Lanang	20.5	20	8.1-9
5.	Siraman Wedhok	19	21	8.1-8.4
6.	Siraman Candi	21	20.5	8
7.	Kali Kuning	22	20.5	7.8-8.3

Tadpoles inhabit aquatic habitats that extremely varied both the temperature and pH of water. Temperature plays an important role in the physiology, ecology and behavior of tadpoles, such as the level of development and body size at metamorphosis (McDiarmid and Altig, 1999). According to Goin *et al.* (1978), temperature tolerance for amphibians is in the range 3.0 to 41.0 ^{o}C. Low pH can inhibit the growth of larvae and metamorphosis. Based on research conducted by Pierce (1985) on Amphibian tolerance to acidity habitat, most species die at pH value close to 4. Measurements of water temperature at the time of sampling ranged from 19 to 22 ^{o}C, while the air temperature ranged from 20.5 to 21 ^{o}C. Measurement of water pH at the research site ranged from 7.8 to 9. Based on measurements of water temperature, air temperature and pH of water at the research site,

can be seen that the area of southern slopes of Mount Merapi still have habitat or breeding sites that suitable for Anuran tadpoles development after an eruption in 2010.

Eruption that occurred in 2010 caused significant damage to the ecosystems in the southern slopes of Mount Merapi. The damage caused a threat to the Amphibian, among others, dry springs, ponds and rivers. Dryness of water source is a real threat for Anuran, because they need water to complete their life cycle. Remaining water sources can trigger the gathering of Order Anuran members to breed in that area. This will increase the risk of predators and tadpoles competition to get the nutrients. Volcanic material that accumulates in water bodies also tends to increase the acidity of water, so the tadpoles cannot develop properly. Loss or damaged tress caused the microclimate on the southern slopes of Mount Merapi increased and caused the loss of shelter for adult Anuran.

The next threat comes from human activity. Local citizens tend to build water installation in the spring to meet the daily water needs. Construction of this water installation generally use plastic gutter systems and enclosed container, thus minimize the opportunity for Anuran to breed in water bodies. In addition, plastic gutter systems does not allow for recycling nutrients from the environment that required for tadpoles growth. Water installation with plastic gutter systems and enclosed container can be found in Siraman Lanang, Telaga Muncar and Kali Kuning.

Based on this research, it is necessary management needs to support life cycle of Order Anuran members in the southern slopes of Mount Merapi. Activities that can be done include maintaining the cleanliness of the nature reserve and natural park from inorganic waste, keeping the water flow and water installation on the springs periodically and apply ecology-based water education to maintain the continuity and sustainability of the Order Anuran in the southern slopes of Mount Merapi.

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O-SE15

The Structure of Snake's Skin and Its Role on Locomotion

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ABSTRACT

The skin of the snakes has many important functions including in its locomotion. During snake's locomotion, the skin produces friction along its body against the substrate so the snake's body can move forward. For this purpose, snake's skin have to be strong but pliable. The aims of this research are to study the structure of snake's skin which lives in two different habitat (terran and arboreal) and also to determine the importance of these structure during their locomotion in each habitat.

Two species of snakes were obtained from snakes' seller in Imogiri. Snake skin were observed macroanatomically (scale shape and scale attachments) and microanatomically (tissue composition particularly in dermal layer). The result showed that terran snakes have thicker skin than the arboreal snake particularly in its dermal layer. The differences also can be found in the composition of the dermal layer and the arrangement of collagen fibers in each species. Terran snakes have more collagen fibers than arboreal snakes hence the thicker skin in terran snakes. Collagen fibers in terran snakes were arranged into three different layers which are, longitudinal, transversal and diagonal while in arboreal snakes the collagen fibers only can be found in transversal form. This structure allows arboreal snake to have more maneuverability so arboreal snakes can move freely in its environment. In terranian habitat, velocity is more important than maneuverability hence the thicker, stronger and less pliable skin is advantageous for terran snakes.

Keywords : Snake's skin, Terran, Arboreal, Collagen Fibers, Locomotion

O-SE16

SKELETON OF CAUDAL FIN IN SKIPPER, ROCKSKIPPER AND MUDSKIPPER

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ABSTRACT

Skipper is one group of fish in the intertidal zone who spent most of their life on the terrestrial. Skipper is divided into two groups, rockskipper and mudskipper. Rockskipper use the crevices of coral as habitat while mudskipper using mud as a habitat. Differences between skipper habitat of these two groups caused a structural and functional adaptation in the skeleton structure of the caudal fin. Caudal fin is one organ that is used by most of the fish to help the movement. This research aims to determine differences in the structure of the caudal fin skeleton mudskipper and rockskipper as an adaptation to terrestrial. Rockskipper species used are *Andamia reyi* while mudskipper was *Periophthalmus gracillis*. Staining results of Alcian blue-Alizarin red's showed that *Periophthalmus gracillis* caudal fin has 14 branched rays (7 upper lobe, 7 lower lobe), 6 (upper lobe) and 6 (lower lobe) segmented rays, and 5 (upper lobe) and 10 (lower lobe) unsegmented rays. Six rays on the lower lobe have a branch more than six rays in the upper lobe. In *Andamia reyi* caudal fin have 6 segmented rays in each lobe and 6 unsegmented rays, 3 on the upper lobe and 3 on the lower lobe. In rockskipper rays do not have a branch at the tip. In both skipper, hypural divided into two parts, the superior and inferior.

Keywords : skipper, skeleton of caudal fin, *Periophthalmus gracillis, Andamia reyi*

O-SE17

DIVERSITY OF MAMMALS IN KALIKI LOWLAND, MERAUKE – PAPUA

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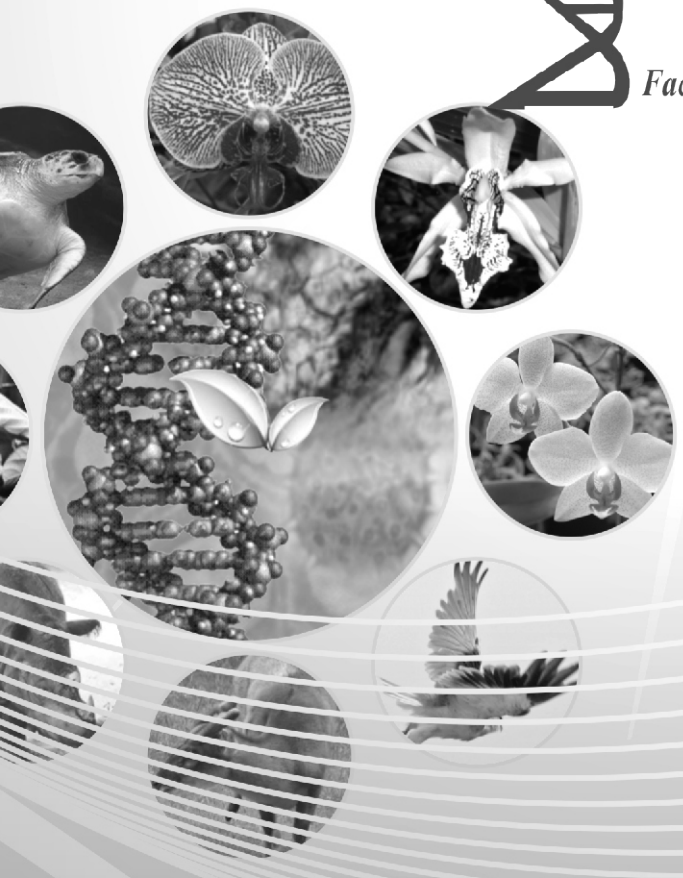
ORAL - TOPIC 4

Physiology and Developmental Biology (O-PD)

ICBS 2011

Faculty of Biology Universitas Gadjah Mada



FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

O-PD01

THE EFFECT OF PLANT SUBMERGENCE AT DIFFERENT GROWTH STAGES AND LEVEL OF NITROGEN FERTILIZER ON GROWTH AND GRAIN YIELD OF RICE (*Oryza sativa* L. cv. Sintanur)

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ABSTRACT

Rice is a crop that can grow in flooded conditions. Water management and nutrients enrichment are important factors on increasing rice production. Submergence of plants for varying duration and depth that occurs at different growth stages resulting in unpredictable yield losses. Nitrogen is an essential nutrient to increase plant growth and productivity. An adequate supply of nitrogen to the plants during their early growth period is very important for the initiation of leaves and tillers. We examined the effect of plant submergence at different growth stages for various durations and level of nitrogen fertilizer application on growth and grain yield of rice (*Oryza sativa* L.cv. Sintanur). The experiment was arranged in complete randomized design with five replicates for each treatment. The plants were grown in pots and were subjected to nitrogen fertilizer at concentration 0, 0.5, 1 and 1.5 g/pot applied before submergence. Submergence treatment was conducted at different growth stages i.e. without submergence (control); 10 days submergence at the vegetative phase; 10 days submergence at the reproductive phase; 10 days submergence at the vegetative and reproductive phase. The submergence was 4-cm in depth from the surface. The observed parameters were plant height, number of tillers, flowering age, number of filled and unfilled-grains per panicle, precentage of filled-grain, 1000-grain weight, plant biomass, root shoot ratio, pH, and the nitrogen contents in the soil. The result showed that nitrogen fertilizer treatment had significant effect on the plant growth. The number of filled-grains increased with increasing the level of nitrogen fertilizer application. Submergence treatment on the vegetative and reproductive phase with 0.5 g/pot nitrogen dosage increased the growth and productivity of rice (*Oryza sativa* L.cv.Sintanur) include number of tillers, precentage of filled-grain, dry weight of root, dry weight of shoot, and shortened of flowering time.

Keywords : rice (*Oryza sativa* L.cv. Sintanur), nitrogen fertilizer, submergence period

INTRODUCTION

Plant growth in agricultural soils is influenced by many abiotic and biotic factors. There is a thin layer of soil surrounding plant roots that is an extremely important and active area for root activity and metabolism. Plant roots are crucial for the absorption and translocation of water and nutrients. An adequate supply of nitrogen to the plants during their early growth period is very important for the initiation of leaves and florets primordia (Tisdale and Nelson, 1984). Nitrogen encourages vegetative growth and improve yield and grain quality by increasing the number of tillers, leaf area development, the formation of grain, grain filling and protein synthesis (Below, 2002; Barker & Pilbeam, 2007). Earlier studies reveal that

proper use of nitrogen fertilizer can markedly increase the yield and improve the quality of rice (Weeraratna,1981).

Among agricultural crops, rice has the ability to germinate and grow under extremely low oxygen conditions. Submergence of crops for varying duration and depth occurs at different growth stages resulting in unpredictable yield losses. Use of nitrogen fertilizer has led to significant improvement in crop yield (Chaturvedi, 2005). Submergence-induced internode elongation and the maximum length that adult plants can reach varies amongst the different rice cultivars (Keith *et al*., 1986; Das *et al*., 2006). Nitrogen and phosphorus availability and assimilation can influence submergence responses and have implicated in differences in tolerance between cultivars (Jackson & Ram, 2003).

During submergence, plant survival is greatly affected by depth of water and by its physico-chemical characteristics (oxygen and carbondioxide concentration, pH, degree of turbidity, temperature, etc (Sarkar *et al*., 2006). Submergence effect is very complex phenomenon that varies with genotype and pretreatment, carbohydrate status before and after the submergence, development stage of the plant when submergence occurs, the level and duration of submergence and degree of water turbidity. Morphologically and physiologically, the submergence effect can be characterized by leaf chlorosis, stunted growth, and the death of the whole plant tissue (Jackson and Ram, 2003; Das *et al*., 2005).

Rice grow well in flooded soils due to the ventilation efficiency that is acquired by formation of air spaces within the tissue to improve the exchange of gases between the submerged plant part and the atmosphere. Moreover, submergence induces the formation of adventitious root mediated by ethylene that also appeared to facilitate aerenchyme formation (Justin and Armstrong, 1991). In this study, we examined the effect of plant submergence at different growth stages for various durations and level of nitrogen fertilizer application on growth and grain yield of rice (*Oryza sativa* L. cv.Sintanur).

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of Faculty of Biology UGM, Yogyakarta on April 2010 to August 2010. The rice seed (*Oryza sativa* L. cv. Sintanur) was obtained from Assessment Institute for Agricultural Technology, Daerah Istimewa Yogyakarta (AIAT DIY), Indonesia. Urea was used as a source of nitrogen fertilizer. Physico-chemical properties of the soil were measured by the standard methods of soil chemical analysis. The experiment was arranged in a complete randomized design with five replications for each treatment.

Rice seeds were sown in tray containing a mixture of soil and farmyard manure in a 3:1 ratio. Fourteen day-old seedlings were transplanted (two seedlings per pot) in circular

plastic pots (30 cm height and 24 cm diameter) contaning 5 kg of soils. Ten days after transplanting, plants were thinned to 1 seedlings per pot. The urea as nitrogen source at different doses : (1) 0 g/pot, (2) 0.5 g/pot, (3) 1.0 g/pot and (4) 1.5 g/pot was applied before transplanting. Plants were then completely submerged and the water depth was maintained at 4 cm from the soil surface. The submergence treatment were (A) control, in which the soil relative water content was maintained at 70–80% of pot holding capacity / not submerged; (B) 10 days submergence at the vegetative phase; (C) 10 days submergence at the reproductive phase; and (D) 10 days submergence at the vegetative and reproductive phase. After submergence treatment, the availability of water during the growth of rice maintained at field capacity conditions.

The observed parameters were plant height, number of tillers, flowering time, number of filled and unfilled-grains per panicle, precentage of filled-grain, 1000-grain weight, plant biomass, root shoot ratio, pH, and the nitrogen contents in the soil. Plant height and number of tillers were observed every week. Number of filled and unfilled-grains per panicle, precentage of filled-grain, 1000-grain weight, plant biomass and root shoot ratio were determined after harvest. While the nitrogen contents in the soil were determined before and after submergence treatment.

Data was statistically analyzed according to Gomez & Gomez (1984) and means were compared between treatments by Duncan Multiple Range Test (DMRT) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Result of the experiment conducted to study the effect of plant submergence at different growth stages for various durations and level of nitrogen fertilizer application on the growth and yield of rice (*Oryza sativa* L. cv.Sintanur) are reported. The submergence and nitrogen fertilizer treatment had significant effect on the plant growth at different growth stages.

The plants subjected to submergence showed higher plant height as compared to those grown in which the soil relative water content was maintained at 70–80% of pot holding capacity and increased with increase in duration of submergence (Table 1). Plant height reveals the overall vegetative growth of the crop in response to various management practices. Submergence enhanced the elongation of the shoots through the increase in shoot length. The increase in plant height in response to application of nitrogen fertilizer is probably due to enhanced availability of nitrogen which enhanced shoot elongation.

Table 1. Plant height of rice (*Oryza sativa* L. cv. Sintanur) at 12 weeks after submergence at different growth stages and level of nitrogen fertilizer

Submergence treatment	Dose of Nitrogen				
	0 g/pot	0.5 g/pot	1.0 g/pot	1,5 g/pot	Average
A	98.17 ± 2.21^{ab}	109.50 ± 9.08^{bc}	109.68 ± 8.17^{bc}	104.60 ± 9.22^{bc}	105.49 ± 5.41^{x}
B	104.62 ± 2.78^{bc}	113.62 ± 8.69^{c}	107.92 ± 1.61^{bc}	106.62 ±13.59^{bc}	103.20±10.89^{x}
C	101.38 ± 3.09^{bc}	107.38 ± 5.04^{bc}	107.12 ± 5.36^{bc}	101.38 ±14.77^{bc}	104.32 ± 3.39^{x}
D	107.50 ± 4.65^{bc}	107.45±10.73^{bc}	99.38 ± 7.11^{abc}	103.50 ±13.43^{bc}	104.46 ± 3.87^{x}
Average	102.92± 4.03^{x}	109.49 ± 2.93^{x}	101.03 ± 9.77^{x}	104.03 ± 2.19^{x}	104.37

Values are means of five replicates ± standard errors. Means within the column and row followed by different letters differ significantly at the 5% probability level DMRT.

From the results obtained can be seen that doses of nitrogen fertilizers are most responsible for driving the growth of plant height in rice varieties Sintanur is the treatment of 1 g/pot in all treatment of submergence (Figure 1). Whereas high level of nitrogen tend to decrease plant height. Our result clearly suggest that high N uptake by plants was partitioned more assimilates to leaves and thus resulting number of tillers (Table 2).

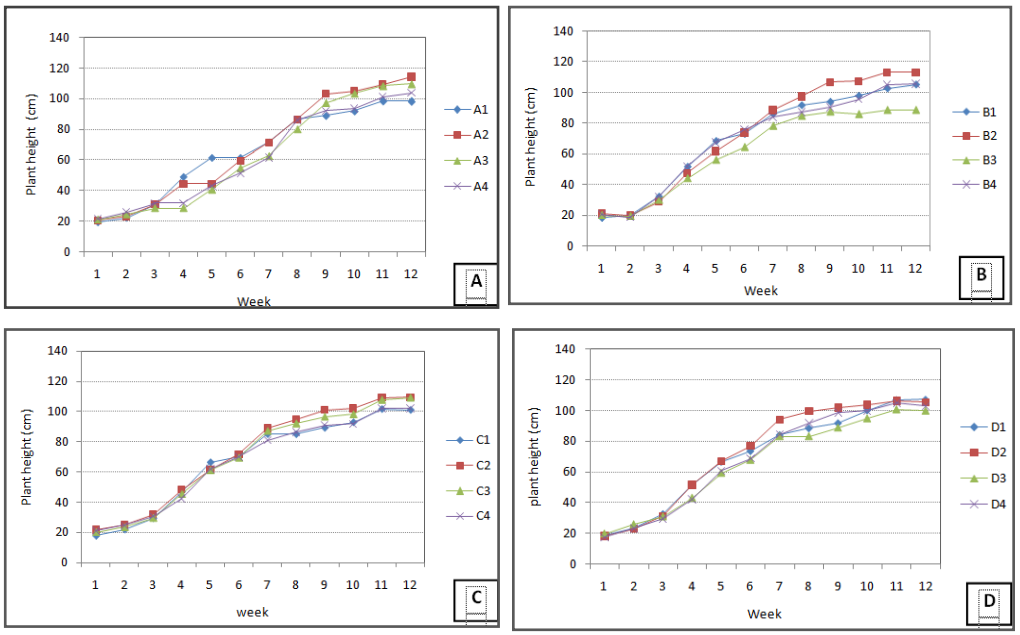

Figure 1. Plant height of rice (*Oryza sativa* L. cv.Sintanur) in submergence treatment. (A). control, (B) 10 days submergence at the vegetative phase; (C) 10 days submergence at the reproductive phase; and (D) 10 days submergence at the vegetative and reproductive phase.

Plant height was positively correlated with plant biomass in the submergence and nitrogen fertilizer treatments as shown in Figure 2. This suggests that the yields of photosynthesis are used for increasing plant height. An increase in plant height is a result of

stem elongation as a response to the submergence tolerance of plants (Jackson and Ram 2003; Kawano *et al*, 2002).

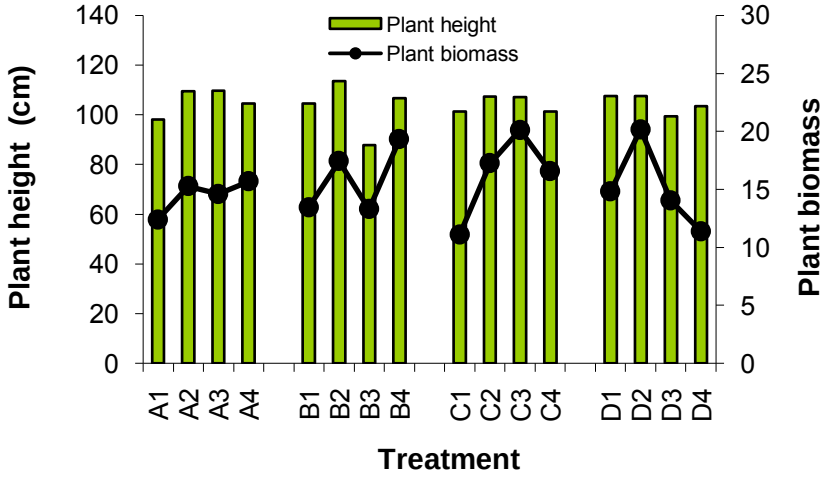

Figure 2. The correlation between plant height and plant biomass of *Oryza sativa* L. cv. Sintanur after submergence treatment at different growth stages and level of nitrogen fertilizer

Nitrogen fertilizer application significantly increased number of tillers (Table 2). Number of tillers per unit area is the most important component of yield. More the number of tillers, especially fertile tillers will increase the yield. Increasing the number of tillers might be due to the increased availability of nitrogen plays an important role in cell division. Nitrogen is a major component in the synthesis of proteins, therefore nitrogen needed in the vegetative phase of plants, especially in the process of cell division. An adequate nitrogen during the vegetative phase of growth encouraged tillering which had a bearing on the number of panicles per plant.

Submergence at the vegetative phase would increase the number of tillers in rice. Water requirements for rice in early vegetative phase is critical because the vegetative phase is the active phase of seedling establishment and maximum tillering (Vergara, 1976). In addition to submergence, the dose of nitrogen fertilizer also affects the number of tillers formed. From the results of this study the maximum tillers formed is on the submergence treatment vegetative and reproductive phases with a dose of nitrogen fertilizer 0.5 g/pot. This suggests that the dose of 0.5 g/pot was the optimum dose in increasing the number of tillers produced in rice (*Oryza sativa* L. cv. Sintanur). Also, it can note that the submergence at the vegetative phase was also influential in increasing the number of tillers.

Table 2. The number of tillers of rice (*Oryza sativa* L. cv. Sintanur) after submergence at different growth stages and dose of nitrogen fertilizer

Submergence treatment	Dose of Nitrogen				
	0 g/pot	0.5 g/pot	1.0 g/pot	1.5 g/pot	Average
A	2.00 ± 0^{ab}	2.00 ± 0^{ab}	2.25 ± 0.5^{ab}	4.00 ± 2.16^{de}	2.56 ± 0.97^{xy}
B	2.50 ± 0.58^{abc}	2.50 ± 0.58^{abc}	4.00 ± 1.15^{de}	3.75 ± 0.96^{cde}	3.19 ± 0.80^{xy}
C	1.50 ± 0.58^{a}	2.50 ± 0.58^{abc}	3.75 ± 0.96^{cde}	3.25 ± 1.5^{bcd}	2.75 ± 0.98^{xy}
D	2.25 ± 0.5^{ab}	4.75 ± 0.50^{e}	3.75 ± 0.5^{cde}	3.25 ± 0.5^{bcd}	3.50 ± 1.04^{xy}
Average	2.06 ± 0.43^{x}	2.94 ± 1.23^{xy}	3.44 ± 0.80^{xy}	3.56 ± 0.38^{y}	3.00

Means within the column and row followed by different letters differ significantly at the 5% probability level DMRT.

The submergence and nitrogen fertilizer gave significant effect on flowering time of rice (*Oryza sativa* L. cv. Sintanur). In the treatment without submergence and submergence on the vegetative phase, the flowering time become longer by increasing the doses of nitrogen fertilizer (Table 3). In the treatment of submergence on the reproductive phase there was no significant differences in flowering time. While the submergence treatment on vegetative and reproductive phases, the application of nitrogen fertilizer 0.5 g/pot had a significant effect in shortening the time of flowering. From the results obtained showed that the treatment of submergence for 10 days at vegetative and generative phases significantly shortened of flowering time. This suggests that rice plant get adequate water supply for flowering. Water needs for rice crop is very important during the formation of tillers and panicle initiation to grain filling (Vergara, 1976).

Table 3. Flowering time of rice (*Oryza sativa* L. cv. Sintanur) with different submergence at different growth stages and dose of nitrogen fertilizer

Submergence treatment	Dose of Nitrogen Fertilizer				
	0 g/pot	0.5 g/pot	1.0 g/pot	1,5 g/pot	Average
A	64.00 ± 1.41^{abc}	64.25 ± 0.50^{bc}	64.50 ± 1.73^{bc}	68.25 ± 3.86^{d}	65.25 ± 2.01^{y}
B	64.75 ± 0.50^{bc}	64.50 ± 0.58^{bc}	68.25 ± 3.50^{d}	65.75 ± 2.22^{cd}	65.81 ± 1.71^{y}
C	65.00 ± 0.82^{bcd}	63.50 ± 0.58^{abc}	65.50 ± 3.11^{cd}	66.25 ± 2.63^{cd}	65.06 ± 1.16^{y}
D	62.00 ± 1.63^{ab}	61.00 ± 1.41^{a}	63.75 ± 0.5^{abc}	62.25 ± 2.22^{bcd}	62.25 ± 1.14^{x}
Average	63.94 ± 1.36^{xy}	63.31 ± 1.60^{xy}	65.50 ± 1.97^{y}	65.63 ± 2.50^{y}	64.59

Means within the column and row followed by different letters differ significantly at the 5% probability level DMRT.

Percentage of filled-grain is an important parameter to determine the yield of rice. The number of filled-grains increased with increasing level of nitrogen fertilizer application. However, excess nitrogen / high level of nitrogen will produced panicles with unfilled-grain therefore the number of filled-grain has decreased. The number of unfilled-grain will also increase with increasing doses of nitrogen fertilizer (Table 4). Shallow submergence (4 cm) will give higher yields than the intermediates (8 cm), or deep submergence (18 cm) (Teare and Peet, 1983). Therefore, the maximum rice production will be achieved by shallow submergence of 4 cm.

Table 4. Number of filled and unfilled-grains per panicle, precentage of filled-grain, 1000-grain weight of rice (*Oryza sativa* L. cv. Sintanur) with submergence at different growth stages and dose of nitrogen fertilizer

Treatment	Number of filled-grain	Number of unfilled- grain	Precentage of filled-grain (%)	1000-grain weight (g)
A1	178.75 ± 55.02^{a}	42.75 ± 5.06^{abc}	79.50 ± 5.01^{bcd}	23.3
A2	236.50 ± 62.79^{abc}	72.75 ± 52.12^{abcde}	77.00 ± 11.96^{bcd}	20.3
A3	256.25 ± 27.11^{abcd}	68.00 ± 21.32^{abcde}	78.75 ± 5.58^{bcd}	20.0
A4	237.75 ± 113.04^{abc}	109.00 ± 20.96^{cde}	71.75 ± 10.55^{ab}	18.7
B1	220.75 ± 58.69^{ab}	39.50 ± 13.40^{ab}	84.00 ± 3.75^{cd}	20.6
B2	322.00 ± 28.42^{bcd}	98.35 ± 40.60^{bcde}	77.00 ± 8.32^{bcd}	20.5
B3	229.50 ± 108.33^{abc}	34.75 ± 22.10^{ab}	86.75 ± 5.47^{d}	21.0
B4	379.00 ± 44.08^{d}	130.50 ± 21.33^{de}	74.00 ± 2.57^{bc}	20.7
C1	174.25 ± 38.35^{a}	26.00 ± 13.74^{a}	86.75 ± 4.31^{d}	20.1
C2	280.00 ± 59.58^{abcd}	70.00 ± 40.16^{abdce}	80.00 ± 10.98^{bcd}	20.8
C3	358.25 ± 70.49^{cd}	64.50 ± 14.82^{abcd}	84.25 ± 0.68^{cd}	20.6
C4	291.75 ± 156.68^{abcd}	78.25 ± 79.08^{abcde}	83.25 ± 10.45^{bcd}	21.9
D1	267.75 ± 31.46^{abcd}	57.75 ± 21.09^{abc}	82.00 ± 5.84^{bcd}	21.7
D2	351.75 ± 30.58^{cd}	53.00 ± 20.31^{abc}	86.75 ± 3.43^{d}	20.5
D3	244.25 ± 51.21^{abc}	52.50 ± 34.41^{abc}	83.25 ± 6.74^{bcd}	21.8
D4	249.00 ± 125.46^{abc}	133.50 ± 41.84^{e}	63.25 ± 7.19^{a}	20.5

Means within the same column followed by different letters differ significantly at the 5% probability level DMRT.

Nitrogen fertilizer and submergence treatment influenced the dry weight of root and shoot, but had no effect on root-shoot ratio (Table 5). In this study a high level nitrogen treatment causes the roots have a greater dry weight of grain than the other treatments. In addition to nitrogen, submergence can also affect the formation of roots. According to Shimamura *et al*., (2007), morphological adaptations of plants in flooded conditions is by forming adventitious roots.

Table 5. Plant biomass and root shoot ratio of rice (*Oryza sativa* L. cv. Sintanur) after submergence at different growth stages and dose of nitrogen fertilizer

Treatment	Plant Biomass		Root Shoot Ratio
	Root (g)	Shoot (g)	
A1	0.975 ± 0.46^{ab}	6.675 ± 1.13^{a}	0.1425 ± 0.06^{a}
A2	1.175 ± 0.51^{ab}	7.550 ± 0.51^{ab}	0.1580 ± 0.07^{a}
A3	0.950 ± 0.44^{ab}	6.950 ± 1.31^{ab}	0.1346 ± 0.04^{a}
A4	1.850 ± 1.24^{b}	8.200 ± 4.19^{ab}	0.2034 ± 0.13^{a}
B1	1.075 ± 0.26^{ab}	7.525 ± 1.73^{ab}	0.1438 ± 0.03^{a}
B2	1.075 ± 0.49^{ab}	8.850 ± 1.10^{ab}	0.1187 ± 0.05^{a}
B3	1.175 ± 0.41^{ab}	6.975 ± 2.52^{ab}	0.1718 ± 0.04^{a}
B4	1.100 ± 0.26^{ab}	9.520 ± 3.23^{ab}	0.1223 ± 0.04^{a}
C1	0.775 ± 0.27^{a}	6.275 ± 2.13^{a}	0.1257 ± 0.03^{a}
C2	1.350 ± 0.51^{ab}	9.125 ± 1.45^{ab}	0.1392 ± 0.04^{a}
C3	1.825 ± 0.72^{ab}	10.200 ± 0.50^{b}	0.1756 ± 0.07^{a}
C4	1.550 ± 1.21^{ab}	8.200 ± 2.87^{ab}	0,1708 ± 0,09^{a}
D1	0.950 ± 0.47^{ab}	7.475 ± 1.62^{ab}	0,1256 ± 0,05^{a}
D2	1.900 ± 0.78^{b}	10.275 ± 2.04^{b}	0,1733 ± 0,05^{a}
D3	0.950 ± 0.26^{ab}	7.400 ± 1.78^{ab}	0,1297 ± 0,03^{a}
D4	0.900 ± 0.52^{ab}	6.325 ± 2.01^{a}	0,1353 ± 0,05^{a}

Means within the same column followed by different letters differ significantly at the 5% probability level DMRT.

In all treatments showed soil pH tends to be stable (range 6-7) during submergence treatment. This is due to submergence treatment in a short time that is 10 days, so that the pH change is not significant. Based on analysis of nitrogen content on soil, NO_3^- content tends to decrease after the application of fertilizer. This indicates that the content of NO_3^- available in the soil have been absorbed by the plants during the acclimatization process. After fertilization, the transformation of NH_4^+ to NO_3^- was slow, so that the content of NO_3^- are measured is still low. After submergence treatment on the vegetative phase can be seen that the content of NO_3^- in submergence treatment will be lower than in treatments without submergence. This is due to the waterlogged soil, the soil becomes anaerobic.

Summarizing the results obtained we could conclude that submergence treatments increase the growth and yield of rice (*O. sativa* L. cv. Sintanur). The best growth of rice (*O. sativa* L. cv. Sintanur) obtained in submergence treatment for 10 days at vegetative and reproductive phases with fertilizer dose of 0.5 g/pot which includes number of tillers, precentage of filled-grain, dry weight of root, dry weight of shoot, and shortened of flowering time.

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O-PD02

Innovation Rice Cultivation with Bio Organic Fertilizer to Increase Productivity of Three Rice Cultivars (*Oryza sativa* L.) In Rainfed Rice Land Beji Village, Ngawen, Gunung Kidul

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ABSTRACT

Chemical fertilizers are widely used by farmers since the early 1980s, known to cause problems in the soil, plants, and the environment in general, although the yields obtained an improvement. The aims of the study were to obtain the right of bio organic fertilizer dose to increase the productivity of rice plants and improve soil quality in rain fed wetland Beji Village, Ngawen, Gunung Kidul. Increased productivity of rice crop is expected to increase farmers' income, improve the quality of life and maintain sustainable development by improving the quality of paddy soil Bejii Village.The research conducted in the wetland rainfed using three commonly planted by farmers of this village (IR-64, Ciherang and Slegreng). Each of these rice cultivars will be planted on rainfed rice fields, each field and rice cultivar given variation fertilization (organic bio fertilizers). The doses were a dose of 15 liters / ha, 10 liters / ha, 5 liters / ha and 0 liters / ha. Each dose was given in the form of basal fertilizer dose of NPK ½ (75 kg / ha). Bio organic fertilizer and NPK fertilizer were given in 3 stages (ages 7, 21 and 35 days after planting) and the manure was given prior to planting. The study design used was Completely Randomized Design in factorial with two factors, there are variation of bio organic fertilizer dose (15 liters / ha, 10 liters / ha, 5 liters / ha, 0 liters / ha) and crop varieties of rice (IR-64, Ciherang and Slegreng). Parameters measured were the growth parameters, environmental parameters, levels Prolin, Levels of Chlorophyll and productivity parameters. Results Data were analyzed with ANAVA followed by DMRT test. The results showed Ciherang varieties with bioorganic fertilizer dose of liquid of 10 liters / ha has the highest productivity in land Tegalrejo. IR 64 rice varieties with a dose of liquid fertilizer bioorganic 15 liters/ha has the highest productivity in land Daguran. IR 64 rice varieties with a dose of liquid fertilizer bioorganic 10 liters/ha has the highest productivity in land bejono.

Keywords: productivity, rice, organic bio fertilizers, Beji Village.

O-PD03

EFFECTS OF SALT STRESS AND CYTOKININ ON GROWTH AND PRODUCTIVITY OF MELON (*Cucumis melo* L. cv Gama Melon Basket) GROWN HIDROPONICALLY.

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ABSTRACT

Melon (*Cucumis melo* L.) is one of cultivated fruits that can be grown hydroponically. The fruit growth and its nutritional value normally can be improved by hormones and stress treatment respectively. The purpose of this research was to evaluate the effect of salt stress and cytokinin on growth and productivity of *Cucumis m*elo L. cv Gama Melon Basket grown hydroponically.

The research was conducted by using factorial design (2 x 4). The first factor was salt stress at of two levels, (EC = 2.5 mS / cm and EC = 5 mS/ cm). The second factor was cytokinin concentration at four levels (control. 0.5 ppm. 1 ppm and 2 ppm). Parameters observed included vegetative and generative growth, total chlorophyll content, sucrose levels, reducing sugar, total carotenoids, vitanim c content and water content of the fruits. Data were analyzed using Anova and Duncan's Multiple Range Test at significancy level of 5%.

The results showed that cytokinin application can maintain the chlorophyll content. In addition, combination of salt stress (EC= 5mS / cm) and cytokinin of 1 ppm increased the fresh weight, sugar reduction, and sucrose content of the fruits, whereas total carotenoids, vitamine C and water content of the fuits were not affected.

Keywords : *Cucumis melo*, salt stress, cytokinin

O-PD04

Effect of Giving Borax *(Diantrium Tetraborate Decahydrate)* During the Period of Organogenesis Against Skeleton, Brain and Kidney Development of Mice Fetuses *(Mus musculus)* strain Balb/C

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ABSTRACT

This study was aimed to identify the effect of borax on the fetal *Mus musculus* strain Balb/C development during organogenesis period. A number of 25 pregnant mice which 2.5 months age was administered with borax by *gavage* 4 times during 6th to 15th day of pregnancy. There are five treatment dose groups consist of: 0; 140; 280; 420; and 560 mg/kg body weight/day. Observations by fetal surgery were done on the 18th day of pregnancy. The observation of this research are: 1) fetal viability, 2) fetal morphology and fetal hemorrhage, 3) skeletal ossification, 4) head development, 5) kidney histology. The result of this research is borax interfere the development of mice fetuses on organogenesis period ($p<0,05$) significantly. To begin at 140 mg/kg of body weight/day dose, borax took effect on fetal developmental disorders such as death and resorption of fetuses, morphological abnormalities such as: oval head, eye do not uppermost, developmental defect on fetal length and weight. At this dose, borax can also cause development defect on eyeballs, hydrocephalus, development defect on skeleton ossification and structural abnormalities of kidney histology such as: enlargement of glomerulus, Bowman's capsule, proximal convoluted tubules and distal convoluted tubules diameter. Whereas 280 mg/kg of body weight/day dose, borax cause bleeding under the skin.

Keywords: borax, development, fetus, organogenesis, mice

INTRODUCTION

Borax *(Diantrium tetraborate decahydrate*) or $Na_2B_4O_7 \cdot 10H_2O$ used in glasses industry such as fiberglass, borosilicate glass, enamel, and glaze. Borax also is used in soap industry and detergent, nuclear installation, low antiseptic, cosmetic, pH buffer, pesticide, and fertilizer[11]. But now, borax is added in food as food addictive because it can repair the texture of food better[3]. Approximately 80%, borax in non production code food[1].

Borax have teratogenic potency with the many character such as: water solution at 100^0C or biological liquid with pH 6-11 counted 65,64%, the molecular weight is 61,83 dalton (<600 dalton), LD_{50} equal to 4500-5000 mg/kg of body weight in rat[4], easy accumulate in tissue of body like heart and ren[9].

Borax can cause riboflavin deficiency[2]. Riboflavin have important function as coenzyme in respiration enzyme system, development and fetal growth, looking after the epithelial mucosa and tissue of the eyes[8]. Borax is fastening ribitil side of riboflavin to form

the complex which water dilution and as an active metabolic. This matter can cause riboflavin deficiency, so that the energy to cell growth was decreasing, because riboflavin is required to produce energy[6]. Borax can also cause death of cell so that is formed necrosis zone. Necrosis zone can disturb organogenesis and cause fetal anatomy retardation[5].

The central nervous system is the first system which develop and differentiation but last in finish. Brain of mouse began to develop at the 7th day of pregnancy. At the 18th day of pregnancy, brain is completely in the form of telencephalon which apart become 2 cerebral lobes, or hemisphere, olfactory lobes, and cerebellum began to form fold character[7]. Vertebrae of mouse is began to develop since 10th day of pregnancy[10]. The origin of vertebrae is schlerotome which consist of an anterior zone and cranial with the low density, and a posterior zone and caudal with high density. The centre of chondrification is looked the first time at early 11th day of pregnancy. The correlation or interrelation is the integral of nervous development network. Chondrification were done actively and some centre ossification appear at 14.5th day of pregnancy. Ossification expand at 16th day of pregnancy[7].

The kidney of mouse development is began at 8th day of pregnancy, with appearance of nephrogenic string. At the 10th day of pregnancy, pronephric tubule and collecting pronephric duct is covered up with coelom. At the 14th day of pregnancy, metanephric consist of collecting tubule, glomerulus and Bowman's capsule. In early of mice development, the kidney is differentiation actively. Kidney is mostly composed of mesenchyme and adkidney glands. The kidney differentiate fully during organogenesis and has functioned, but for a while, the excretion of fetus through the placenta[7].

MATERIAL AND METHODS

Preparation

Both of the 2.5-month-old male and female parents of mice were mated. The average weight of female mice was 20 $\pm$ 2 g. The female parents who were mated were in estrus period. To determine the estrus period of the female mice was performed by using *lavage* method by observing their vaginal cells. A total of 25 pregnant female mice were ready for treatment, divided into five treatment dose groups and for each were 5 repetitions. Borax solution was made by dissolving borax powder into distilled water. Borax solution was given to the mice taken from the stock solution.

Treatment

The treatment was done by giving borax solution to the 25 pregnant mice using *gavage* syringe with a volume of 0.5 ml/20 g body weight, with the dose of: 1) 0, 2) 140, 3) 280, 4) 420 and 5) 560 mg/kg body weight/ day. The treatment dose was determined from the conversion of human daily consumption, conversion of the previous studies and LD_{50}. Borax solution was given during the organogenesis period of the mice (the 6^{th} to 15^{th} day of pregnancy) with 4 times *gavage* that was: the 6^{th}, 9^{th}, 12^{th} and 15^{th} day of pregnancy.

Observation

The mice who have reached the 18th day of pregnancy were dissected by surgery. The observations made include: 1) viability of the fetus, 2) fetus morphology and bleeding under the skin, 4) development of the fetus skeleton ossification, 5) development of the fetus head, and 6) histology structure of kidney fetuses. The data obtained then been analyzed with a statistical analysis of anova (analysis of variance) with significance level of 5%. If the result of the anova showed a significant effect, then was followed by LSD test.

Viability of fetus

The observation of fetal viability was the count of live fetus, dead fetus, and fetus resorption

Morphology and Haemorrhage of Fetus

The observations of morphology included: 1) the length and weight of the fetus, 2) the organs on the head part, 3) the organs on the body and limbs part, and 4) other morphologies. Haemorrhage or bleeding is a discharge of blood from the blood vessels and was accompanied by the accumulation in the body space or in the body tissue. Observation of the bleeding of the lower skin could be observed with the presence of blood accumulation on the lower skin and there would be the frozen red color.

Development of Fetus Skeleton Chondrofication

Observations on the development of the skeleton ossification was done with the skeleton coloring technique using Alizarin red s. Skeleton coloring technique consists of: 1) Evisceration of fetus at 0.9% of NaCl, 2) Fixation of fetus in 96% alcohol for a week, 3) Immersing the fetus in 1% KOH until transparent for 24 hours, 4) Coloring by using alizarin red s 0.01% in 1% KOH for 24 hours, 5) Leaching in KOH 1%, 6) Purification consists of 3 stages, they were: a) KOH + 1% glycerol (3:1) during 24 hours, b) KOH + 1% glycerol (1:1) à 24 hours, and c) KOH + 1% glycerol (1: 3) during 24 hours. Next is storing in the pure glycerol. Bones of vertebrae and members that have undergone ossification (formation of

hard bone) were indicated by red in color. Parameters observed include the development of ossification on the vertebrae, metatarsus and metacarpus, and also the presence of other ossification developmental abnormalities.

Development of Fetus Head

Observation of the development of the head was done by using the razor blade sectioning technique that was immersing the fetuses in the Bouin solution for 1 week. After 1 week of immersion, then there would be series slices on the fetal head by using a razor knife and dividing it into 4 slices. The first slice was the slice that passes through the eyeball; the second through the palate, the third through the cerebrum, and the fourth through the hindbrain. Observations were made by using a stereo microscope.

Histology Structure of Kidney's Mice Fetuses

The observation of histology was by observing histology structure of kidney slides which was made using the paraffin technique and coloring with haematoxylin eosin.

RESULTS AND DISCUSSION

Base on Anova test is known that borax can cause some abnormal development significantly ($p<0,05$). LSD test was used to determine the first dose that giving effect to abnormal development, and is shown on table 1 until 5.

Table 1 Percentage of total life, death and resorption fetuses from parent who has given borax

Dose (mg/ kg of body weight/ day)	∑ Parent	∑ Implantation	∑ Fetus			%Fetus			Body long rate of fetus (mm)	Weigh rate of fetus (g)
			life	death	resorption	life	death	resorption		
0	5	45	45	0	0	100 [a]	0 [a]	0 [a]	19,62 [a]	0,76 [a]
140	5	38	20	6	12	52,63 [b]	15,79 [b]	31,57 [b]	14,05 [b]	0,53 [b]
280	5	35	15	6	14	42,86 [bc]	17,14 [b]	40 [bc]	12,23 [c]	0,46 [bc]
420	5	31	9	7	15	29,03 [c]	19,35 [b]	48,38 [c]	11,08 [c]	0,38 [c]
560	5	28	4	6	18	14,28 [d]	21,42 [b]	64,28 [d]	8,37 [d]	0,30 [d]

**) Anova with significance level 5%, is continued with LSD test. The difference notation in same column show that differences significantly.*

Table 2 Percentage of total oval head, not uppermost eye, and haemorrhage fetuses from parent who has given borax

Dose (mg/ kg body weight/ day)	∑ Parent	∑ Implantation	∑ Fetal Object	∑ Fetus			%Fetus		
				Oval head	Not uppermost eye	Haemorrhage	Oval head	Not uppermost eye	Haemorhrage
0	5	45	45	0	0	0	0 [a]	0 [a]	0 [a]
140	5	38	26	4	5	1	15,83 [b]	19,23 [b]	3,85 [ab]
280	5	35	21	7	7	1	33,33 [bc]	33,33 [b]	4,76 [b]
420	5	31	16	9	7	3	56,25 [c]	43,75 [b]	18,75 [bc]
560	5	28	10	10	8	5	100 [d]	80 [b]	50 [c]

**) Anova with significance level 5%, is continued with LSD test. The difference notation in same column show that differences significantly.*

Table 3 The Average of ossificated thoracic, lumbar, costae, metacarpus and metatarsus and percentage of costae bridge.

Dose (mg/ kg of body weight/ day)	∑ Parent	∑ Implantation	∑ Object fetus	% Costae bridge	Average of ossificated skeleton				
					Thoracic	Lumbar	Ribs	Metacarpus	Metatarsus
0	5	45	45	0 [a]	13 [a]	6 [a]	13 [a]	4 [a]	4 [a]
140	5	38	26	1,47 [b]	11,29 [b]	3.35 [b]	11,34 [b]	2,78 [b]	2,46 [b]
280	5	35	21	2,186 [c]	10,98 [c]	2.84 [c]	10,8 [c]	1,82 [c]	2,14 [c]
420	5	31	16	-	0 [d]	0 [d]	0 [d]	0 [d]	0 [d]
560	5	28	10	-	0 [d]	0 [d]	0 [d]	0 [d]	0 [d]

**) Anova with significance level 5%, is continued with LSD test. The difference notation in same column show that differences significantly.*

Table 4 The Average of fetus eyeball and percentage of fetal hydrocephalus.

Dose (mg/ kg of body weight/ day)	∑ Parent	∑ Implantation	∑ object fetus	Average of eyeball	∑ Hydrocephalus	% Hydrocephalus
0	5	45	45	2 [a]	0	0 [a]
140	5	38	26	1,66 [b]	16	61,53 [bc]
280	5	35	21	0,95 [c]	15	71,42 [c]
420	5	31	16	0 [d]	13	81,25 [c]
560	5	28	10	0 [d]	10	100 [d]

**) Anova with significance level 5%, is continued with LSD test. The difference notation in same column show that differences significantly.*

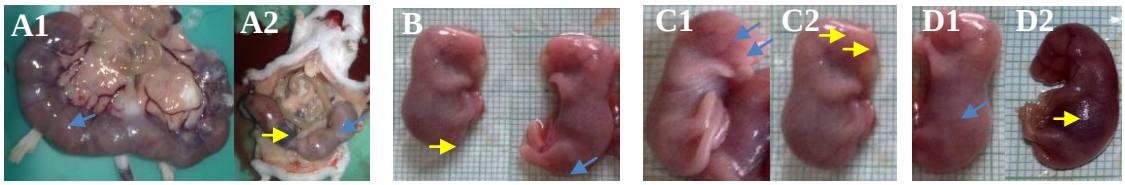
Table 5 The Average of glomerulus, Bowman's capsule, proximal and distal convoluted tubule Diameter.

Dose (mg/ kg of body weight/ day)	∑ Parent	∑ Implantation	∑ Object fetus	Average of diameter (µm)			
				Glomerulus	Bowman's capsule	Proximal convoluted tubule	Distal convoluted tubule
0	5	45	45	12.23 [a]	16,71 [a]	4.02 [a]	12,81 [a]
140	5	38	26	16,12 [b]	20,86 [b]	4,31 [b]	16.09 [b]
280	5	35	21	20,03 [c]	24,35 [c]	4,89 [c]	19,21 [c]
420	5	31	16	24,09 [d]	32,45 [d]	5,61 [d]	25,60 [d]
560	5	28	10	28,14 [e]	36,08 [e]	5,82 [e]	28,82 [e]

**) Anova with significance level 5%, is continued with LSD test. The difference notation in same column show that differences significantly.*

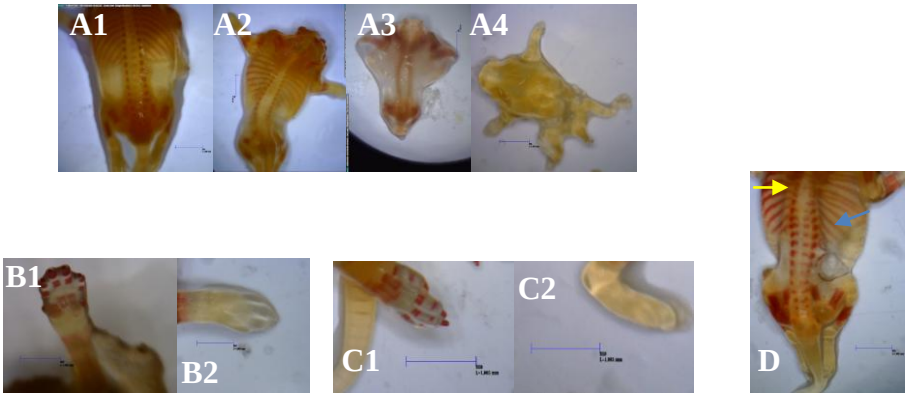
Base on LSD test is known that dose 140 mg/kg of body weight/day is began to effect on some abnormalities on mice fetuses, such as:1) low viability, 2) low body weight and body lenght, 3) abnormal morphology: oval head and not uppermost eye, 4) retardation of ossification, 5) eye do not be formed and hydrocephalus, 6) enlargement of glomerulus, Bowman's capsule, proximal and distal convoluted tubule. Whereas on the dose 280 mg/bg of body weight/day, borax is began to effect on haemorrhage on mice fetuses.

The picture 1 show that abnormalities on mice reproduction appearance and morphological development.



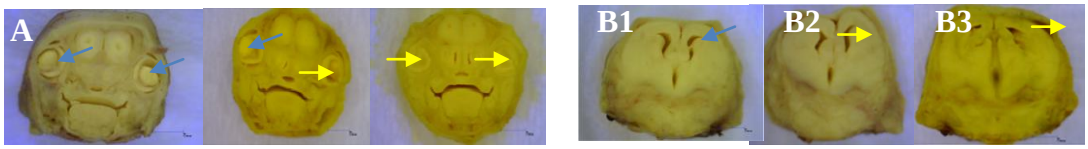
Picture 1 Abnormalities forms on mice reproduction appearance and : A1 Life fetus, A2.death and resorbtion fetus, B. Body long fetus, C1. Oval head fetal and non uppermost eye, C2. Normal head and eye, D1. Non Haemorrhage, D2. Haemorrhage fetus: blue arrow show that normal form, yellow arrow that abnormal form.

The picture 2 show that abnormalities on vertebrae development.



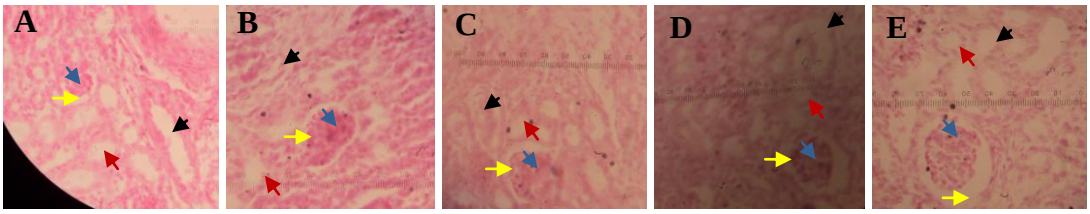
Picture 2 Forms of the mice reproduction appearance abnormalities: A. Thoracic, lumbar and costae vertebrae, 1: normal (13 internode of thoracic and ribs, 6 internode of lumbar vertebrae, 26 vertebrae body), 2,3 & 4: abnormal, B. Metacarpus, 1: normal (there are 4 internode in each digity), 2: abnormal (non internode in digity, C. Metatasus, 1: normal (there are 4 intenode in each digity), 2: abnormal (non internode in digity), D.Costae bridge (blue arrow: normal vertebrae, yellow arrow: costae bridge)

The picture 3 show that abnormalities on eye anh hydrocephalus.



Picture 3 Forms of abnormalities on eyeball and hydrocephalus: A. Eyeball, B. Hydrocephalus (B1: normal, B2: internal hydrocephalus, B3: external hydrocephalus): blue arrow show the normal form, yellow arrow show the abnormal form)

The picture 4 show that expand diametry of glomerulus, Bowman's capsule, proximal and distal convoluted tubule.



Picture 4: A. Dose 0 mg/kg of body weight/day, B. Dose 140 mg/kg of body weight/day, C. Dose 280 mg/kg of body weight/day, D. Dose 420 mg/kg of body weight/day, E. 560 mg/kg of body weight/day (blue arrow: glomerulus, yellow arrow: Bowman's capsule, black arrow: proximal convoluted tubule, red arrow:distal convoluted tubule)

Based on the result of observation known that to begin at dose 140 mg/kg body weight/day, borax took to effect on development retardation of mice fetuses and cause intrauterine death such as fetus resorption and postnatal death. This fact presumed that borax can passed through the placental barrier and then affect to mice fetus developmental. Generally the development anomaly caused by borax are the retardation of organ's growth and development, the defect of tissues, and anomaly of organs structurally. The anomaly in growth retardation showed from the weight and long of fetuses under normally, oval shape of head, and retardation of vertebrae, ribs, metacarpus and metatarsus ossification. The defect of tissues were showed as the enlargement of kidney cavities, not forming of eye balls, haemorrhage or the bleeding under the skin, and hydrocephalus phenomena. Whereas the defect of organ shape showed as the formed of costae bridge.

At the dose of 140 mg/kg body weight/day borax has potency to accumulate in the tissue. It was proved that borax interfere as embryotoxic potent. Borax was proved to retardate the growth and development of fetuses. It was presumed that at the dose of 140 mg/kg body weight/day borax has caused the riboflavin deficiency so that interfere the growth and development. In other sides, riboflavin deficiencies caused the damage of the cells composing eye ball so that to result in eye ball defect. The accumulated borax can interfere physiological processes in fetus so that cause the damage of certain tissues and organs. Borax presumably cause the damage of cellular function by riboflavin deficiency so that interfere dividing and growing of the cells, and defeat cell to synthesize the enzymes for growing and developing because of its receptor function disturbance by decreasing of cell integrity. This cell function disturbance then can retarded the growth of fetus length and weight and osteogenesis of skeletal cells. The disturbed cells in their function and integrity by a toxic agent will become necrosis. It presumably to increasing diameter of glomerulus and the happened of cell's necrosis.

To begin at 140 mg/kg of body weight/day dose, borax presumed to accumulate in placenta and amnion fluid, so that interfere transfer of nutrition in fetus. Maybe the borax can

accumulate too in blood vessels so that increase it's osmotic pressure and cause vasoconstriction which make the bleeding finally. The accumulated borax in the brain presumed to stop the cerebrospinal fluid circulation in several place like aquaduct silvii. This phenomenon cause the imbalance of producing cerebrospinal fluid and the circulated one. It cause internal hydrocephalus, that is the incident of accumulating fluid in lateral ventricle and the third ventricle of the brain. Beside that, borax presumable to accumulate in another places at the brain like in the small vessels in piamater, ventricular wall and arachnoid villy so that cause the interfere of cerebrospinal fluid absorption by the veins. It was resulted in external hydrocephalus, that is cerebrospinal fluid accumulation in the cavity between the brain an duramater.

CONCLUSION

To begin at the dose 140 mg/kg body weight/day, borax took effect on some abnormalities on mice fetuses, such as: 1) low viability, 2) low body weight and body length 3) abnormal morphology: oval head and not uppermost eye, 4) retardation of ossification 5) eye do not be formed and hydrocephalus, 6) enlargement of glomerulus, Bowman's capsule, proximal and distal convoluted tubule diameters. Whereas to begin at the dose 280 mg/ body weight/day, borax cause haemorrhage on mice fetuses.

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O-PD05

Taurine Effects on Growth and Gonad Maturation in Cobia (*Rachycentron canadum*)

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Abstract

The aim of the study was to elucidate the effect of taurine amino acid dietary on cobia growth rate and reproductive status. The growth was determined by their body weight and morphological changes included the length and the width of their body. The reproductive status was determined by measuring estradiol concentration of the blood samples by using enzyme immune assay (ELIZA) and fish gonadosomatic indexes (GI). Four experimental groups were chosen, two groups with commercial fish food and two groups with natural fish food. Taurine given within the groups consisted of 0 and 0.5% (0.06 gram/fish/day). Complete randomized design with factorial 2 x 2 was applied to this study and 40 premature cobia (@ approximately weight was 2.5 kg) were used for the experimental units. Analysis variance and SNK at 5% were used to determine the differences among experimental groups. The study was conducted in 4 months. The results indicated that groups with taurine in their diets increased their body weight for 25 – 50% compared to the control (0 % of taurine in their diet) as well as their body length and width ($p \leq 0.05$). When the premature cobia of the taurine groups reaching 4000 – 6000 grams in their body weight they were able to spawn and this also indicated by estradiol concentration ranging from 108 – 393 pg/ml. At that time the GSI of female cobia ranged from 2.34 – 7.91 and male were 0.40.

Keywords: Taurine, cobia, gonadosomatic index, estradiol

Introduction

The growth of cobia (*Rachycentron canadum*), as well as others marine fishes in general, depend on the ability of the fry dealt with the common stresses occurred in the seawater during their development reaching up to juvenile. One of the common stresses is the osmotic pressure of surrounding water. The ability of the cobia larvae to coup with this stress could be improved by giving supplement such as mannan oligosaccharide (MOS) (Salze, *et al.*, 2008). On the other hand, if the cobia fry was maintained in the fully circulated tank their ability to survive was low (Webb, Jr., Hitzfelder, Faulk, and Holt, 2007). It was assumed that the MOS affected on the development of the intestinal villi of cobia, consequently, this well development increase the food absorption which necessary for the fry development.

Meanwhile, the study had been done on juvenile red sea bream (*Pagrus major*) also indicated some increase in development and efficiency on food and consumption rate (Matsunari, *et al*, 2008).

On the other hand, some study conducted on the grouper fry (*Epinephelus fuscoguttatus*) indicated that the experimental group giving either taurine or/and inositol increased their survival rate compared to the control group Widiastuti, *et al*, 2005). This assumed that the accumulation of taurine and/or inositol might decrease the osmotic stress of the fry. From this study also was expected that the accumulation of these organic compound could take place by using ion calcium for the mechanical transport into the body. Taurine is amino acid derivative and in form of very simple compound, so that it is assumed it can be transported easily. If the taurine as organic compound known for osmolite organic could increase the survival rate of grouper or sea bream juvenile, then it was expected that this compound might also affect on cobia juvenile as well. This study then attempted to elucidate the effect of taurine on juvenile cobia, particularly on their growth and reproductive status.

Materials and Methods

Forty juveniles cobia collected from hatchery/supplier in southern part of Lampung Province were used for experimental units. They were in almost 8 months old and the body weight average was 2.5 kg. They were acclimated in the ocean using floating net. They were grouped into four different groups and were tagged individually. Each of groups received different treatments of food and taurine, such as one group was fed with fish and squib (2% of their body weight) with/without taurine added (0.06 gram/ind/day), other group was fed with commercial fish (2% of their body weight) with/without taurine added (0.06 gram/ind/day). Taurine used was commercial dietary taurine.

The body weight, length as well as the width (which was measured surrounding their stomach area) was determined. FSH and estradiol concentration of the blood samples were also determined by using enzyme immune assay (ELIZA) in Reproductive Rehabilitation Unit of Bogor Agriculture Institute (IPB).

Complete randomized design with factorial 2 x 2 was applied to this study. Analysis variance and Student-Newman Klaus at 5% were used to determine the differences among experimental groups. The study was conducted in 4 months.

Blood collection

Blood samples were collected from the gill arches intravenously with the syringe filled with as EDTA 0.1 mg. After centrifugation with 10.000 rpm blood plasma was collected and put under 10^{o} C for further determining of FSH, testosterone and estradiol levels.

Morphological samples

Before the body weight, length and width were determined, animal samples were anesthetized individually by using 25 ml clove oil diluted in 50 L of seawater with temperature of water 24 - 26^{o} C. The animals were weighted to the nearest 0.1 g, and were measured in the length and width to the nearest 1 mm.

Gonadosomatic Index

Gonadosomatic indexes (GSI) of the animal samples were calculated by using formula of:

GSI = [GW / (TW – GW) X 100] (Brown-Peterson, *et al*, 2001)

GW : gonad weight, TW : total body weight

Results and Discussion

Both taurine treatment groups (0.06 g/ind/day) either in natural or commercial fish food showed significantly increase in body weight compared to the control groups (0.00 g/ind/day) for about 33% - 58% (Table 1) ($p \leq 0.05$). Beside the body weight, the length and the width of the fish body were also determined and the length of the body showed significantly increase for about 20 – 51%, while the width was significantly for about 40 – 56%. This indicated that taurine given in the diet could affect the cobia growth.

Taurine was known to have high effect on mammalian metabolism, either in the skeletal muscle or in the cardiac muscle (Bakker and Berg, 2002; Goodman, *et al* 2009). Taurine also had an effect on the kidney (Mozaffari and Schaffer, 2001), eyes (Militante and Lombardini, 2002; El-Sherbeny, *et al*, 2004), as well as the brain (Gelder, 2005; Schurr, *et al* 1987) and white blood (leukocyte) function (Wang, *et al*, 2009). Therefore, we assumed that the taurine also had an effect on the premature cobia, especially for their muscle growth. Taurine was expected also had an insulin-like action, or it could modulate the insulin work (Baianchi, *et al*, 2006). If this true, then it was expected that the insulin-like modulation might affect on converting nutrient into developing new tissues or depositing nutrient in tissues just like those of adipose tissues formation. Yet, this statement should be elucidated more.

Tabel 1. The difference of body weight, length, and width each treatment on juvenile cobia

Treatment group (n)	Weight X ± SEM (g)	Length X ± SEM (cm)	Width[1] X ± SEM (cm)
A1 (10)	1972.7 ± 170.6[b]	19.80 ± 1.53[b]	8.00 ± 1.07[b]
A2 (10)	2633.3 ± 352.8[a]	29.92 ± 4.01[a]	11.17 ± 1.08[a]
B1 (10)	960.0 ± 180.6[d]	11.82 ± 1.78[c]	5.20 ± 0.73[c]
B2 (10)	1520.0 ± 174.4[c]	14.54 ± 1.52[c]	8.10 ± 1.08[b]

A1: Fish+squib diet
A2: Fish+squib diet + taurine
B1: Comercial fish diet
B2: Comercial fish diet + taurine
[1] the difference of each individual was taken at the last month of observation

were also expected that taurine may affect the growth and development of many different tissues supporting animal maturation, especially for gonad maturation. In order to determine the level of gonad maturation, we determined the FSH, testosterone and estradiol level of the premature cobia and the result was shown in Table 2.

Attempted to measured the level of FSH and testosterone was unsuccessful; however, the level of estradiol seemed to increase for those groups given taurine on their diets. The estradiol level almost double for those of taurine groups (0,06 g/ind/day) compared to the control groups (0.00 g/ind/day).

Table 2. The body weight and estradiol concentration after 4 months of treatments*

Treatment group (n)	Body Weight X ± SEM (kg)	Estradiol X ± SEM (pg/ml)[1]
A1 (7)	5.30 ± 0.32	45.43 ± 17.17
A2 (5)	5.54 ± 0,25	108.68 ± 31.76
B1 (5)	3.78 + 0.19	54.96 ± 27.42
B2 (7)	4.49 ± 0.20	117.43 ± 55.11

* We attempt to determine the FSH level, however, it was unable to be detected at this time.

Few days after the blood samples were collected, the groups of taurine added on diet were spawning, however; we were unable to indicate which individual fish was able to spawn since they were kept together in a group. But, if we looked at Table 2, it indicated that the level of estradiol few days before spawning was 100 to 390 pg/ml, and the range of body weight was 4.40 to 5.70 kg, with the age of animals less than 12 months. Unlike the sea-

culture cobia in Taiwan, the fish reached their gonad maturation when they reached 2 years old (Gothreaux, 2007). It was claimed that the cobia was able to spawn throughout the years as long as the temperature of water was maintained on 23 – 27^{o}C. The eggs would hatch at 21 – 37 hours after released and the larvae stayed 3 days or until the yolk was disappear and started to feed on microcrustasea, like capepoda or artemia.

Gonadosomatic index of the premature/mature cobia can be seen in Table 3 below. We only could indicate that there was some increase in the GSI number, however, this data should be elucidated more with more number of samples either for males and/or females.

From the previous information which we stated that the taurine groups showed spawning activity at the end of observation made, we could see that the GSI number for female might be in the range of 2.34 – 7.91, and the GSI for male was 0.40. Once again, it was stated that that the male cobia could reach its gonad maturity when it was reaching 1 – 2 years old, while the female could reach its gonad maturity when it reached 2 – 3 t years old and could reach weight of 60 kg (FAO-Fishery and Agriculture Department).

Tabel 3. Gonadosomatic Index (GSI) of the premature cobia

No	Sexes	Age (months)	Body weight (gram)	Gonad weight (gram)	GSI
1	Male	8	3000	2.15	0.07
2	Female	8	2400	121.84	4.38
3	Male	8	2700	52.91	2.00
4	Female	9	3800	210.54	5.87
5	Male	9	3500	8.45	0.24
6	Male	9	3200	10.88	0.34
7	Male	9	3300	5.72	0.17
8	Male	10	7500	369.64	5.18
9	Male	10	4400	37.62	0.86
10	Male	10	3800	20.93	0.55
11	Male	10	4900	36.88	0.76
12	Male	11	5000	19.51	0.39
13	Female	11	4900	35.23	7.91
14	Female	11	5400	123.62	2.34
15	Female	11	6500	415.36	6.83
16	Male	11	4100	16.36	0.40

Conclusion

We can conclude that taurine on fish diets has an effect on cobia growth for about 25 – 50% and its gonad maturation. Based on their age, the cobia either female or male in age of less than 1 year was able to spawn. The spawning female was indicated by estradiol concentration ranging from 108 – 393 pg/ml and body weight ranging of 4.0 – 6.0 kg. The GSI of spawning female was assumed ranging from 2.34 – 7.91 and male was 0.40.

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O-PD06

Measurement of Testosterone and Cortisol in Faecal of JAVA DEER (*Cervus timorensis* Mul. & Schl 1844)

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ABSTRACT

This research will develop analytical techniques of hormones derived from the wildlife, Java deer (*Cervus russa timorensis* Mul. & Schl 1844). In general, ferocity of wildlife is a problem of handling them because of its wild and frightened if approached by humans. Usually, hormone analysis conducted using blood samples. Development of this technique using a faeces sample, so there is no direct contact with wildlife, but we can obtain data or learn the physiology status of wildlife such via hormones concentration.

Data of reproductive hormones is importance in wildlife or endangered species conservation through reproductive technology; release of the hormone testosterone in male animals show that testicular function is an essential thing to be studied for successful breeding. Methods of hormone analysis with non-invasive method, which uses faeces sample was developed for wild animals. Although this method is rather complicated and only inactive metabolites are measurable but the method analysis will be very useful in knowing the reproductive status of breeding animals for the purpose.

Two adult male deers were isolation into a separate cage. Samples of feces were collected in the morning and evening for 30 days. Sample is processed through the stages of lyophilization, pulverisasi, solvent extraction with methanol and centrifuged to obtain supernatant which will then be used in ELISA. ELISA test results showed that hormone content of both testosterone and cortisol, although levels are still low. This shows that there in-active metabolites in the faces. With this method the results of hormonal measurements through (in) fases (non-invasive) it is possible to be developed for observation and research on the reproductive status of the Deer.

KEYWORDS: Deer; steroid hormones; testosterone; cortisol; noninvasive monitoring; feces/faeces.

O-PD07

Plasma Magnesium Concentration in Suckling Ettawa Crossbred Goats

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ABSTRACT

This study was aimed to investigate profile of plasma magnesium (Mg) consentration during suckling period in goats. A total of 79 Ettawa crossbred goats were selected to represent a combination of sex and age status. The animals were at the age of 3, 15, 30, and 60 days. They were kept together with their doe fed rhougage and concentrate. Blood was collected by jugular veni-puncture into heparinized vacutainers for Mg analyses. Plasma Mg concentration was determinated spectrophotometrically. Changes in Mg plasma concentrations underlying different age and sex states were monitored. Data analyses showed that the plasma Mg concentrations were not significantly influenced by sex and age difference (P>0.05). The average plasma Mg concentration range 0.60-2.00 mg/dL.

Keywords**:** Age, Ettawa crossbred goats; Magnesium; Sex

INTRODUCTION

Magnesium is required for many metabolic enzyme systems (Haenlein, 1987) and for normal skeletal development and one of the catalyst in over 300 enzyme systems (McDowell *et al.*, 1993). Previous study has indicated that plasma Mg level shows an age related changing in goat. Breibart *et al.* (1960) and Ahmed *et al.* (2000) reported that plasma Mg concentration in young suckling goat is lower than that in adult one. It is known that gastrointestinal tract and kidney play an important role in Mg homeostasis. Walser and Bostedt (1990) reported that the gastrointestinal tract develop rapidly during the suckling period and young goat kid or lamb will start to get roughage and show ruminating activity at the second month of its postnatal life. Widiyono (2003) has found that renal function (glomerular filtration rate) of young suckling goat kid increased rapidly during the first month of its postnatal life and reached its adult level at the age of 1 month. Study in mouse indicated that Mg content of some organs (tibia and muscle) increased significantly during growth and development in suckling neonate (Reis *et al.,* 1991). Morever, Sowande *et al.* (2008) found that there was a sex related difference in plasma Mg concentration in

ruminating sheep but not in goat. The present study was thus initiated with the objective to investigate the profile of plasma Mg concentration in male and female goat kid during the first 2 months of its postnatal life.

MATERIALS AND METHODS

. A total of 79 Ettawa crosbred goats were selected to represent a combination of sex and age status. There were young goats at 3, 15, 30, and 60 days after kidding.The animals were kept naturally together with their doe fed rhougage and concentrate. Blood was collected by jugular veni-puncture into heparinized vacutainers. Plasma was separated and stored at -20^{o}C pending the analysis of Mg. Plasma Mg concentration was determinated spectrophotometrically (Kraft and Duerr, 1999). ANOVA were used to analyze the influence of age and sex on the plasma Mg concentration.

RESULTS AND DISCUSSION

The mean and standard deviation of plasma Mg levels of both sex at different age (3,15, 30 and 60 days) are shown in Table 1. The mean levels of Mg at the age of 3 days was 1.20 ± 0.30 mg/dL in males and 1.30 ± 0.40 mg/dL in female goats. At the age of 15 days, the Mg concentration decreased slightly to the level of 1.10 ± 0.50 mg/dL (male) and to the level of 1.10 ± 0.20 mg/dL (female). The plasma Mg concentrations did not change significantly until the age of 60 days (1.10 ± 0.30 mg/dL in male goats and 1.10 ± 0.30 mg/dL) ($P>0.05$). The average plasma Mg concentration range 0.60-2.00 mg/dL. These levels were lower than that found in 4-6 months old (2.40±0.50 mg/dL) or in 10-12 months old Nubian goat (2.80±0.40 mg/dL) reported by Ahmed *et al.* (2000). It may therefore strengthen the former findings in ruminating goats which underline an age related increase of plasma Mg concentration (the lower Mg concentration in young animals compared to that in the older ones). Breibart *et al.* (1960) stated that the low plasma Mg level in young animals was due to the fact that uptake of Mg by young animals was more rapid than in adult ones. Furthermore, it has been found that the exchange of Mg was five to ten times greater in young than in old animals. Study in mouse also indicated that Mg content of some organs (tibia and muscle) increased significantly during growth and development in suckling neonate (Reis *et al.,* 1991). Another possible reason for this lower plasma Mg concentration in younger animals is that young animals have more water content than older ones so that more water ions are adsorbed on the surface of bone crystal resulting in low Mg ions in the blood (Fontenot *et al.*, 1989).

Table1. Mean and standard deviation of plasma Mg levels (mg/dL) during the suckling period in male and female Ettawa crossbreed goats

Sex	Age (days)				Statistical analysis
	3	15	30	60	
Male	*1.20±0.30 (0.80-1.70)*	*1.10±0.50 (0.70-2.00)*	*1.20±0.30 (0.80-1.90)*	*1.10±0.30 (0.80-1.30)*	*P>0.05*
Female	1.30±0.40 (0.60-2.00)	1.10±0.20 (0.80-1.30)	1.10±0.20 (0.90-1.50)	1.10±0.30 (0.80-1.00)	

Statistical analyses indicated that the plasma Mg concentration of both male and female groups did not differ significantly at any time during the suckling period (P>0.05). No significant sex differences of the plasma Mg concentration were also observed in West African Dwarf goats in either wet or dry seasons (Sowande *et al.*, 2008). In contrast to these findings, sheep showed a sex related difference of plasma Mg concentration, in which the female animals have a higher plasma Mg concentration than the male ones (Sowande *et al.*, 2008). The same case was found in Xenopus and the domestic fowl, in which female animals have a significantly higher plasma Mg level than male ones (Charles, 1930).

CONCLUSION

There is no significant sex and age related changing of plasma Mg concentration in young suckling Ettawa crossbred goat kid.

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O-PD08

Histology of the developing digestive tracts in larval of golden trevally (*Gnathanodon speciosus*)

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ABSTRACT

The survival rates (SR) of golden trevally (*G. speciosus*) larvae in hatcheries mostly depend on living organisms such as phytoplankton (*Nannochloropsis* sp.), rotifer (*B. plicatilis*), artemia nauplii (*Artemia* sp.) and mantis shrimp (*Mysid* sp.) as the main food. The feeding regime should suit to their ages because at the early stages, the organs are still primitive in which they should undergone several changes towards the ages. Few days after exogenous feeding, the internal organs undergo morphological, histological and histochemical changes gradually. A histological observation on the golden trevally or kue fish (*G. speciosus*) digestive tract was conducted to find out the development of the tracts. The observation began from Day 1 (D1) which was counted from the initial stocking date and was concluded at D_{30} by preserving the samples with formalin 5 %, adopting CSIRO (1996). At the final stage, a digital camera (Nicon) that linked to ACT-1 software was used to visualise the sections. The results showed that in general, the digestive tracts of larval (D_1-D_{30}) golden trevally are still primitive. Several important organs such as stomach (S), middle intestine (MI), back intestine (BI) and intestine (I) start to develop at D_4 and it is continued until D_{30}.

Keywords: histology, digestive tracts, larval of golden trevally (*G. speciosus*)

INTRODUCTION

The research on mass production and the technology of golden trevally (*Gnathanodon speciosus*) has been conducted at the Research Institute for Mariculture (RIM) since 2006. The survival rate of the produced juveniles varied due to the critical periods during larval stages that caused high mortality rates [1]. Therefore, several studies have been conducted and published, including several important biological aspects. For instance, the newly hatched golden trevally (*G. speciosus*) larvae was claimed to utilise yolk and oil globule with the volume of 2.27×10^{-1} mm^3± 0.06 and 5.57×10^{-3} mm^3, respectively as their main energy sources. The yolk sac (YS) and oil globule (OG) were totally absorbed in between 35.5-47.5 hrs and 70.5 hrs after hatching. The average absorption rates were 4.64×10^{-3} mm^3/hr for YS and 1.727×10^{-4} mm^3/hr for OG [2]. Subsequently, the growth of the backbone is detected to be positive to the increase in the total length of the larvae. The changing of cartilage started to from D_{30} and there was no deformity on the backbones until this rearing day [3].

Broodstocks of golden trevally (*G speciosus*) spawn in the evening time every dark moon [2]. The eggs experienced several developmental stages after fertilization (AF). The subsequent stages which are morula, blastula and gastrula occur 2 hr 24 sec AF, 5hr 16sec AF dan 6hr 12sec (the completed figures are available in [4]). Larval stage occur 10 hrs AF after the hatching time [3]. The small sized larvae carry yolk sac (YS) that rich with protein and fat, and oil globule (OG) [2]. The embryonic and larval periods involve extensive maturational changes in the internal organs, especially the digestive tract. The tracts soon will undergo morphological, histological and histo-chemical development after the endogenous feeding phase concluded. The feeding habits of fish larvae have been correlated with digestive structure [5],[6],[7]. The first few weeks of development become a critical period for fish larvae, particularly when the yolk-sac (YS) is reabsorbed and exogenous feeding starts. Morphological and physiological changes, including the complete development and functioning of the digestive tract are some of the features to be carefully studied, since an adequate feeding program should be planned based on the knowledge of such changes. The data of the development of digestive tract in kuwe fish (*G. speciosus*) larvae using a simple histological study is hoped it will be useful for the design of the optimal rearing technique to support the culture. This present histological study on the digestive tracts was conducted to fill the gap.

MATERIAL AND METHODS

Larval rearing

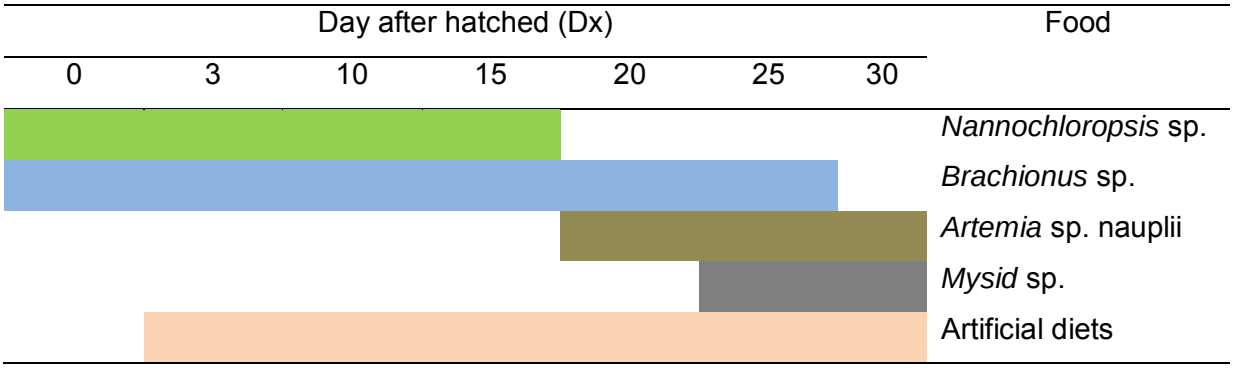
Fertilised eggs of golden trevally (*G. speciosus*) with the size of 600-750 µm were collected from the broodstock tanks at Research Institute for Mariculture (RIM) were distributed at 100,000 eggs/tanks into three yellow 6m^{3}concrete tanks. Feeding regime for the larvae during the culture was set (Table 1.) after hatching. Twelve air-stones were placed in each tank and used to maintain dissolved oxygen at saturation, and to promote a homogeneous distribution of foods.

Sample collection and observation

Specimens of the larvae were randomly taken from the tanks at 5-10 larvae daily. They were fixed by immersion in 5% formalin. After dewaxing and dehydration, serial transfers and sagittal 5µm sections were stained with haematoxylin-eosin (H&E), according to CSIRO method with a few modifications [8]. Histological observation was conducted to the tissue samples of D_1 until D_{30} larvae using a light microscope that linked to a digital camera

ACT-1 to visualise the tissues and enumerate the total length (TL) of the larvae. The results then were presented descriptively.

Table 1. Feeding regime of golden trevally (*G. speciosus*) larval rearing



RESULTS AND DISCUSSION

The average total length of the larvae varied toward the ages (Table 2.). While the visualisation showed several changes in internal organs of the larvae (Fig.1.).

Table 2. Total length (TL) of kue fish (*G. speciosus*) samples

Day after hatched(D)	Average of total length (TL) (mm)
5	2.51 ± 0.25
10	5.34 ± 0.29
15	5.43 ± 0.53
20	8.03 ± 1.27
25	10.12 ± 1.03
30	14.85 ± 5.62

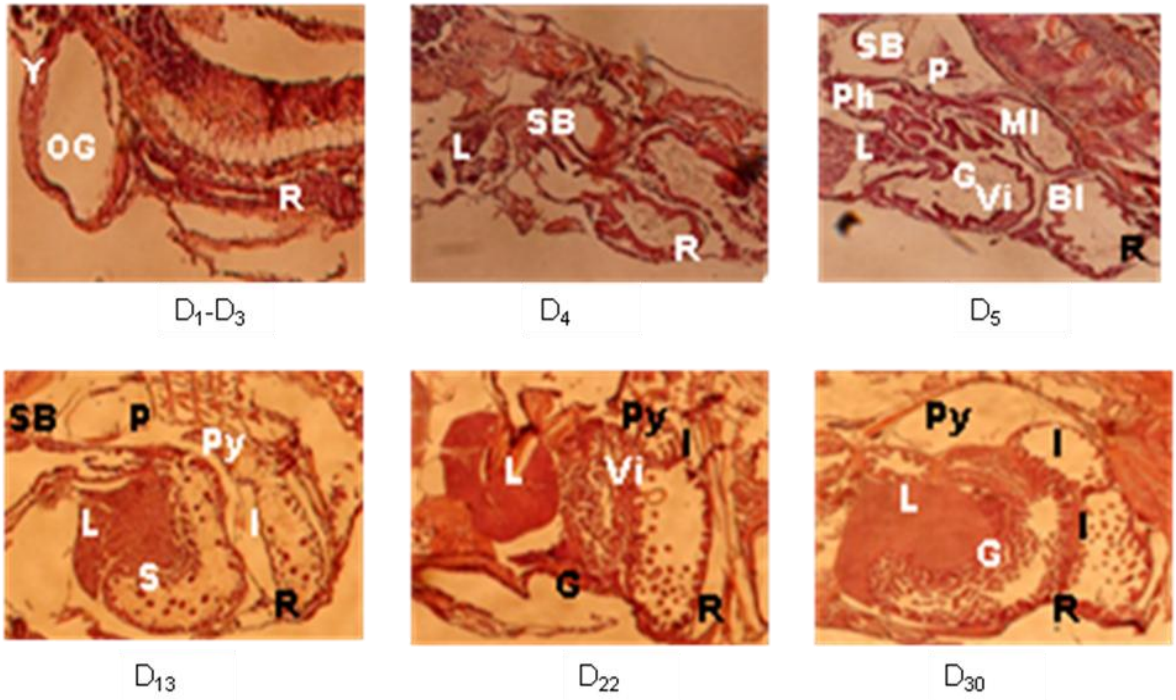


Fig.1. Sections of the developing digestive tracts in larval golden trevally (*Gnathanodon speciosus*). Remarks: Y: yolk; OG: oil globule; R: rectum; L: liver; SB: swim bladder; Ph: pharynx; G: gut; P: pancreas; MI: middle intestine; BI: back intestine; Py: pylorus: Vi: villous; I: intestine (H&E staining).

Developmental phase of fish larvae consists of two divisions, namely pro-larva and post-larva. Pro larva with the transparent body has oval, rounded or cylindrical yolk sac. Like other carangid larvae, newly hatched golden trevally (*G. speciosus*) have the average total length (TL) between 2 and 4.3 mm and big yolk sac (YS). It is posterior to the oil globule (OG) (Fig.1. D_1-D_3). At hatching, the digestive tract was a straight tube which extended posterior to the yolk sac. At this stage, lipids are the most important energy sources for the rapidly developing eggs. Wax esters and triglycerides provide the major consumed energy, whereas glycogen is less than 2% of the energy. Protein consumption increases during the development [9]. At the end of D_1, yolk remains small, shrinking to 2.10^{-2} mm^3. Whereas OG remains $3.05.10^{-3}$ mm^3 in volume [2] even zero (Fig.1.D_1-D_3). The incipient intestine appeared as a straight translucent tubular segment laying dorsally to the yolk sac.

Yolk sac (YS) is totally absorbed at D_4 where the opening process of eye, mouth and rectum taken place. The early extraneous feeding occurs at the time of no OG inside the larvae or 80.5 hrs after hatching to begin post larva phase. At this stage, the larvae that received insufficient nutrition because the outer food is limited will not survive due to their primitive organs [2]. The mouth and anus or rectum (R) open soon after yolk sac is absorbed. Therefore, life food should continually be added during this period.

Following the feeding, the digestive tract became wider. Concordantly, swim bladder (SB) and liver start to develop at D_4 (Fig.1.D_4) until D_{13}. The swim bladder (SB) lay at the dorsal part of the developed body. It is an organ that filled with air to control the lateral balance of the body that keep them buoyant through the arrangement of air pressure without wasting energy during swimming. It also enables accesses for the larvae during exogenous feeding. Liver with lobes lay on anterior of the larvae that distribute the nutrition inside blood and produce spleen. The digested foods then are used in morphogenesis, organogenesis and metamorphosis.

Even though the larvae undergo exogenous feeding, the digestive tracts are still primitive. Newly developed larvae are equipped with primitive intestine as a straight tiny tube in which the structure of the tract is still ambiguous with unconscious stomach and smooth intestine. The histological tissues (Fig.1) demonstrated that at the earlier larval stages (D_5-D_{22}) the layer of the digestive villous cells in the form is still incomplete. The enzymes activities at this stage are suggested to be low. The structure of digestive tracts in the larvae develops towards the age. Intestines start to develop at D_5. Gut (G) is a place where the food is stored and mixed with the gastric glands in the larvae start to differentiate with intestines at D6. The outer layer of the tracts perform villous that increase in number towards the age. The villous widen the surface of intestine canals that transfer food into the tract. Therefore the food became easier to be ingested.

Pancreas that is a vital organ underneath gut that ends up at duodenum star to develop at D_{13}. It produces two glands, endocrine and exocrine glands. Exocrine contains digestive enzyme whereas endocrine produced insulin and glycogen, controlling the metabolism of glucose inside the body. At the same time, pharynx as a junction for oesophagus and oral cavity also develop (Fig1.D_{13}).

The digestive tract of an adult fish consists of mouth, throat, oesophagus, gut, intestine and rectum [10]. At larval stages, the digestive organs are still primitive and the mouth is not open perfectly. Therefore, several points need to be considered during the rearing. Firstly, the development of mouth opening should be observed in order to find out the precise time, size and type of given food. Fish larvae consume food with the size between 30 and 50% of their mouth opening size. Phytoplankton (*Nannochloropsis* sp.), rotifers (*Brachionus* sp.), *Artemia* sp. and mantis shrimp (*Mysid* sp.) with the size of 9-10 µm, 150-360 µm, 500 µm, and 0.5-1 cm, respectively are used as the main food during the larval stages of kue fish (*G. speciosus*). Secondly, the development of digestive enzyme inside the larvae depends on the formation of gut. Therefore, before gut is visible, life food should be continually given as the main food for the larvae. The next point is that, the development of

larval eyes to detect the food inside the tanks. Adequate size of tanks and high density of food inside the tanks should be on top priority.

The length of the digestive tracts development period of each species is different. For example, Gouramy (*Osphronemus* sp.) requires 35 days to complete the tract whereas catfish (*Mystus* sp.) requires shorter time (22 days). Fish with large diameter of eggs have their completed digestive tract soon after the yolk sac totally been absorbed. Fish larvae that already have completed the development of digestive organ that properly work in the digestive process, similarly with those of adult fish start to enter juvenile stages [11]. This present study showed that Golden trevally (*G. speciosus*) with the average oocyte diameter of 150-250 µm tend to entry the juvenile stages after 30 days from hatching, where the organs has been completely formed and worked similarly with that of the adults.

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O-PD09

Fecundity, eggs development and growth of juvenile redclaw crayfish *Cherax quadricarinatus* under laboratory conditions

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not presented

O-PD10

STAGING OF SEMINIFEROUS TUBULES IN 3 to 12 WEEKS OF AGE WISTAR RATS (*Rattus norvegicus* L.)

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ABSTRACT

Research on male reproductive system cannot be separated from spermatogenesis. Staging of seminiferous tubules are one of the important variables to evaluate spermatogenesis. Rats are frequently used as a model in reproductive research, have XIV stages in their seminiferous tubules. However, proportion of each stage in a single age and comparison among ages has not yet reported. The goal of this research was to study staging of seminiferous tubules in 3-12-week-old Wistar rats. Qualitative data consist of staging appearence and development on each age. Semi-quantitative data including comparison of staging composition within age and among ages. Serum testosterone level was also measured to support the finding. Each age group has five replication as sample, which chosen randomly from local animal breeding house. Right testes from each sample were processed for histological preparation using routine paraffin method and stained with Hematoxylin&Eosin. As much as 150 seminiferous tubules from each replication were captured using optiLab® and then observed based on standard spermatogenesis staging map. Results indicated that age is important factor to influence appearance and development of seminiferous tubules in Wistar rats. This is supported by testosterone concentration, which the value is raising along with ages. In 3-5-weeks-old rats (young rats), spermatogenic cells have not fully developed as in 6-12-week-old rats (mature rats). Thus, stages in young rats cannot be well determined. In mature rats, all XIV stages are clearly shown. Shifting in staging appearence were detected, which stage V, VII, and VIII are the most dominant, while stage I-IV are in the lowest proportion. Percentage of each stage among ages was fluctuated and has a specific range.

Keywords: stage, seminiferous tubule, *Rattus norvegicus* L, testosterone.

O-PD11

ACUMULATION AND THE CONTENT OF CAPSAICIN IN THE FRUIT OF *Capsicum frutescens* L., *Capsicum annuum* L. var. *abreviata* Eingerhuth AND *Capsicum annuum* L. var. *longum* Sendt DURING FRUIT DEVELOPMENT

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ABSTRACT

The accumulation of the capsaicin is still debated, whether the capsaicin is accumulated in pericarpium, septum or seed. Moreover, the capsaicin optimum content in the fruit is also still debated. The research aims were to analyse the tissues where capsaicin is accumulated, the optimum content of capsaicin in the fruit, and fruit anatomy of *Capsicum annuum* L. var. *abreviata* Eingerhuth, *Capsicum annuum* L. var. *longum* Sendt, and *Capsicum frutescens* L. during fruit development.

Twenty one old seedling of three chilli papers varieties were grown in the polybag. The samples of chilli papers fruits were collected in the ages of 14, 21, 28 and 35 days after anthesis. The samples were divided in two groups. Ones were used for phytochemical analyses and the second were used for anatomical analyses. The capsaicin contents were analysed using gas chromatography and the anatomical data were prepared using parafin method.

The results showed that septum of *Capsicum annuum* L. var. a*breviata* Eingerhuth and *Capsicum annuum* L. var. *longum* Sendt were the tissue where capsaicin are accumulated. The optimum content of capsaicin in the fruit of *Capsicum annuum* L. var. *abreviata* Eingerhuth and *Capsicum annuum* L. var. *longum* Sendt were 35 days after anthesis. Moreover, the optimum content of capsaicin in the fruit of *Capsicum frutescens* L were 28 days after anthesis. The increase of capsaicin content were not concomitan with the increase of pericarpium and cuticula tickness, and the size and number of giant cells but its related with the increase of septum tickness in the fruit of *Capsicum annum* L. var. *abreviata* Eingerhuth and *Capsicum annuum* L. var. *longum*. Meanwhile, the increase of capsaicin content in the fruit of *Capsicum frutescens* L concomitan with fruit development.

Keywords. *Capsicum annuum* L. var. *abreviata* Eingerhuth, *Capsicum annuum* L. var. *longum* Sendt, *Capsicum frutescens* L, capsaicin, fruit anatomy.

INTRODUCTION

One of the characters in the chili is spicy taste which caused by the presence of capsaicinoid in the fruit. Location of capsaicin in the fruit of chili is still debated. Lewis (1984) said that capcaisin is accumulated in pericarp and little on the seed, in the septum (Claus, 1956), or scattered unevenly on pericarp but higher concentration in the placenta (Morrow, 1999). According to the opinion of the people capsaicin accumulated in the seeds. Anatomically Capsicum fruit are composed of several tissues such as pericarp, seed, and the placenta. The deepest part of the mesocarp consists of cells that are very large, so-called giant cell (Youngken, 1950).

Research of capsaicin content in chilies have been carried out. Megawati (2009) analyzed the content of capsaicin in small green chili. Planting medium treated with

cow urine (0, 7.5 mM / L, 15 mM / L and 22.5 mM / L). The result showed at the highest capsaicin at 35 DAA (Days After Antesis) treated with 22.5 mM /L cow's urine. Aisyah (2009) examined the content of capsaicin in big red chili and curly red chili. Planting medium were treated with organic fertilizer (concentration of cow's urine : 5 ml, 10 ml, 15 ml, 20 ml and without fertilization). The content of capsaicin and the anatomy of the fruit was observed when fruit was 40 DAA. The content of capsaicin in curly red chili is higher than a big red chili. No anatomical differences both of chili fruit.

In this study the fruit of small green and small white chili, as well as a big red pepper and curly red pepper were used. Each fruit was analyzed their capsaicin content and fruit anatomy in developmental phases. The development stage of fruit were : 14 DAA, 21 DAA, 28 DAA, and 35 DAA. This study aims were to analyse the tissues where capsaicin is accumulated, the optimum content of capsaicin in the fruit, and fruit anatomy of *Capsicum annuum* L. var. *abreviata* Eingerhuth, *Capsicum annuum* L. var. *longum* Sendt, and *Capsicum frutescens* L. during fruit development.

MATERIALS AND METHOD

A. Material

In this research fruit of small chili, big red chili, and curly red chili, growing medium (soil), compost for seeding, and NPK fertilizer were used. Some chemical for anatomical preparations were : ethanol, distilled water, razor blades, objects glass and cover glasses; for analysis of capsaicin were methanol, chloroform, HCl, NaOH and distilled water. Tools : a) for making anatomical preparations were rotary microtome, microscope, glassware, light microscope, microphotograph, an object micrometer, ocular micrometer, and digital cameras; b) for detection of capsaicin content : oven, centrifuge, vortex, sonicator, and a set of Gas Chromatography (GC) apparatus.

B. Method

a) Planting and maintained of chili plants

Chili seeds were grown in the field (soil mixed with compost). After 20-30 days, seedlings were transferred into polybags (26 cm in diameter). Plants treatment were watering and fertilizing routinely by NPK fertilizer a dose of 15 ml/L (Nugroho *et al.,* 2006).

b) Sampling, anatomical preparations and capcaisin analysis

Sampling was done when the age of fruit was 14, 21, 28 and 35 DAA. Samples prepared with 5 replications. At each stage of harvesting samples were divided into two groups. The first group for the observation of anatomy and the second group for the detection of capsaicin content. Sample of chili was separated into 4 groups, namely pericarp, septum, seeds and placenta. Each group was detected the capcaisin content to determine which tissues that contains the highest capsaicin. To analyzed the content of capsaicin in the tissue of small chili fruit were not broken down like red chili because the size of fruit very small. For anatomical observation, paraffin method was used (Ruzin, 1999). Before the content of capsaicin was analyzed by GC, the first step is capsaicin extraction using alkaloids extraction method (Nugroho et al., 2002). The extract dissolved in 0.5 ml of methanol and then analyzed by GC (Harborne, 1996).

C. Data Analysis

The data of anatomy were analyzed descriptively. The number, length, and width of the giant cell, pericarp thickness, placenta thickness, and seed coat thickness of red chili and small chili for each stage of fruit development, analyzed statistically. Quantitative data

about the content of capsaicin was statistically analyzed using CRD ANOVA and then DMRT test to detect a real difference at every stage of development.

RESULTS AND DISCUSSION

Fruit anatomy

Fruit anatomy were observed of four chili varieties were pericarp, giant cell, septum,and seed. Pericarp of chili composed by epicarp / exocarp as outermost part, mesocarp as middle part, and endocarp as the inner layer of pericarp. Epicarp consists of one layer of cells; mesocarp consists of parenchyma cells and giant cells. Endocarp composed of parenchyma cells and sclerenchyma cells (Esau, 1978). Pericarp in small chili is thinner than red chili. Pericarp thickness were increase during fruit development in four varieties of chili. The pericarp thickness of four chili varieties presented in Table 3.

Table 1. Pericarp Thickness (µm) of four chili varieties during fruit development.

Kind of chili	Age of fruit (DAA/days after anthesis)			
	14	21	28	35
Big red chili (BR)	652,8[a]	1156,8[c]	1460[d]	1856[e]
Curly red chili (CR)	717,6[a]	804,8[b]	1089,6[c]	1112,4[c]
Small white chili (SW)	541,20[a]	692,40[c]	777,60[d]	810,00[f]
Small green chili (SG)	618[b]	818,4 [f]	862,8[g]	1040,40[h]

* Note: Figures followed by the same letter indicate no significance difference based on analysis of DMRT at 5% significance level.

From Table 1. can be seen that pericarp thickness of red chili and small chili fruit at each developmental stage was significantly different. According to Sumardi (1990) that increasing fruit size caused by two processes, namely cell division and cell enlargement. The most thickness of pericarp was owned by big red chili (1856) and then successively followed by a curly red chili (1112.4), small green chili (1040.4) and small white chili (810.0) at 35 DAA. Increasing of pericarp thickness possibly related to it's function as protected tissues especially for the seeds. After 14 DAA, pericarp thickness of four varieties showed quite different. According to Salisbury and Ross (1992), the genetic and environmental factors such as macro and micro nutrients can influence the pericarp thickness.

The number and size of Giant Cell

Giant cell is a cell which located in the deepest part of mesocarp, very large cell and different with the cells around it. These cells are characteristic features of *Capsicum* genus (Wallis, 1946). According to Rygol and Luttge (1983), generally giant cell is succulent, and the central vacuole is very large. The presence of these cells is strongly associated with environmental conditions, and allegedly is a form of adaptation to drought condition. The statistical analysis of giant cell presented in Table 2.

Table 2. The number and size of giant cell (µm) of four chili varieties during fruit development.

Parameter	Kind of chili	Age of fruit (DAA/ days after anthesis)			
		14	21	28	35
Number of *giant cell*	BR	44[c]	49[d]	62[f]	58[e]
	CR	30[ab]	31[b]	30[b]	29[a]
Length of *giant cell* (µm)	BR	184,13[a]	403,73[cd]	445,2[d]	394[bcd]
	CR	126,4[a]	307,2[abcd]	256[abc]	218,8[ab]
Width of *giant cell* (µm)	BR	118,4[a]	382,4[c]	369,6[c]	366,4[c]
	CR	212[ab]	374,4[c]	285,2[bc]	220,8[ab]
Number of *giant cell*	SW	31,20[a]	32, 60[a]	34,60[b]	38,00[c]
	SG	23,20[d]	30,00[f]	29, 20[f]	27,40[e]
Length of *giant cell* (µm)	SW	254,80[a]	381,60[a]	441,40[a]	443,6[a]
	SG	254,80[a]	381,60[a]	441,40[a]	443,6[a]
Width of *giant cell* (µm)	SW	156,40[a]	229,20[ab]	264,67[ab]	299,60[ab]
	SG	303,2[ab]	354,4[b]	366,40[b]	400,00[b]

* Note: Figures followed by the same letter indicate no significance difference based on analysis of DMRT at 5% significance level

Table 2. showed that the number and size of the giant cell in the four chili varieties increased significantly during fruit development until 28 DAA, after that time declined, except for the small chili ones. An increasing number of giant cell, caused by the cell division, while increasing the length and width of the giant cell due to cell enlargement. The lowest amount found in small green chili (27.4), followed by curly red chili (29), and highest in big red chili (58) respectively. According to Rygol and Luttge (1983), giant cell has a greater vacuole ratio and is thought available to accommodate many metabolism, but the action of the enzyme which affect cell division process will decreased. This enzyme knew as sucrose synthase.

Septum Thickness

Septum on chili generally serves as a place for seed attachment. According to Diaz *et al*.(2004), septum is the site of capsaicin accumulation. Septum epidermis is the primary part of the biosynthesis of capsaicin during fruit development. Septal thickness of four chili varieties increases during the fruit development. This is due to cell division and cell enlargement. According Hartanti (2004) in line with the maturity of the fruit, the parenchyma of septum growing larger in size than the parenchyma in mesocarp. At 14 DAA epidermal cells of septum is still compact and homogeneous. At 21 DAA, the size of parenchyma cells of septum is increasing. Septum epidermal increasingly stretched at 28 DAA and 35 DAA. The statistical analysis of septum thickness in the four varieties of chilies presented in Table 3.

Table 3. Septum thickness (µm) of four chili varieties during fruit development phase.

Kind of chili	Age of fruit (DAA/ days after anthesis)			
	14	21	28	35
BR	584,4[a]	954[e]	1192,8[f]	1386[g]
CR	552[a]	650,4[b]	724,8[c]	786[d]
SW	628,80[b]	726,40[d]	768,00[e]	741,60[d]
SG	459,60[a]	674,40[c]	764,40[e]	789,60[f]

* Note: Figures followed by the same letter indicate no significance difference based on analysis of DMRT at 5% significance level.

Table 3. showed that the septum in the four varieties of chili significantly increased during fruit development up to 35 DAA, except for white chili. The thickest septum is owned by big red chili (1386), followed by small green chili (789,6), curly red chili (786), and the thinnest is the small white chili (741.6). As mentioned earlier, increasing septal thickness at the four varieties of chili related with their functions as a place of seed attachment. During the development of fruit, the seed will grow bigger, increasing their size, followed by increasing the septum thickness.

The difference of septal thickness between the two types of chili should be influenced by the internal factors (genes and hormones) and the environment. According to Ayesha (2009), the availability of nutrients in the soil also affects the thickness of the septum, because nutrients in the soil available absorbed by the plants to support the process of cell division and cell enlargement in the septum. Salisbury and Ross (1992) states that cytokinins estimated enhance the cell division and cell enlargement, particularly improving the transition of G2 phase to mitosis phase.

Thickness of Seeds coat

Generally mature seed composed of seed coat, endosperm/ food storage, and embryo (Fahn, 1991). Seed coat is the outermost part of the seed. The seed coat of chili consist of radially elongated cells that resemble the palisade, and called macrosclereid, because the shape and the thickening walls of these cell. According to Fahn (1991) cell wall thickness due to uneven overall. The thickening occurs on the long side of seed and consist of cellulose or lignin substances. Seed coat thickness of the four varieties of chilli significantly increased with the age of fruit (Table 4.)

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Table 4. Seed coat thickness (µm) of four varieties of chili during fruit development.

Kind of chili	Age of fruit (DAA/ days after anthesis)			
	14	21	28	35
BR	31,2[a]	38,4[ab]	90[c]	120[e]
CR	44,4[b]	82,8[c]	108[d]	129,6[f]
SW	92,40[a]	181,20[de]	190,80[b]	224,40[f]
SG	147,60[b]	163,20[c]	174,00[cd]	189,60[e]

* Note: Figures followed by the same letter indicate no significance difference based on analysis of DMRT at 5% significance level.

Seed coat thickness of four varieties of chili has increased significantly during fruit development (Table 4.). The thickest seed coat found in small white chili (224.4), followed by the small green chili (189.6). Curly red chili seeds have thicker coat (129.6) compared with big red chili (120.0).

Capsaicin content

The highest levels of capsaicin (100%) obtained in the septum of curly red chilies at 35 DAA. The lowest content of capsaicin (0.31%) obtained in the pericarp of big red chilies at of 21 DAA.

Table 5. Capsaicin contain (%) at the pericarp, septum and seed of big and curly red chili during fruit development phase.

Age of fruit (DAA/ days after anthesis)	Capsaicin content (%) of big red chili			Capsaicin content (%) of curly red chili		
	Pericarp	Septum	Seed	Pericarp	Septum	Seed
14	0	0	0	0	0	0
21	0.31	0	0	0	45.24	0.97
28	0.37	0.86	1.86	1.96	58.57	16.76
35	1.99	6.98	0.99	1.16	100	8.88

Based on the Table 5. can be seen that the highest content of capsaicin obtained in septum at 35 DAA, while in pericarp and seed coat is very low. This result accordance with the opinion expressed by Claus (1956), that the capsaicin found in septum. In addition, based on observations of histology and histochemistry, Otha (1962) concluded that the tissues which secreted capcaicinoid is dissepiments of septum. Iwai *et al*. (1979) also reported that capcaisin accumulated in septum, specially in cells of epidermal septum. whatever plant chili pepper were grown in the same environmental conditions, but the content of capsaicin produced were different. Differences of capsaicin content of two varieties of chillies (big red and curly red) is caused should be by genetic and physiology factors.

At 14 DAA, no detected of capcaicin content in all varieties. Stewart *et al*. (2005), reported that the synthesis of capsaicin is estimated began in 20 DAA. After 14 DAA septum and seed of curly red chili showed early accumulation of capsaicin in pericarp and seed. Wuryaningsih (1998) suggested that the capsaicin synthesis increases in line with fruit maturity, while at 35 DAA the capsaicin content decrease. According to Padilla *et al.* (1998), capsaicin synthesis is controlled by peroxidase activity. Increased levels of capsaicin is always followed by the reduce of peroxidase activity. Peroxidase is the most influential in epidermis cells of septum. Therefore, capsaicin content is highest in the septum than the other tissues. Capcaisin was increased in big red and curly red chili at 21 DAA to 28 DAA, then decrease at 35 DAA. This result suggests that capsaicin were transfered from seed to other parts such as the septum and pericarp.

Table 6. Capsaicin content (%) of small white (SW) and small green (SG) chili during fruit development.

Kind od chili	Age of fruit (DAA)			
	14	21	28	35
SW	1,37	37,16	65,88	39,35
SG	-	13,83	17,14	37,72

At 14 DAA capsaicin were synthesed in small white and green chilies (1.37%), increased at 21 DAA (37.16%) and 28 DAA (65.88%), then decreased at 35 DAA (39.35 %). The decrease of capsacin at 35 DAA estimated that capcaisin has been oxidized. As well as red chili, capsaicin content in small chili increased during fruit development. The content of capsaicin in small chili is higher than red chili.

Conclusion

The results of this research summarized : that capcaisin in four varieties of chili were accumulated in septum. The highest content of capsaicin in big red chili, curly red chili, and small green chili founded at 35 DAA, while the small white chili at 28 DAA. The increased of capsaicin levels was not followed by increasing of pericarp thickness, seed coat thickness, the number and size of the giant cell, but followed by increasing of the septum thickness during fruit development.

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O-PD12

FLOWER DEVELOPMENT OF *Aeschynanthus tricolor* Jack.

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not presented

O-PD13

HORMONES CONTENT IN THE DEVELOPING COCOA PODS (*Theobroma cacao* L.)

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Abstract

The development of cocoa pods was controlled by hormones, namely gibberelin, auxin, cytokinin and abscisic acid. This experiment was aimed to determine gibberellin, auxin, cytokinin and abscisic acid content in the developing cocoa pods. Two cocoa clones namely KW 163 which represent self compatible clone and KW 165 which represent self incompatible clone were used. Several plants for each clone were selected and hand pollination was conducted before anthesis. Samples of healthy pods were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 week old following hand pollination. Gibberellin, auxin, cytokinin, and abscisic acid content were determined by HPLC (High Performance Liquid Chromatography).

The results showed that there was a certain composition in the content of gibberellin, auxin, cytokinin and abscisic acid at each stage of the development of cocoa pods. The composition regulated the metabolism in pods, determined the process of embryogenesis. The results revealed the role of hormones in determining the progress of cocoa pod development.

Keywords: *Theobroma cacao* L., gibberellin, auxin, cytokinin, abscisic acid.

Introduction

Many flowers are produced on cocoa plants, but only 1-5% of those flowers can develope into pods[1;2]. Cocoa pods grow slowly for 40 days due to the first division of the zygote that occurred about 40 days after pollination. The embryos grow very slowly compared to the growth of pericarp and other tissues until the age of 70 – 75 days[1;3]. The development of pods is regulated by hormones, such as gibberellin, auxin, cytokinin and abscisic acid[1;4]. There is an interaction amongst hormones which determine the development of the pod[5]. The content of hormones in the pod play a role in determining sink strength and thus affect the ability of the pod in the photosynthate competition between pods.

Auxin and cytokinin have synergic function on the development of embryonic root meristem on *Arabidopsis* fruit. Auxin stimulates meristem cell elongation while cytokinin

stimulates cell division[6]. Abscisic acid has antagonic effect with auxin. Abscisic acid regulates fruit aborsion process[7]. The aimed of this study was to know the content of gibberellin, auxim, cytokinin and abscisic acid in the developing young cocoa pods

Methods

Hand pollination was used to produce pods from KW 163 and KW 165 cocoa clones. The research was conducted at the Indonesia Coffee and Cocoa Research Institute in Jember Indonesia. Laboratory research was carried out in Chemistry and Biochemistry Laboratory, Center for Food and Nutrition Studies, Gadjah Mada University. Hormone analysis was carried on healthy young cocoa pods aged 1 to 10 weeks. Gibberellin, auxin, cytokinin and abscisic acid content were determined by HPLC of Beckman (USA) 515 type. 26% acetonitril was used as mobile phase with the addition of 30 mM phosphoric acid, with pH 4. Column ODS / C (18), wavelength 254 nm, flow rate 0.9 mL / min[8].

Result and discussion

Gibberellin, auxin, cytokinin and abscisic acid contents can be seen in Figure 1, 2, 3 and 4.

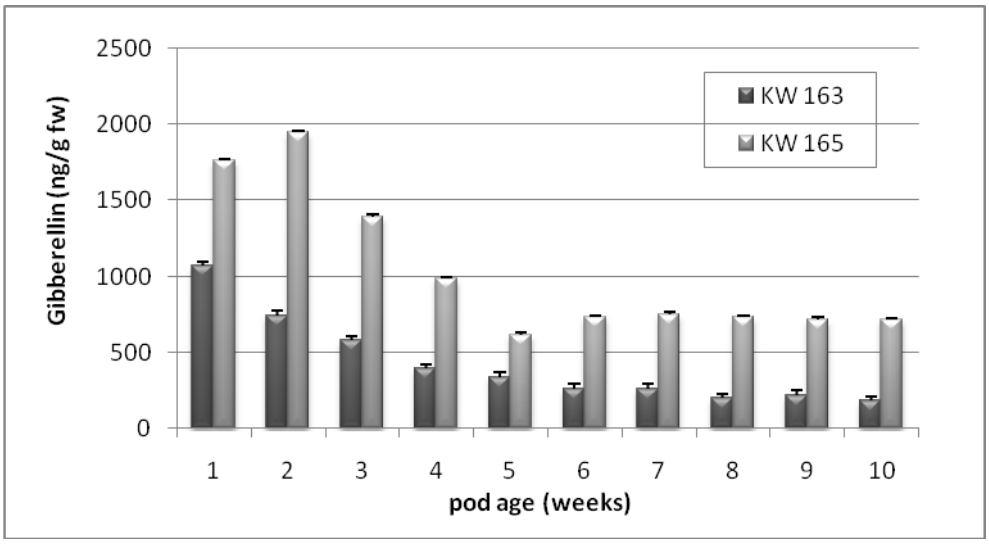

Figure 1. Gibberellin content in a various physiological age of cocoa young pods

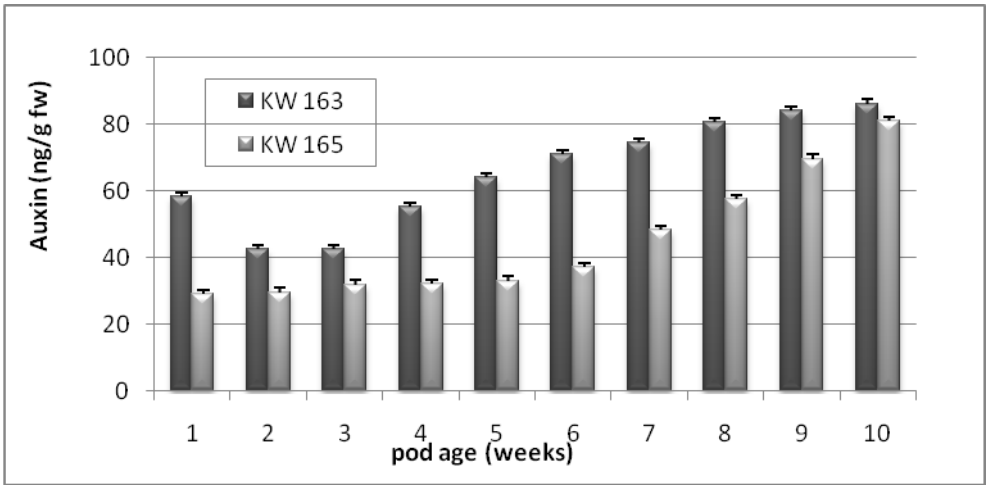

Figure 2. Auxin content in a various physiological age of cocoa young pods

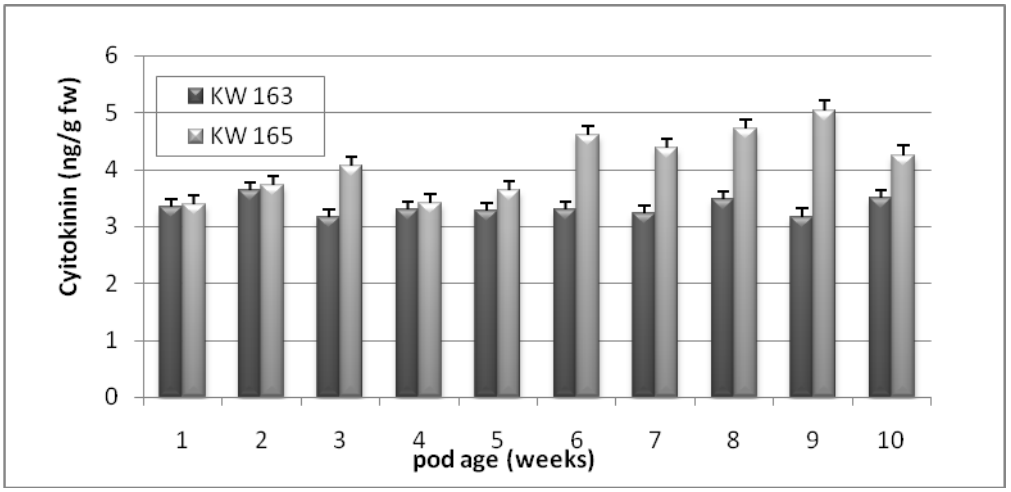

Figure 3. Cytokinin content in a various physiological age of cocoa young pods

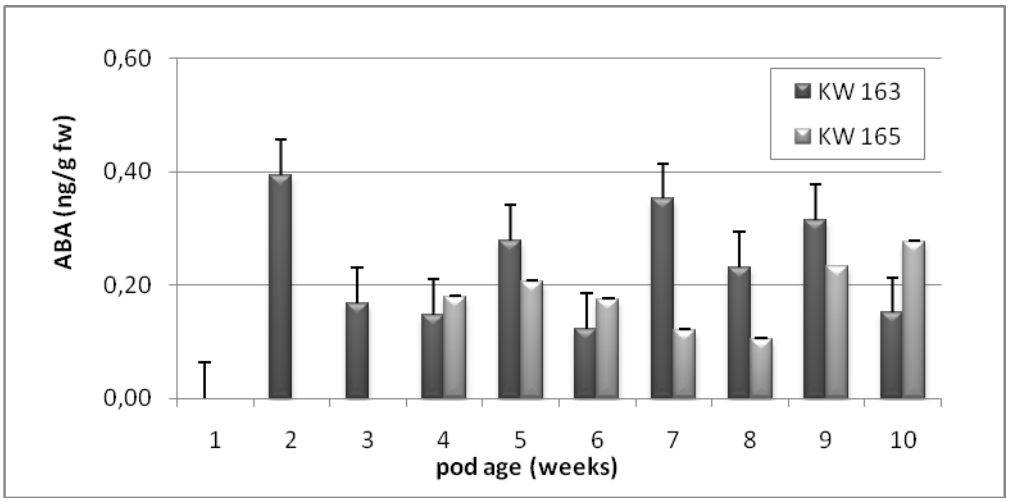

Figure 4. Abscisic acid content in a variious physiological age of cocoa young pods

The content of gibberellin in the pod of KW 163 and KW 165 cocoa clones was high at the aged 1-2 weeks, but then decreased to age 10 weeks. Gibberellin are synthesized when the zygote is formed, besides gibberellin has a role in regulating embryonic and pod development[9; 10; 11; 12; 13].

The content of auxin on young pod tends to increase with age of pod, but the content of auxin is lower than the gibberellin. Gibberellin and auxin have a role in pod development. Auxin stimulates cell elongation by increases the osmotic pressure, beside stimulates the biosynthesis of gibberellins (14).

The content of cytokinin in the pod tends to constant at variety of age and the content of cytokinin was lower than gibberellin and auxin. On the pod of KW 163 cocoa clone, abscisic acid was not detected at the age of a week and detected only at the age of 2-10 weeks. In pod of KW 165 cocoa clone, abscisic acid was not detected at the age of 1 to 3 week and was detected at the age of 4-10 weeks.

The content of gibberellin, auxin, cytokinin and abscisic acid at different ages showed that there was a certain composition of hormones according to the metabolism of stage of developing young cocoa pod. This suggests that hormones play a role in the growth and development by their interaction. Auxin and gibberellin have a positive interaction in organ development. Auxin affects the synthesis of gibberellin in the seed[10; 13]. There is an interaction between gibberellin and auxin in various stage of pod development. Auxin induces the expression GA20ox and GA3ox. Auxin plays a role in the process of morphogenesis[15]. Cytokinin has a role of plant growth and development. Cytokinin affects the differentiation of meristem cells. Auxin and cytokininin play a role in regulating the cell cycle in plants[16]. In cocoa young pod of various ages, the content of gibberellin is very high compared to the abscisic acid. There is an antagonistic relationship between gibberellin and abscisic acid in regulating the growth and development of cocoa pod. These results illustrate that there is a certain composition between the content of gibberellin, auxin, cytokinin and abscisic acid at each stage of the development of cocoa pods. This hormones composition regulates the metabolism in the pod and determine the process of embryogenesis.

Conclusion

The conclusion that can be drawn from the results and discussion in this study was: the content of hormones play a role in determining the survival of cocoa pod development. There was a certain composition in the content of gibberellin, auxin, cytokinin and abscisic acid at each stage of the development of cocoa pods. The hormones composition regulated the metabolism in pods and determined the process of embryogenesis and viability of pod

development. This revealed the role of hormones in determining the progress of pod development.

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O-PD14

Expression of Ligninolytic Enzyme of *Pleurotus djamor* EB9 and *Hohenbuehelia petaloides* EA4 on Sengon-Wood Liquid Substrate and Partial Purification of Manganese peroxidase

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not presented

O-PD15

The growth rate of three selected microalgae (*Dunnaliella tertiolecta, Nannochloropsis sp and Scenedesmus sp*) at different temperature cultured condition

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Abstract

The growth rate of two marine microalgae (*Dunnaliella tertiolecta and Nannochloropsis sp*) and one freshwater microalgae (*Scenecesmus sp*) cultured at two different temperatures condition, were studied. Microalgae were grown in laboratory using batch culture in phK and MLA medium for marine and freshwater microalgae, respectively at 18°C and 25°C (photon flux density 150 – 170 µmol photon $m^{-2}s^{-1}$; 24 hours in light condition). Cell density was measured daily to determine growth rate and cell division rate.

The optimum temperature for growth was 18°C for *Dunnaliella tertiolecta and Nannochloropsis sp* (specific growth rate, µ = 0.47/day and 0.52/day for *Dunnaliella tertiolecta and Nannochloropsis sp,* respectively) and 25°C for *Scenedesmus sp* (specific growth rate, µ=0.47/day). Every microalgae cultured had different time of cell density peak. *Dunnaliella tertiolecta* shown an early peak of cell density which was occured at Day 4 of cultured, while *Nannochloropsis* sp *and Scenedesmus* sp shown a peak of cell density at Day 9 and Day 6 of cultured, respectively. Interestingly, *Nannochloropsis* sp and *Scenedesmus* sp had a highest cell division rate at Day 2 of culture, while *Dunnaliella tertiolecta* showed a decreasing dramatically of cell division rate since Day 1 of cultured at their optimum temperature for growth.

The results indicated that three selected microalgae which were cultured at two different temperature condition responded differently on cell density, growth rate and cell division rate. Those results indicated that three selected microalgae was spesies specific responded on temperature cultured condition.

Keywords : Growth Rate, *Dunnaliella tertiolecta, Nannochloropsis* sp *and Scenedesmus* sp,

1. Introduction

Temperature is one of the major environmental factors and plays a critical role in growth, reproduction, migration, succession pattern and metabolism of organisms and communities [1]. In general, elevated water temperature causes changes in species composition, species dominance, standing crop and productivity of biota including phytoplankton communities in any aquatic ecosystem [2].

Every organism has a range of temperature that it can tolerate, which is known as tolerance levels. As temperatures get too far above or below this preferred range, the number of individuals of the species decreases until finally there are few, or none. The range

of temperature tolerated by the life form is completely wide but each species shows characteristic-limited temperature preference and tolerance [3-4].

The effect of temperature on algal growth rate indicated that, as temperature increased, the algal group with the highest growth rate changed from diatom to green algae to blue green algae (cyanobacteria) [5]. Temperature alone is a major factor in determining the occurrence of a particular algal species [6]. Growth rates of marine phytoplankton fall in the same range of values as those for freshwater algae, and there are no obvious distinctions between marine and freshwater unicellular algae with respect to the variation of specific grwoth rate (μ) with temperature [7].

Many laboratory studies have been conducted to determine the effect of temperature on the rate of phytoplankton growth and photosynthesis [8-9] and has been implicated to explain patterns of chlorophyll a – specific photosynthetic rate in the ocean [7]. Phytoplankton culture fully adapted to lower temperature exhibit decrease in the chl a-specific photosynthetic reate at light saturation, whereas little variability is observed in the light-limited rate [10-13].

The focus of this study was to investigate the impact of temperature on cell density, specific growth rate and cell division of three selected microalgae (*Dunnaliella tertiolecta, Nannochloropsis sp and Scenedesmus sp*) which were culture using batch culture method under laboratory condition.

2. Material and method

2.1. Microalgae and culture medium

Three microalgal species were used in this study, specifically Dunaliella tertiolecta, Scenedesmus sp and Nannochloropsis sp (all species from culture collection of Algae, Algal Physiology Laboratory, Biological Science, Monash University). All microalgae are eukaryotic photosynthesic microorganisms that grow rapidly as a consequence of their simple structure (Li et al, 2008). *Dunaliella tertiolecta and Nannochloropsis sp* are marine microalgal were cultured in PhK medium, consisting of 2L of pasteurized artificial seawater which has the following composition (per liter): 22 g NaCl, 5.0 g $MgSO_4.7H_2O$, 0.6 g KCl, 0.5 g $NaNO_3$, 0.25 g $CaCl_2.2H_2O$, 0.2 g tris-base, 0.165 g $NaHCO_3$, 1.0 mL of 3% solution KH_2PO_4 and 6.0 mL of trace elemental solution. The trace elemental solution (per liter) includes 0.02 g $CuSO_4.5H_2O$, 0.0125 g $NaMO_4.2H_2O$, 9.0 g Fe citrate, 9.0 g Citric acid, 0.046 g $ZnSO_4.7H_2O$, 0.289 g $MnCl_2.4H_2O$, 0.0081 g $CoCl_2.6H_2O$, 0.1001 g $FeSO_4.7H_2O$, 2.0027 g EDTA, 2.2877 g H_3BO_3, 0.010 g vitamin B12, 0.005 g Biotin and 0.02 g Thiamine

HCl. *Scenedesmus sp* is freshwater microalgal was cultured in MLA medium, consisting of 2 L pasteurized distillated water which has the following composition (per liter) : 49.4 g $MgSO_4.7H_2O$, 85 g $NaNO_3$, 6.96 g K_2HPO_4, 2.47 g H_3BO_3, 0.00129 g H_2SeO_3, 16.9 g $NaHCO_3$, 29.4 g $CaCl_2.2H_2O$, 10 mL micronutrients. The micronutrient solutions (per liter) includes 4.36 g Na_2EDTA, 1.58 g $FeCl_3.6H_2O$, 0.6 g $NaHCO_3$, 0.36 g $MnCl_2.4H_2O$, 1.0 g $CuSO_4.5H_2O$, 2.2 g $ZnSO_4.7H_2O$, 1.0 g $CoCl_2.6H_2O$, 0.6 g $Na_2MoO_4.2H_2O$, 0.010 g Biotin, 0.010 vitamin B12 and 0.010 g Thiamine HCl.

2.2. Culture system

Growth experiments were done at different temperatures conditions in 500 mL-Erlenmeyer flasks. The medium and flasks were sterilized in an autoclave for 20 mins at 121^oC in order to prevent any contamination during the early stages of growth.

The cell cultured was kept at incubator room at 18^oC and 25^oC equipped with artificial lightining. Each autotrophic batch cultivation was carried out in triplicate for 9 and 10 days at a continous photon flux density of 180 µmol m^{-2} s^{-1}, which was measured by a light intensity meter (LICOR Model LI-1400 data logger) for 24 hours.

Temperature in the medium was selected as a treatments (independent variables). Two different temperatures condition were selected, such as 18^oC and 25^oC.

2.3. Microalgal cell counting and growth rate

A direct microscopic cell count was performed daily with Brightline Hemocytometer (Neubauer, Weber England) and a Olympus CHS model microscope (Olymphus Optical Co. Ltd, Japan).

Specific growth rate (µ d^{-1}) was calculated as follows :

$\mu = (\ln N_2 - \ln N_1)/(t_2 - t_1)$

where N_2 and N_1 are the average values of cell numbers at times t_1 and t_2 [14]. (Ono and Cuello, 2007).

2.4. Statistical analysis

All the experiments were run in triplicate and teh results were presented as means and. In all cases, comparisons that showed a p value <0.05 were considered significant.

3. Results and discussion

3.1. The effect of temperature on growth rate and specific growth rate

The growth behavior of *Dunaliella tertiolecta, Scenedesmus sp and Nannochloropsis sp* under different temperature conditions showed a varied trend (Fig. 1). Cell growth in terms of cell density was higher at normal temperature than temperature stress. The peak of cell density all microalgals cultured were occurred at day 6 or day 7. The effect of temperature on cell density of microalgae varied within species. Cell density was higher at ambient temperature for marine (*Dunaliella tertiolecta* and *Nannochloropsis sp*) and freshwater species (*Scenedesmus sp*), such as 18^{o}C and 25^{o}C, respectively. *Dunaliella tertiolecta* showed a higher cell density at 18^{o}C than 25^{o}C account for 1.48 x 10^{6} cell/ml (Fig. 1a). Eighteen degree of temperature for *Dunaliella tertiolecta* showed an optimum temperture for cell growth. *Dunaliella tertiolecta* has a lower temperature for optimum growth than others species (*Dunaliella salina and D. viridis*), such as 22^{o}C and 26^{o}C, respectively [15]. Like others species of Dunaliella, *D. tertiolecta* showed a positive response on cell growth in terms of cell density to increase of temperature. Figure 1(a) showed that there was a significant decrease in cell density of *D. tertiolecta* to increase temperature. This result was supported by Garcia et al [15] who found that growth of *D. salina* and *D. viridis* decreased significantly with incresing temperature.

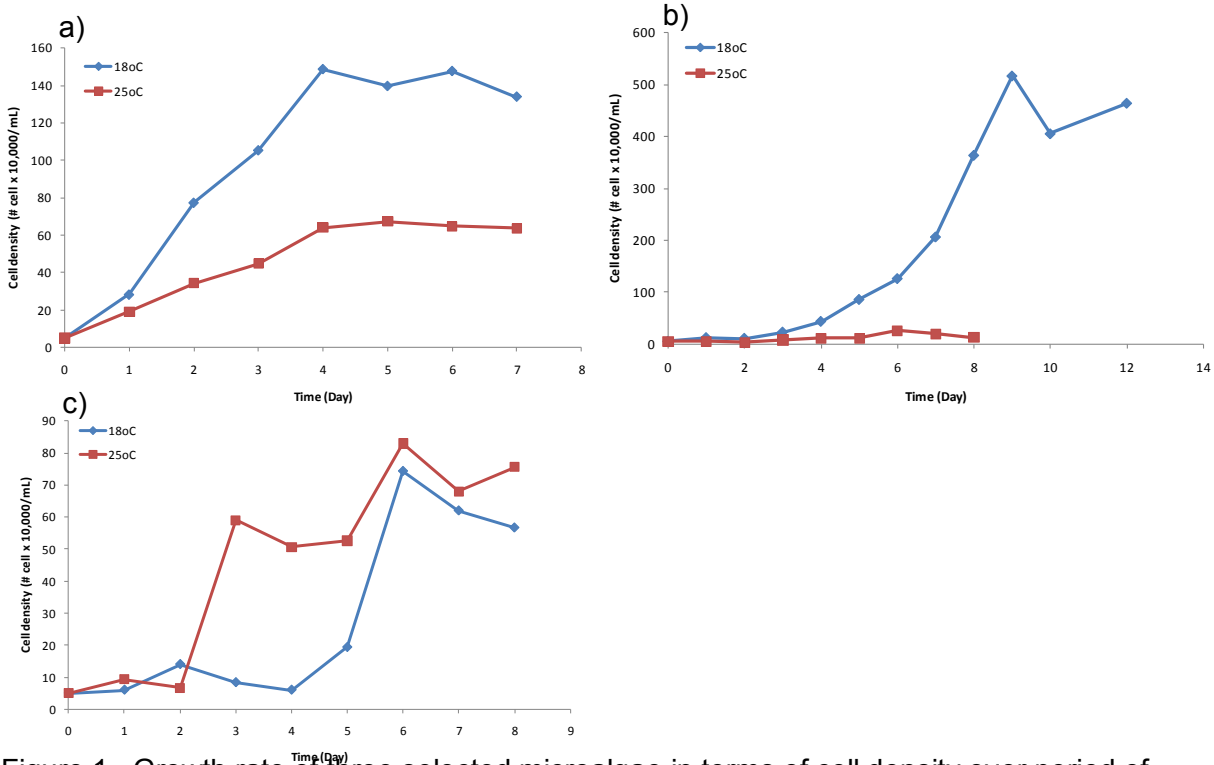

Figure 1. Growth rate of three selected microalgae in terms of cell density over period of culture. a) *Dunaliella tertiolecta*, b) *Nannochloropsis sp* and c) *Scenedesmus sp*

Nannochloropsis sp growth appeared to be affected at temperatures above 18°C (Fig. 1b). The highest cell density was occurred at Day 9 on the temperature of 18°C account for $5.17x10^6$ cell/ml. At 25°C, this microalgae exhibited a very low and almost no grow in terms of cell density, this temperature led to an abrupt interruption of microalgal growth and later the cell dead on further period of cultivation. Previous studies by Sayegh and Montagnes [16]; James et al [17] and Brown and Jeffrey [18] found that *Nannochloropsis sp* grown optimally at temperature around 20°C.

The growth curves of *Scenedesmus sp*. in the growth medium for different cultivation temperature was shown in Fig. 1(c). After 6 days of cultivation *Scenedesmus sp*. grew into the decreasing phase and reached a maximum cell density account for $8.4x10^5$ cell/ml at day 6 of cultivation for all temperature treatments. The study found that the optimal temperature for *Scenedesmus sp* growth was 25°C with the highest number of cell was $8.4x10^5$ cell/ml. Through Paired-samples t-test, microalgal densities in the cultivation process at the temperature of 25°C was significantly higher than at 18°C ($p < 0.001$). At 18°C *Scenedesmus sp*. grew very slowly at the beginning till the end of cultivation. The peak of cell density at low temperature was one day later than higher temperature. The result was supported by previous study by Wagner et al. [19] who found that at 25°C Scenedesmus sp. LX1 got the maximum specific growth rate and at 30°C it had the maximum carrying capacity K and population growth rate Rmax.

Figure 2 illustrated the effect of temperature on specific growth rate of three selected microalgae. Specific growth rate based on number of cell was calculated for all temperature level for each microalgal tested. Maximum specific growth rate (μ_{max}) were 1.734, 0.981, and 2.180 for *Dunaliella tertiolecta, Nannochloropsis sp and Scenedesmus sp*, respectively. For *Dunaliella tertiolecta* and *Nannochloropsis sp,* μ_{max} was occurred at temperature of 18°C at Day 1 and Day 2 of cultured, respectively (Fig 2 a and b). While *Scenedesmus sp* μ_{max} was achieved at Day 2 for the temperature of 25°C (Fig. 2c). Interestingly, *Scenedesmus sp* had a fluctuative value of specific growth rate over period of cultured. This species had a higher μ_{max} than two others tested species (*Dunaliella tertiolecta and Nannochloropsis sp*). The value of μ_{max} in this study was higher than previous study which was found by Zargar et al [2]. The higher value of μ_{max} in this study compare to previous study was probably due to different of species was used. Temperature above 25°C for *Dunaliella tertiolecta and Nannochloropsis sp* and below 25°C for Scenedesmus sp appeared to be unfavourable for proper growth of the alga. Temperature up to 18°C and below 25°C may considered as the limit of safe exposure for thermal stress of *Dunaliella tertiolecta,Nannochloropsis sp and Scenedesmus sp*, respectively. This finding was supported by previous study of Zargar et al

[2] who found that the growth rate of *Scenedesmus quandricuada* was inhibited at temperature up to 36°C. This level of temperature may considered as the limit of safe exposure for thermal stress of this species.

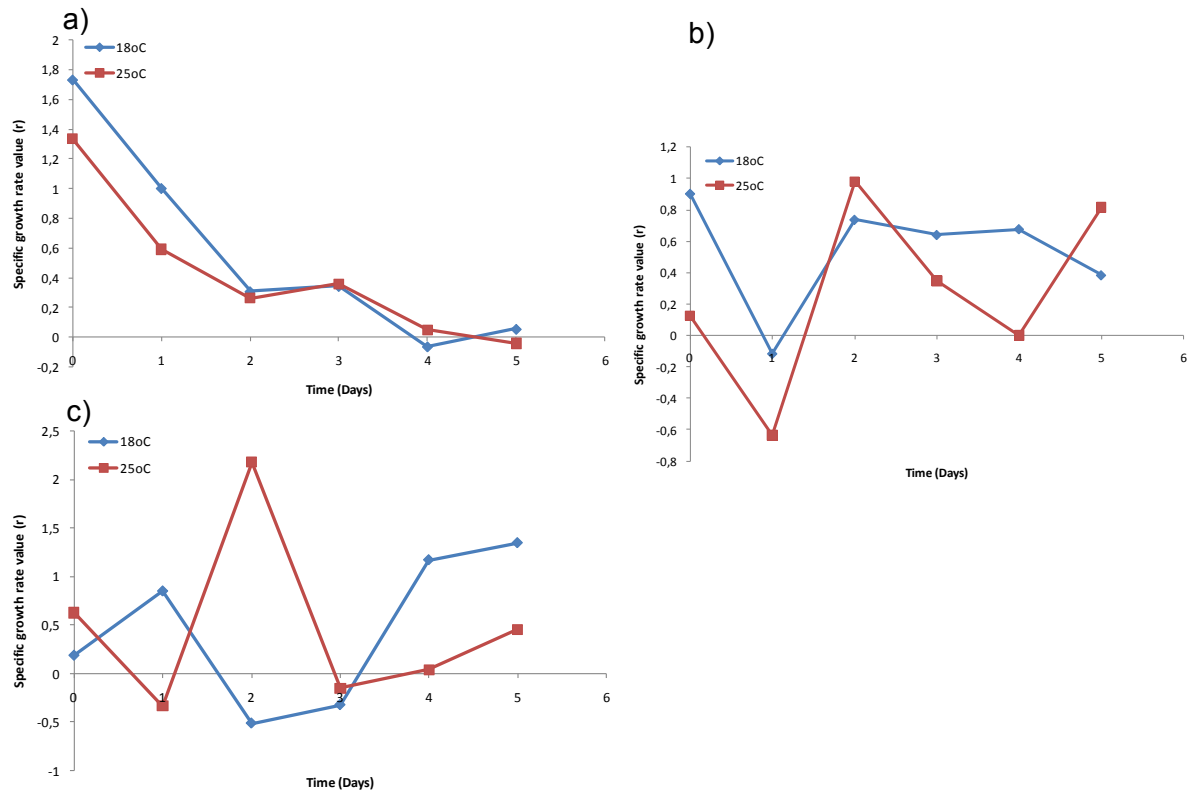

Figure 2. The specific growth rate of three selected microalgae (*Dunaliella tertiolecta, Nannochloropsis sp and Scenedesmus sp*) at two different temperature culture condition. a) *Dunaliella tertiolecta*, b) *Nannochloropsis sp* and c) *Scenedesmus sp*.

3.2. The effect of temperature on cell division

Temperature showed a direct influence to cell division rate of phytoplankton. Figure 3 illustrated the effect of temperature on cell division three selected microalgae. The pattern of cell division rate was varied within species. *Dunaliella tertiolecta* had higher cell division rate at the begining of cultured period and decreased dramatically at Day 2 of cultured period and remain steady till Day 5 of cultured period for both temperature treatments (Fig. 3a). The highest of cell division was accounted for 5.7 and 3.8 at 18°C and 25°C, respectively.

Cell division rate of *Nannochloropsis sp* increased sharply at Day 2 and achieved the highest division rate at Day 2 at 25°C account for 2.7 (Fig. 3b). Cell division rate then decreased significantly at Day 3 of cultured period. Interestingly, at 25°C *Nannochloropsis sp* showed other peak of cell division rate, which was at Day 5 of cultured period account for

2.3. On the other hand, at 18°C, *Nannochloropsis sp* only have one peak of cell division rate, which was at the beginning of culture account for 2.4 and cell division rate tended to decrease over period of cultured.

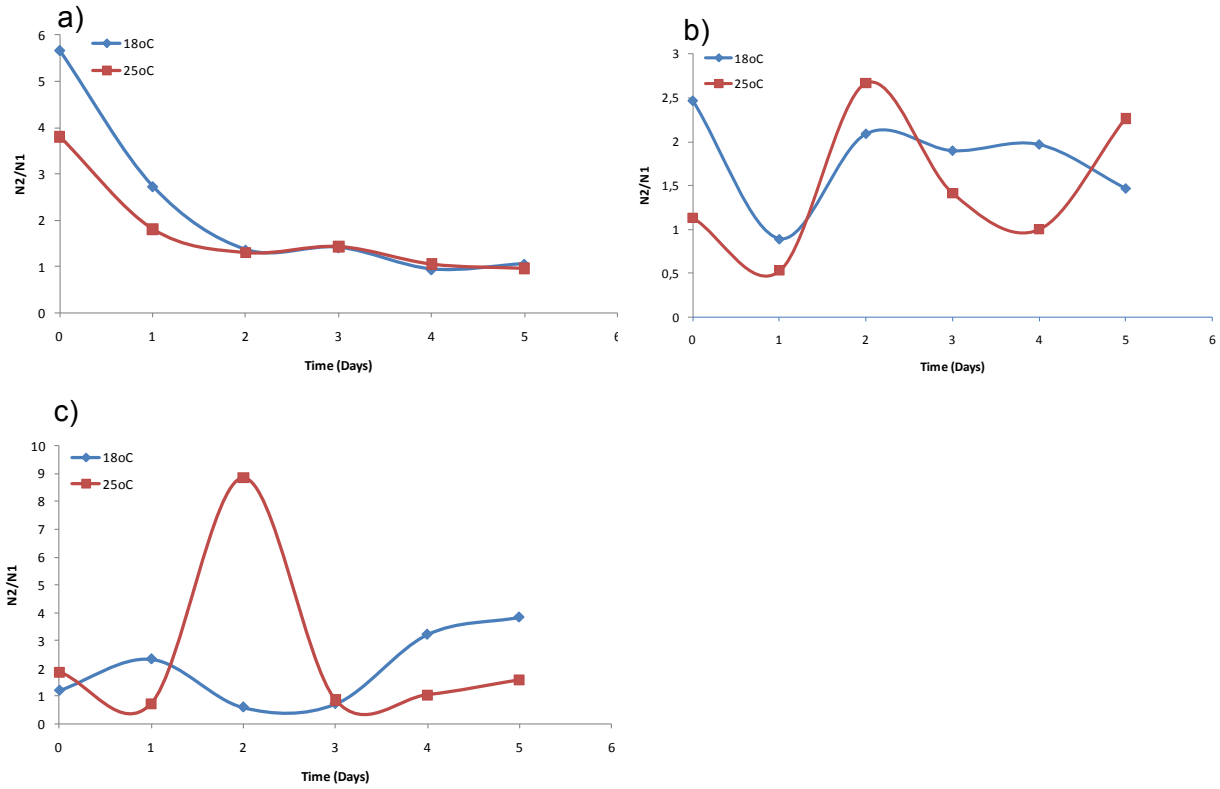

Figure 3. Cell division rate of three selected microalgae (*Dunaliella tertiolecta, Nannochloropsis sp and Scenedesmus sp*) at two different temperature culture condition. a) *Dunaliella tertiolecta*, b) *Nannochloropsis sp* and c) *Scenedesmus sp*.

Scenedesmus sp showed a peak of cell division rate at Day 2 of cultured period account for 8.9 for the temperature of 25°C (Fig. 3c). The cell division rate decreased sharply in the following day and got the lowest cell division rate at Day 3 of cultured period, with the value of cell division was 0.86. At 18°C, cell division rate increased gradually and achieved the maximum value at Day 5 with the value of cell division rate 3.8. Cell division rate was higher at 25°C than at 18°C. This result was supported by previous study of Jogersen [20] who found that in S, costatum lowered temperature led to lowered division rates but higher rates of carbon and nitrogen assimilation.

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O-PD16

Isolation of endophytic microorganisms from upland rice and the potential to plant growth and health stimulation

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Not presented

O-PD17

Diversity and Distribution of Bivalves at Modung Beach Madura

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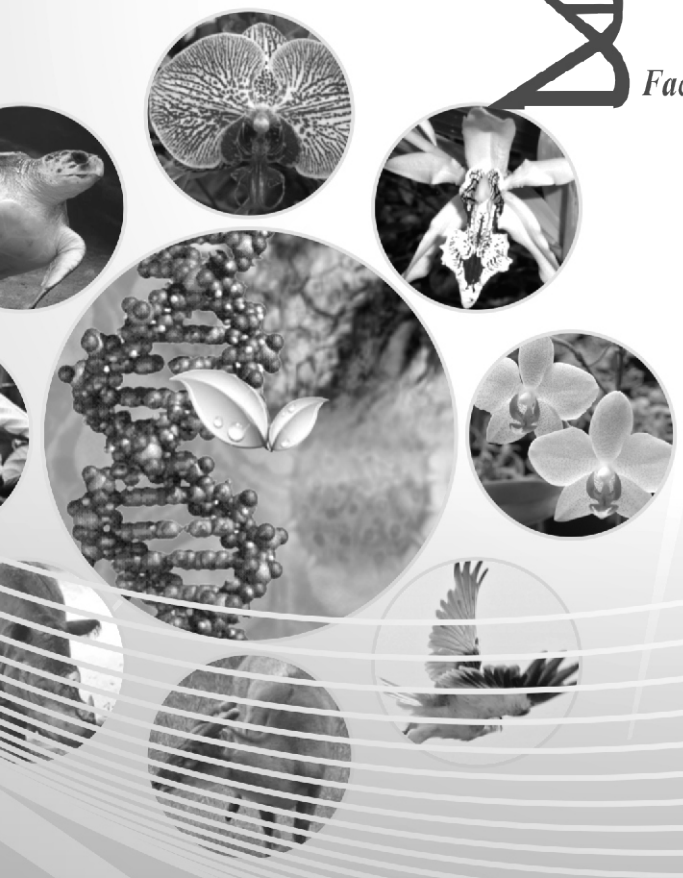
not presented

ORAL - TOPIC 5

Biomedics (O-BM)

ICBS 2011

Faculty of Biology Universitas Gadjah Mada



FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

O-BM01

Growth Inhibition Activity of *Citrus maxima* (Burm.) Merr. Peel Essential Oils on HeLa Cervical Cancer Cells

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Abstract

Citrus peel essential oils have been known as having anticancer properties indicated by their capacity in inhibiting the growth of various cancer cell lines. In this study, the growth inhibition activity of *Citrus maxima* essential oil on HeLa cell line was examined to determine its potential as anticancer compound. Essential oils from fruit peel of three *C.maxima* cultivars, i.e. Nambangan, Sri Nyonya, and Gulung, were extracted using destilator. GC-MS analysis showed that these essential oils were consisted of monoterpenes, sesquiterpenes, and diterpenes. The growth inhibition activity was tested using MTT assay using six concentrations of peel essential oils: 100, 200, 400, 600, 800, and 1,000 µg/mL, respectively. Results showed that the growth inhibition activity of peel essential oils from Nambangan dan Gulung cultivars increased with oil concentrations, while the essential oil from Sri Nyonya cultivar had no clear pattern in which all concentrations resulted in < 55% inhibition. The IC_{50} of peel essential oils from Nambangan and Gulung cultivars were 77,845 µg/mL and 158 µg/mL, respectively. It could be concluded, therefore, that peel essential oil of Nambangan cultivar had a potential as natural anticancer compound.

Keywords: essential oil, *Citrus maxima*, anticancer, HeLa cells

Introduction

Cancer is one of the most cause's deaths in Indonesia. Continuous efforts have been done in order to find the more effective and cheaper drug for cancer treatment. Herbal plants are one of the natural resources contain a lot of bioactive compounds which are potential as anticancer. Some research showed that *Citrus* is one of plants which having potency as anticancer.

Maslorava & Henonen (2001)[1] reported that *Citrus* extract have antioxidant properties. It is potential to inhibit growth of cancer. Besides, some epidemiological research showed that *Citrus* consumption has protective effect against cancer. Those effect might be caused by bioactive compounds of *Citrus* such as vitamin C, folat, carotenoids, and flavonoids[2]. Juice of Pamelo, Lemon, and the others economical *Citrus* were known having cytotoxic effect against Caco-2 cells monolayers[3]. *Citrus* essential oils were known as compounds that can inhibit proliferation of human colon cancer cells[4], human mouth epidermal carcinoma cells line, and murine leukemia cells line[5].

Citrus maxima peels contain essential oils and have not used optimalically yet. This research was studied the potency of essential oils of *Citrus maxima* peel to inhibit cervical cancer cells growth.

Materials and Methods

a. Materials

Three cultivars of *Citrus maxima* (pamelo): Nambangan, Sri Nyonya, and Gulung. Chemical for essential oils extraction, analysis, and compounds identification was diethyl ether. Chemicals for cancer inhibition growth assay were HeLa cells obtained from LPPT, Universitas Gadjah Mada, RPMI 1640 media, FBS, Penstrep, fungizon, MTT, trypsin 0,25 %, SDS 10% in 0,01 N HCl, PBS, DMSO.

Apparatus for *Citrus* collection were plant scissors, collection bottle, and GPS. Apparatus for essential oils extraction were mortar, petri dish, and reaction tube. Apparatur for inhibition growth assay were inverted microscope, laminar air flow hood, waterbath, beker glass, flask culture, 96 wells microplate, micropipet, CO_2 incubator, pipet, and ELISA reader.

b. Methods

1. Sample collection of *Citrus maxima* was done in *Citrus* production center, Magetan (Dukuh and Duwet villages, Kecamatan Bendo). This research used mature fruits (ready to harvest).
2. Essential oils extraction and analysis of *Citrus maxima* peel followed the method of Merle *et al.* (2004)[6].
   a. Essential oils extraction

      Twenty grams of *Citrus maxima* peels at base point for each cultivar were pressed manual until excretes oils. The oils were collected on petri dish. Those oils were removed in the reaction tube and then centrifuge 15.000 rpm for 10 minutes to seperate the oils and water phase. Oil phase (supernatan) was removed in another reaction tube and added diethyl ether (1:99) for gas chromatography analysis.

   b. Essential oils analysis

      Sample of essential oils were analyzed by gas chromatography using capillary column with length 25 m and diameter 0,25 mm, film thickness 0,25 µm. Gas chromatography program used column temperature 60°C for 6 minutes and the temperature increased 5°C every minute until reach 180°C. Helium was used as carrier gas with a flow rate set at 2 mL/minutes. The injector and detector temperature were held at 225 and 250°C.

c. Compounds identification

Every peak was identified using gas chromatography-mass spectrophotometry. The results of chromatogram were compared with the data in GC-MS library.

3. Growth inhibition assay

HeLa cells were cultured in 96 wells microplate ($2x10^4$ cells/100 μL/well). Each treatment was done 5 times. The groups of this research were:

a. Media control group (HeLa cells suspension incubated in RPMI 1640 + FBS 10%).
b. Treatment groups, HeLa cells suspension were given test compounds at the concentration of 100, 200, 400, 600, 800, and 1000 μg/mL respectively. Test compounds were added 100 μL/well. Media were used to dilute the test compound. Test compounds were essential oils from three cultivar of *Citrus maxima* (Gulung, Nambangan, and Sri Nyonya) peels.

HeLa cells were incubated at 37°C, CO_2 5% for 24 hours. Test compound were given in each well as have been set in the treatment group and were incubated again at 37°C, CO_2 5% for 24 hours. Inhibition growth assay was done using MTT assay method as follow: in the end of incubation period, each of well was added by MTT (5 mg/mL) 10 μL. Cells suspension were incubated at 37°C, CO_2 5% for 4 hours. The reaction was stopped using SDS 10% in 0,01 N HCl. Optical Density (OD) was read using *ELISA reader* at a wavelenght 550 nm[4,5,7,8,9,10]. Growth inhibition percentage was calculated using formula:

$$\% \text{ Growth Inhibition} = \frac{(\text{OD media control} - \text{OD treatment})}{\text{OD media control}} \times 100\ \%$$

Notes:

OD media control = OD media control – OD negative control of media.

OD treatment = OD treatment – OD negative control of each treatment.

4. Data analysis

Growth inhibition assay was analyzed using one way ANOVA followed by *Tuckey's HSD test*. IC_{50} of test compounds were analyzed using probit analysis.

Results and Discussion

Results

a. Sample collection of *Citrus maxima*

Sample collection was done in May, 14-16, 2009, in Dukuh and Duwet village, Kecamatan Bendo, Kabupaten Magetan. There were three cultivars which have been collected: Nambangan, Sri Nyonya, and Gulung. Sampling collection was in close area, with range 30-200 m for one *Citrus* plant to the others. The mature fruits were collected. The fruits were kept in refrigerator until essential oils analysis would be done.

b. Essential oils extraction and analysis

Based on the chromatogram from GC was known that essential oils of *Citrus maxima* peels from three cultivars contain the same compounds. There were 32 compounds. Quantitatively the essential oils of Nambangan *Citrus* peels have the highest concentration, followed by Gulung and Sri Nyonya. Each peak from GS-MS was compared by the library from MS for identification. Based on this research there were some compounds that could be identified. Those compounds could be categorized in monoterpenes (limonene; citronella; trans-1,2-diisopropenyl-cyclobutane; cyclobutane; naphthalene (CAS) white tar; cyclohexene; 1-methyl-4-(1-methylethenyl)-(CAS)1-p-mentha-1,8-Diene; 2-pentanone-4-hydroxy-4-methyl-(CAS) diacetone alcohol; octane,2-bromo-(CAS)2-bromooctane); butane,2,2-dimethyl-(CAS) 2,2-dimethylbutane), sesquiterpenes (hexadecanoic acid (CAS) palmitic acid; tetradecanoic acid (CAS) myristic acid), and diterpenes (10-octadecenoic acid, methyl ester (CAS) methyl octadec-10-enoate; 10-octadecenoic acid, methyl ester (E)-(CAS) methyl elaidate; 11-octadecenoic acid, methyl ester (Z)-(CAS) methyl cis octadec-11-enoate; methyl 9,9 dideutero-octadecanoate; 12-octadecenoic acid, methyl ester (CAS) methyl octadec-12-enoate).

c. Growth inhibition assay

The results showed that essential oils of *Citrus maxima* peel from three cultivars have different potency to inhibit the growth of HeLa cells. For Nambangan cultivar, growth inhibition percentage on HeLa cells increased as the increasing of concentration. However, growth inhibition percentage at the concentration of 200 μg/mL and 400 μg/mL did not show any significant differences as well as the concentration of 800 μg/mL did not show any significant differences with the concentration of 1000 μg/mL (Table. 1).

For Gulung cultivar, growth inhibition percentage on HeLa cells increased as the increasing of concentration. However, growth inhibition percentage at the concentration of 400 μg/mL, 600 μg/mL, and 1000 did not show any significant differences. Growth inhibition percentage at the concentration of 800 μg/mL did not show any significant differences with the concentration of 1000 μg/mL (Table. 1).

Sri Nyonya cultivar did not show the clear pattern on growth inhibition of HeLa cells. In this cultivar the growth inhibition tend having the same value in any given concentration (from 100-1000 μg/mL) (Table. 1).

Table 1. Average of growth inhibition percentage of essential oils of *Citrus maxima* (Burm.) Merr. peel on HeLa cells.

Cultivar	Essential oils concentration (μg/mL)	Growth Inhibition (%)
Nambangan	100	53,211 ± 2,912[a]
	200	72,945 ± 3,346[b]
	400	77,005 ± 4,018[b]
	600	86,189 ± 6,144[c]
	800	100[d]
	1000	100[d]
Gulung	100	34,239 ± 2,218[a]
	200	62,193 ± 5,019[b]
	400	71,947 ± 1,25[c]
	600	75,057 ± 5,235[c]
	800	86,448 ± 6,296[d]
	1000	80,491 ± 5,986[cd]
Sri Nyonya	100	52,438 ± 2,899[c]
	200	46,832 ± 7,104[bc]
	400	40,796 ± 3,791[ab]
	600	33,234 ± 5,359[a]
	800	42,345 ± 4,196[b]
	1000	39,270 ± 1,856[ab]

Note: the number which was followed by different letter in the same column have significant differences ($p<0,05$)

Based on probit analysis, IC_{50} value of essential oils of Nambangan cultivar was 77,845 μg/mL and IC_{50} value of essential oils of Gulung cultivar on HeLa cells was 158,439 μg/mL. Whereas IC_{50} value of essential oils of Sri Nyonya cultivar could not calculated because the result did not show linier pattern.

Discussion

This research studied the growth inhibition of essential oils of three cultivars (*Citrus maxima* (Burm.) Merr.) peel on HeLa cells. The results showed that along with increasing of the essential oils concentration of Nambangan and Gulung cultivar the effect of growth inhibition on HeLa cells were higher. At the concentration of 800 and 1000 μg/mL growth inhibition percentage of Nambangan essential oils on HeLa cells was 100% whereas growth inhibition percentage of Gulung essential oils on HeLa cells was > 80%. IC_{50} value of Nambangan essential oil was 77,845 μg/mL and IC_{50} value of Gulung essential oils was 158,439 μg/mL. These IC_{50} value depend on the cultivar of *Citrus maxima*. Manosroi *et al.*

(2006)[5] classify essential oils sample with IC_{50} < 0,125 mg/mL have a high potency to develop as anticancer agent. So essential oils of Nambangan cultivar have a high potency as anticancer agent. Whereas IC_{50} value among 0,125-5 mg/mL have a moderate potency to develop as anticancer agent. So essential oils of Gulung were in this category. The results of compounds analysis and identification using GC-MS showed that qualitatively and quantitatively essential oils composition from the highest to the least were essential oils of Nambangan, Gulung, and Sri Nyonya peel. Major compounds of those essential oils were limonene and citronella. Nambangan have the highest growth inhibition percentage followed by Gulung and Sri Nyonya. This potency was relevant with the highest composition and concentration of essential oil of Nambangan peel compared with Gulung and Sri Nyonya peel. This is not in agreement with Lim *et al.* (2009)[8] who stated that there was no corelation between IC_{50} value with major flavonoid and limonoid from four fractions organic solvent. Lim *et al*. (2009)[8] showed that the analysis results of major flavonoid and limonoid from hexane fraction significantly lower that the other 3 organic fractions. However, hexana fraction was the most potent as anticancer agent compare with the other three fractions especially to induce apoptosis of histiocytic lymphoma cells (U937). Those anticancer activity might be influenced by the minor componen from hexane fraction. Bakkali *et al.* (2008)[11] stated that a lot of componens of essential oils play role as antioxidant. Those cytotoxic effect depend on the kind and concentration of the compounds. However essential oils did not show genotoxic effect.

Based on the identification results, the compounds of essential oils of *Citrus maxima* peels were included in category monoterpenes, sesquiterpenes, and diterpenes. Minor componens which was played role in growth inhibition of HeLa cells could be monoterpenes or sesquiterpenes. Patil *et al.* (2009)[4] studied that growth inhibition on colon cancer was caused by monoterpene compounds of lime essential oils. Sylvestre *et al.* (2006)[12] said that volatile extract which have cytotoxic effect majority are sesquiterpenes and a little of monoterpenes. In this research for knowing definitely the kind of compounds in the essential oils which is play role in growth inhibition have to be done using fractination and structure elucidation to the componens of essential oils of *Citrus maxima* peel of three cultivars.

In this research the growth inhibition mechanism on HeLa cells of *Citrus maxima* peels essential oil could not known yet. However, from some research were known that antiproliverative effect on human leukemia cells (U937) caused by apoptosis induction and involved signaling pathway which was mediated by mitochondria[8]. Patil *et al*. (2009)[4] indicated essential oils could cause the lost of membrane integrity on human colon cancer cells. This lost of membrane integrity may be caused by the high lipophylicity of its compounds. It indicated that essential oils could induce cytotoxicity through membrane

disintegration. Essential oils of *Citrus maxima* peels from three cultivars might be given effect through the same mechanism.

Conclusion

It could be concluded that essential oils of *Citrus máxima* (Burm.) Merr. peel of Nambangan and Gulung cultivar have potency to develop as therapeutic agent for cáncer treatment especially for cervical cancer.

Acknoledgement

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O-BM02

The potency of *Wnt5a* gene for cancer therapy

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ABSTRACT

Wnt5a is a member of Wnt family gene which encodes secreted glycoproteins that play essential roles in many aspects of organ development. This gene performs its function through the activation of non-canonical signaling pathways via planar cell polarity pathway or Ca^{2+} pathway. Some studies had reported that *Wnt5a* also act as an antagonist of canonical *Wnt*s which widely known their involvement in cancer development. Our previous study also suggested that *Wnt5a* as of other Wnt family members is a mesenchymal factor yet does not induce cell division which may resulted on organ enlargement. The inhibition of *Wnt5a* pathway resulted on the disruption of tissue arrangement. Its potency as a candidate of cancer regulator had been reported. Study on Parkinson's disease using *Wnt5a*-bearing mouse ventral midbrain (VM) neural stem-cells unveiled that *Wnt5a* improve the differentiation and functional integration of stem cell-derived dopaminergic (DA) neurons in vivo, and define a safe source of DA neuron for replacement therapy since there is no sign of tumor formation. Furthermore, it is also reported that *Wnt5a* is important for survival of patient with breast cancer, down regulation causes a higher probability of metastasis. Since gene therapy is one of hopes in cancer administration, *Wnt5a* might give a contribution in the regulation of cancer. This paper discusses the possibility of Wnt5a gene for future cancer therapy.

Keywords: *Wnt5a*, cancer therapy

Introduction

Cancer is a generic term for a large group of diseases that can affect any part of the body. Other terms used are malignant tumors and neoplasms. One defining feature of cancer is the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs. This process is referred to as metastasis. Metastases are the major cause of death from cancer. As a leading cause of death worldwide, it is accounted for 7.6 million deaths (around 13% of all deaths) in 2008 caused by cancer. More than 70% of all cancer deaths occurred in low- and middle-income countries. Deaths from cancer worldwide are projected to continue to rise to over 11 million in 2030 [1].

Cancer arises from one single cell. The transformation from a normal cell into a tumor cell is a multistage process, typically a progression from a pre-cancerous lesion to malignant tumors. These changes are the result of the interaction between a person's genetic factors [1,2] and three categories of external agents, including: physical carcinogens, such as ultraviolet and ionizing radiation; chemical carcinogens, such as asbestos, components of tobacco smoke, aflatoxin (a food contaminant) and arsenic (a drinking water contaminant); and biological carcinogens, such as infections from certain viruses, bacteria or parasites [1].

The genetics study on cancer

Currently, the study on cancer regulation in genetic level is highly done. The research in this field began to robust when it has been known that cancer can be considered as a genetic disease of cells [3] and there are different genes expressed in the different cases of cancer [4,5], whilst multiple tumor suppressor are along [6,7]. Since then prevention of cancer in patients with a hereditary disposition to malignant tumors became possible [8].

Among those has been known as oncogenes, *Wnt* family gene is one of which widely studied. This gene family consists of 19 genes encoding lipid-modified secreted glycoproteins that show a highly regulated pattern of expression and has distinct roles during development and tissue homeostasis [8,9,10]. This gene performs its function through the activation of cellular processes well known as *Wnt* signaling pathways. At least three distinct pathways activate by *Wnt* genes, those are: canonical pathway, planar cell polarity pathway and Ca^{2+} pathway [13,14,15], these last two pathways are refer to as non-canonical pathways [12].

***Wnt* genes in cancer**

Wnt gene family previously is well known as oncogene which involved in various cases of cancer [15,16,17,18,19]. Recent study unveiled that 11 of 19 *WNT* family members are expresses in various carcinoma cells [16]. The involvement of this gene family in cancer is mostly by activation of canonical β-catenin signaling pathway (Fig. 2), which results on the accumulation of β-catenin and its nuclear translocation [10], such as in oral carcinogenesis [16,20,21,22], head and neck squamous cell carcinoma (HNSCC) [23,24], T-cell-acute Lymphoblastic leukemia (T-ALL) [19], and colorectal cancer [15].

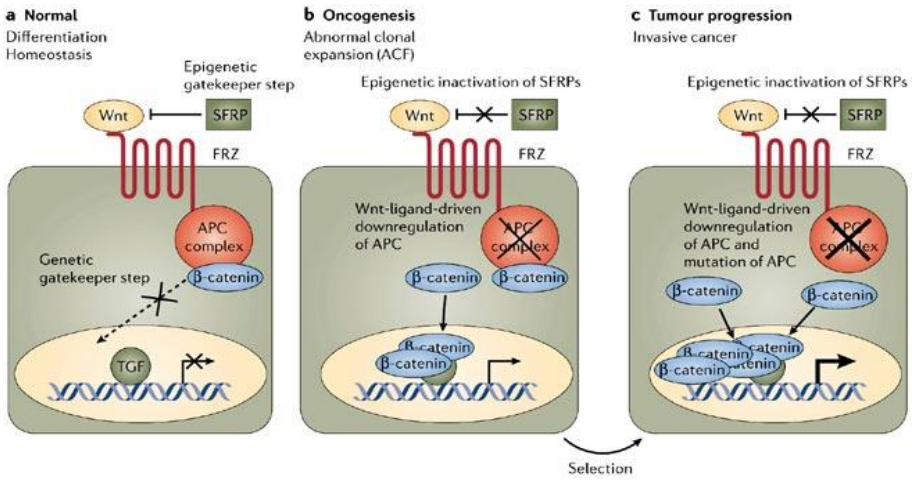

Fig. 1: Wnt Signaling in development and cancer. (Courtesy of Nature Publishing Group, Nature Reviews/Cancer) [10]

In only few cases the activation of *Wnt/β-catenin* signaling result on the inhibition of cancer, such as in myeloma bone disease [25,26] and melanoma [27]; whilst non-canonical *Wnt*, such as *Wnt5a*, induces the occurrence of cancer [28,29,30]. By the time being, many studies unfold that some members of *Wnt* family plays the opposite roles, or even both, the oncogenic and tumor suppressor, as well [11,31,32].

Regardless the controversy of *Wnt5a* role in cancer, some studies had reported that *Wnt5a* also act as an antagonist [33,34,35] or inhibitor of canonical *Wnt* genes [36,37,38]. Our previous study revealed that *Wnt5a* might be acted in fine tuning of organ development since there was no sign of organ enlargement as a result of *Wnt5*a overexpression [39]. In agreement with Schultze *et al*. [40], Castelo-Branco *et al*. [41], and Parish *et al.* [42], precocious expression of some marker genes as a result of *Wnt5a* overexpression leads to the assumption that this gene induces cell differentiation instead of cell division. On the other hand, tissue disruption as a result of *Wnt5a* signaling inhibition [43] leads us to assume that the inhibition of *Wnt5a* allows canonical *Wnts* to run down and induce uncontrolled cell division, and in turn might promote cancer development [18].

Wnt5a as cancer regulator

Taking a consideration that the role of *Wnt5a* in cancer regulation, in same ways, working in opposing the canonical *Wnts* [37], hereby we examine the involvement of *Wnt5a* in the development and regulation of some important cancers such as breast cancer, leukemia, and midbrain dopaminergic (DA) neuron development to seek a wider view for the possible application of *Wnt5a* in cancer therapy.

In breast cancer, melanoma, and Non-Small-Cell-Lung-Cancer (NSCLC) there are controversy on the possible influence of *Wnt5a* in cancer regulation. Some authors reported a significant overexpression of *Wnt5a* in the metastasis-derived finite-life span breast cancer/non-mortalized breast cancer, while a studied canonical Wnt (*Wnt10b*) was underexpressed [30]. In human melanoma *Wnt5a* expression correlated with melanoma progression. *Wnt5a* transfection results on increasing of cell motility and invasion, while disruption of *Wnt5a/Frizzled-5* pathway results on reduction of cell invasiveness [28]. In NSCLC overexpression of *Wnt5a* could produce more aggressive NSCLC especially in squamous cell carcinomas, during tumor progression [29].

Other reports from breast cancer studies show the opposite; *Wnt5a* is important for survival of patient with breast cancer [44,45]. Lost of *Wnt5a* reduce the survival of the patient [44] and increase relapse and death as a result of recurrent ductal breast cancer [46]. Down regulation of this gene cause in higher probability for metastasis [45,47] as a result of significant lower level of membrane-associated β-catenin which related to the establishment

of cell-cell binding through β-catenin/E-cadherin complex formation [45]. According to this finding [45], it seems reasonable that, in some cases [25,26,27], the activation of *Wnt/β-catenin* signaling might result on the inhibition of cell invasion, since (limited amount of) β-catenin is needed to form cell-cell binding, yet it need careful studies in various types of cancer. Concurrent with this finding, the interference with *Wnt* signaling, at the ligand level, may improve the efficiency of breast cancer treatment [48].

In Leukemia, many authors reported that the occurrence of this case is caused by uncontrolled Wnt signaling [18] which positively down-regulated by *Wnt5a* [49] through repression of *Cyclin D1* [37,50] as a result of suppression of β-catenin/TCF-dependent transcriptional activity [37]. The repression of *Cyclin D1* in turn results on inhibition of cell proliferation. This activity is similar as of antagonizing activity against (canonical) *Wnt* signaling which occurs in colorectal cancer [15,35].

One more important finding is the role of *Wnt5a* in the development of DA neurons [51,52]. DA neurons of the ventral mesodiencephalon are affected in significant health disorder such as Parkinson's disease, schizophrenia, and addiction [52]. Since human embryonic stem (hES) cell-derived DA neuron either poor survival [42,53], risk of teratoma formation [42], or their DA phenotype is unstable [53], cell replacement therapy that will successfully promote the specific differentiation of stem cell into DA neuronal phenotype is required [52]. Experiment with parkinsonian mice using mouse ventral midbrain (VM) neural stem cells expanded with *FGF2*, differentiated with *Shh* and *FGF8*, and transfected with *Wnt5a* (VMN-*Wnt5a*) resulted in the significant development of DA neuron; it gave 10-fold more DA neuron compared to conventional *FGF2*-treated VMNs, and significant cellular and functional recovery following the transplantation into parkinsonian mice. This result also shows that *Wnt5a* improve the differentiation [40,41,42,51] and functional integration of stem cell-derived DA neurons in vivo [42,51], and define a safe source of DA neuron for replacement therapy [54], because there is no sign of tumor formation [42]. A further study on the mechanism of DA neuron developmental regulation suggest that Wnt5a protein interact with dopamine D2 receptor (D2R) and working in association with extracellular signal-regulated kinase (ERK) pathway [55].

Conclusion

Taking all together, the involvement of *Wnt5a* in various types of cancer and its role both in the stimulation and the inhibition of particular cancer development has shed the light for the possibility of *Wnt5a* application in genetic therapy of cancer. References already provided on the mechanism running by *Wnt5a* giving a rich foundation on the development of therapy mechanism which applicable and safe for human life in the future.

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O-BM03

TWO MUTATIONS ASSOCIATED WITH OCA4 IN INDONESIAN PATIENTS

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not presented

O-BM04

Early Detection and Serotyping of Dengue Viruses Clinical Isolates Using RT-PCR 2 Primers

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ABSTRACT

Since Dengue Haemorrhagic Fever (DHF) has a very broad clinical spectrum, the rapid, cheap and accurate laboratory diagnosis is absolutely needed. Commonly used diagnostic methods is expensive and time consuming and can not be reached by common peoples. Recently several methods for confirming Dengue Virus have been developed involve virus isolation, detection of virus antigen, and nucleic acid using PCR. It has been reported that rapid detection method for confirming DHF by Multiplex RT-PCR had been successfully developed. It was more simple and rapid than the other methods with a high sensitivity and specivicity were 100% at the early phase (day 1-3). This study was designed to develop rapid detection and serotyping methods for Dengue Virus using RT-PCR 2 primers (Dcon and preM) with annealing temperature was 57^{o}C. The whole blood samples were collected from suspected dengue fever patients that had been confirmed with NS1 kit from R.S. Persahabatan DKI Jakarta and R.S. Prof. Dr. Sardjito DI Yogyakarta during Februari-August 2009. The PCR products showed that in 12 samples, 100 % were postitive with different pattern among the serotypes especially for DEN1 and DEN2, but not for DEN3 and Den4. This method was also able to confirm the double infection DEN2-DEN3, but not for the other ones because of the unspecific pattern. From the results, it indicated that the 2 primers can be a promising rapid detection and serotyping method of Dengue Virus which infected the DHF patients.

Keywords: Dengue Virus, DHF, early detection, serotyping, RT-PCR 2 primers.

O-BM05

HUMAN T LYMPHOTROPIC VIRUS-2 DETECTED IN MSM (MEN WHO HAVE SEX WITH MEN) COMMUNITIES IN SURAKARTA, INDONESIA

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ABSTRACT

MSM (Men Who Have Sex with Men) communities are being associated with high risk of blood borne virus infection, including that of Human T-lymphotropic virus-1/2 (HTLV-1/2). However, for the best of our knowledge, at present there is no molecular epidemiological data of HTLV-1/2 in MSM communities in Indonesia yet. To address the questions, blood samples collected from 143 MSM in Surakarta in September-October 2009 were addressed for serological assay. The nucleic acid was extracted from the anti-HTLV-1/2 positive samples. The nested RT-PCR was performed to detect part of LTR region of the HTLV-1 and HTLV-2 genome, respectively. The positive PCR products were directly sequenced and phylogenetic analyzed. Anti-HTLV-1/2 positive was found in 1.4 % (2/143) of total samples. The HTLV-2 RNA was detected in both of the positive samples. Phylogenetic analysis based on 666 nucleotides of the LTR region revealed that both HTLV-2 isolates were homolog with HTLV-2 isolated in Taiwan. To the best of our knowledge, this was the first molecular data of HTLV-2 circulated and isolated in Indonesia.

Keywords: HTLV-2, MSM, Surakarta, Indonesia

INTRODUCTION

Human T cell lymphotropic virus type 1 and 2 (HTLV-1/2) were the first human retroviruses to be discovered. HTLV-1 is associated with adult T-cell leukemia (ATL), HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV associated uveitis (HAU) (1). Phylogenetic studies suggest that HTLV-1 arrived in South America through fairly recent, multiple introductions, probably during African slavery. All South American countries have reported the presence of HTLV-1, but the prevalence varies greatly between and within countries and population groups. The reasons for HTLV-1 clustering remain unknown. Classically, a pattern of age and sex dependence occurs for HTLV-1 seroprevalence among blood donors, with prevalence higher in females and increasing

steadily with age, similar to patterns observed in endemic areas of southern Japan and the Caribbean basin (1).

HTLV-1 has been estimated infected 15 to 20 million people worldwide. The virus is endemic in southern Japan, the Caribbean basin, central Africa, Central and South America, the Melanesian Islands in the Pacific basin, and in the aboriginal population in Australia. The seroprevalence of HTLV-1 varies from 0.1 % to 30 % within endemic populations. Sporadic HTLV-1 infection occurs among at-risk groups from non endemic regions, including metropolitan areas in the United States and western European countries (2). No molecular epidemiology data of HTLV-1/2 based on men who have sex with men have (MSM) ever been published, for the best of our knowledge.

Little is known about Human T cell lymphotropic virus types 1 and 2 (HTLV-1/2) infections in Indonesia especially that of in MSM community. The seroprevalence of HTLV in the healthy blood donors in Jakarta area, which was also reflected in the western part of Indonesia, was very low; however, there was no evidence of the presentation of the viruses. No seropositive individuals were found in healthy Irian Jaya people (3, 4). Thus, the present study aimed to add information concerning HTLV-1/2 in Indonesia, especially that of the MLM community. We searched for the presence of HTLV-1- and HTLV-2-specific antibodies followed by molecular detection to detect the presentation of the viruses.

MATERIALS AND METHODS

Study population

We conducted a cross-sectional molecular epidemiology study in MSM communities in Surakarta Indonesia between September 2009 and October 2009 by respondent driven sampling method. In total 143 MSM were enrolled in the study. After the written consent was signed, the questionnaire was administered by the interviewer. Approval was obtained from the institutional ethical committee review boards of the Faculty of Medicine of Sebelas Maret University and DR. Moewardi General Hospital Surakarta Indonesia.

Data collection

Data were collected at the time of blood drawing by questionnaires prepared by the investigator in order to determine socio-demographic characteristics and risk factors related to blood borne virus infection. A face to face verbal interview was conducted in a private room with a trained interviewer. The questionnaire was administered anonymously and confidentially. After each interview, participants were asked to provide blood samples.
Questionnaires included questions on socio-demographic characteristics, travel, incarceration and medical histories, drug use and sexual behaviors, especially that of related

to risk factors for HTLV-1/2 infection. Other transmission routes were also assessed, such as receipt of therapeutic injections.

Serological assay

A 5 ml blood sample was collected from willing participants and then fractionated, aliquoted, and stocked in -80 °C until further study. Specimens were tested at Biomedical Laboratory Faculty of Medicine Sebelas Maret University Surakarta Indonesia, analyzed for the presence of specific antibodies to HTLV-1/2 using an ELISA kit that employs HTLV-1/2 viral lysates plus recombinant proteins from HTLV-1/2 as antigens (HTLV-I/II ELISA 4.0, MP Diagnostics, Durhan, DC). The reactions were conducted according to manufacturer instructions.

Molecular biology approaches to confirm HTLV-1 and -2 infections

Samples that presented reactive or in determined results on ELISA assay were confirmed by molecular assay. The peripheral mononuclear blood cells (PBMC) were used for nucleic acid extraction and nested reverse transcriptase polymerase chain reaction (nested RT-PCR). LTR segments of HTLV-1 and HTLV-2 were amplified to confirm HTLV-1 and HTLV-2 infection, as previously described (5). The PCR products were subjected to the determination of nucleotide sequences directly for HTLV-1 LTR region and or for HTLV-2 region, respectively. The tested sequences were aligned with the ones retrieved from Genbank/EMBL/DDBJ database by ClustalW with subsequent inspection and manual modification. The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications. The phylogenetic tree was constructed using the MEGA version 4 software package.

Statistical analysis

All analyses were performed using SPSS version 16 (SPSS, Chicago, IL, USA). Descriptive statistics were generated. For univariate analysis, frequencies and medians of all variables and measures were calculated. Comparisons between groups were performed using the Chi-square tests for proportions and the Student's t-test for proportional and continuous variables.

RESULTS AND DISCUSSION

Anti-HTLV-1/2 positive was found in 1.4 % (2/143) of total sample by ELISA HTLV-1/2. For the two reactive samples in the serologic screening, it was possible to perform PCR

in all cases (100 %), of which all samples confirmed the presence of DNA/HTLV-2 in the PBMC for the LTR regions of the genome. Phylogenetic analysis based on 666 nucleotides of the LTR region revealed that both HTLV-2 isolates were homolog with HTLV-2 isolated in Taiwan.

There no difference in demographic characteristics was observed. In addition, no relationship with factors associated with blood borne virus infection was detected (data not shown). No significant contribution of socio-demographic characteristics, travel, incarceration, medical histories, drug use and sexual behaviors, especially that of related to risk factors for HTLV-1/2 infection.

HTLV-1 and -2 are transmitted in three ways: (i) between sexual partners, mainly from man to woman; (ii) through blood transfusion with HTLV-infected cells; and (iii) from mother to child during prolonged breastfeeding. In areas where the virus is highly endemic, mother-to-child transmission is sometimes the predominant route. In Japan, an area where HTLV-1 infection is highly endemic, antenatal screening and a recommendation for formula feeding of infants of HTLV-1-seropositive mothers have been instituted successfully since 1987 (6). Similar recommendations were proposed in Europe and the Caribbean (7). The seroprevalence varies considerably by sex, age, and region (5% in urban adult's areas, 8.5 to 10.5% in rural adults) (8, 9). The huge number of HTLV-1-infected men among blood donors could be a consequence of the high number of donors of this gender. An age-and-gender-cohort effect due to the characteristics of blood donors worldwide could not be excluded (10).

The prevalence of HTLV-1 infection among pregnant women was estimated to be 5.5 to 6.8%. Most previous studies, however, have been carried out in only one region of the country, the southeast, and the results may therefore not reflect the national prevalence, due to possible regional foci, a hallmark of HTLV-1 infection. Furthermore, the reported rate might be under- or rather overestimated, as in most casesHTLV-1 detection by confirmatory testing with strict Western blot criteria and/or PCR was not done.

Since 2009, our group began screening the high risk communities in Indonesia for human blood borne viruses' infection (HIV, hepatitis B, hepatitis C, hepatitis D, Torque Teno Virus, and HTLV-1/2). In our present study, the seroprevalence of HTLV-1/2 was low. A possible explanation for the low prevalence is the improvement of ELISA test quality, with reduction in the number of false positive tests. The low return rate for the confirmatory test demonstrated the need to reevaluate the current methods of dealing with reactive samples.
Another improvement could be the use of WB testing in the first ELISA positive screening sample. However, we did not use the Western Blot test since the variations verified in the WB test indeterminate results can be caused by the different methods used during the

period, by the possibility of cross reaction with new varieties of HTLV-1/2, cross reaction with other viruses, such as the dengue virus, chickenpox and herpes simplex, severe acute respiratory syndrome virus (SARS), malaria and by the presence of individuals in the initial stages of HTLV infection (11-15). Regarding the sensitivity and specificity of the HTLV screening kits employed in the present study, all samples positive by ELISA kits were confirmed by PCR, therefore the kits was highly sensitive in detecting HTLV-1 and HTLV-2 antibodies. However, this work was limited by its cross-sectional design, and follow-up of such individuals was not done. Finally, the main result that emerges from this study is the HTLV-2 was detected among MSM in Surakarta Indonesia. The correct diagnosis of HTLV-2 is important for prompt attention and counseling these individuals to avoid virus transmission. Indeed, the results obtained suggest that HTLV-1 has probably not yet been introduced in healthy persons in Indonesia.

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O-BM06

Differential Diagnosis Method for Avian Influenza Virus and Newcastle Disease Virus Based on Single Step Multiplex Reverse Transcriptase-Polymerase Chain Reaction

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ABSTRACT

Avian Influenza (AI) and Newcastle Disease (ND) are poultry disease with high mortality rate which caused by a viruses with genetic material RNA. The etiologic agent of AI disease is a virus belonging to family *Orthomyxoviridae* in the genus Influenzavirus type A which is actually known to infect only poultry. Recently, a variety of influenza virus subtypes, such as H7N7, H7N3, H9N2 and H5N1 are zoonotic which also reported to infect humans. Newcastle Disease is also caused by RNA virus belonging to *Paramyxoviridae* family in the genus of Avulavirus. Both the diseases are endemic and strategic disease in Indonesia poultry industry. Generally, the diagnosis of AI and ND have been done conventionally by isolation and viral characterization. Serologically diagnosis of AI and ND viruses are performed by using Agar Gel Immunodiffusion (AGID) test, Haemagglutination Activity test (HA), Haemagglutination Inhibition test (HI) and ELISA. However, these diagnostic methods are less specific and not able to identify the virus in more detail. A method for rapid diagnostic and all at once for appropriate differential diagnosis are required for controlling and surveillance these diseases. The objective of this work was to develop a molecular diagnosis tool to amplify the matrix (M) gene of the AI virus and proteins Fusion (F) gene of ND virus simultaneously based on a single step multiplex reverse transcriptase-polymerase chain reaction (RT-PCR). The results showed that the differential diagnosis method for infectious agent of AI and ND could be performed by single step multiplex RT-PCR that simultaneously amplify the M gene of AI virus and F gene of ND virus by following of RT-PCR condition: reverse transcriptase at a temperature of 48ºC for 30 min, initial denaturation at a temperature of 95ºC for 13 min, denaturation at 94ºC for 1 min, annealing at a temperature of 53ºC for 1 min, extension at 72ºC for 1 min and final extension at 72ºC for 10 min, the PCR reaction run in 35 cycles.

Keywords: differential diagnosis, AI virus, ND virus, single step multiplex RT-PCR

Introduction

Avian Influenza (AI) and Newcastle Disease (ND) are two highly contagious viral disease in the poultry which affecting most of the avian species. These diseases are characterized by impairment of respiratory, gastro-intestinal and central nervous systems [1]. AI is caused by type A influenza virus, which is an enveloped, single stranded, negative RNA virus of the *Orthomyxoviridae* family. Influenza A virus frequently causes widespread and fatal disease in birds and mammals, including humans. Influenza A viruses can be classified into various subtypes on the basis of antigenic differences between the two surface

glycoproteins, hemagglutinin (HA) and neuraminidase (NA). It have been reported, there are 16 subtypes of HA (H1-16) and 9 subtypes of NA (N1-9) [2].

The ND virus (NDV) belong to the *Paramyxoviridae* family, genus Avulavirus, which has a single stranded, non-segmented RNA genome with a negative polarity in size of 15 kb. RNA genome of NDV encodes six major structural and non-structural proteins [3], namely: nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion protein (F), hemagglutinin–neuraminidase (HN), and the RNA-dependent RNA polymerase (L) [4]. According to their pathogenicity in chickens and these correspond to strains of high, moderate, and low virulence, isolates of ND virus are categorized further into velogenic, mesogenic, and lentogenic strains [5]. The F glycoprotein that mediates fusion of the viral and cellular membranes is synthesized as an inactive precursor, Fo. The major molecular determinants for the pathogenicity of ND virus are the amino acids of the F protein cleavage site [6].

AI and ND diseases are endemic and strategic disease in Indonesia poultry industry. Generally, the diagnosis of AI and ND have been doing conventionally by isolation and viral characterization. Serologically diagnosis of AI and ND viruses are performed by using Agar Gel Immmunodiffusion (AGID) test, Haemagglutination test (HA), Haemagglutination Inhibition test (HI) and ELISA. However, these diagnostic methods are less specific and not able to identify the virus in more detail. A method for rapid diagnostic and all at once for appropriate differential diagnosis are required for controlling for surveillance these diseases. A single step multiplex RT-PCR was developed for the detection and differentiation of AI and ND viruses. The objective of this study was to develop a molecular diagnosis tool to amplify the matrix (M) gene of the AI virus and proteins Fusion (F) gene of ND virus simultaneously based on gene amplification using single step multiplex RT-PCR.

Materials and Methods

Virus Collection

Sample of AI and ND viruses were obtained from isolates collection of Virology Laboratory in Animal Disease Investigation Center (ADIC) Wates, Daerah Istimewa Yogyakarta Province. Based on the serological tests, the 24 research samples are classified into 3 categories: 10 samples are AI positive test, 9 samples are ND positive test and 5 samples are AI and ND positive test.

Virus Propagation

Isolate of AI virus and ND virus were propagated in the allantoic cavities of 9-11 days old of specific pathogen free (SPF) embryonated chicken eggs by standard procedures.

Then they were identified using the standard HA and HI tests. The allantoic fluids were harvested and kept at 4ºC in the refrigerator before viral RNA extracted.

Viral RNA Extraction

Viral RNA of AI virus and ND virus were extracted using PureLinkTM Viral RNA/DNA (Invitrogen) according to the manufacture procedures. A total of RNA viruses in 200 μl sterile 1x PBS solution was extracted to obtain the final volume of 50 μl viral RNA.

Primer Design

For amplification by single step multiplex RT-PCR, the specific oligonucleotides primers for amplification of M gene for AI virus was designed by [7] and F gene for ND virus was designed based on [8]. Sequence of oligonucleotides primers are presented in Table 1.

Table 1. Sequence of specific primers for amplification of M gene for AI virus and F gene for ND virus.

Gene Target	Oligonucleotide Sequence	RT-PCR Product	Reference
Matrix (M) AI virus	MF: 5'-GCACTTGAATTGTGGATTCTTAGTC-3' MR: 5'-AGTAGAAACAAGGTAGTTTTTTACTCC-3'	200 bp	AAHL., 2004
Fusion (F) ND virus	FF: 5'-TACACCTCATCCCAGACAGGGTC-3' FR: 5'-AGGCAGGGGAAGTGATTTGTGGC-3	532 bp	Kho *et al*., 2000

Agarose gel electrophoresis

A total of 12,5 μl RT-PCR product, loading buffer and H_2O were loaded onto 1,5% agarose gel. Then it was run in Tris-Buffer-EDTA (TBE) buffer at a voltage 80 volts for 45 min. After that the DNA fragments of RT-PCR products were stained with ethidium bromide and visualized by UV transilluminator in the dark room.

Results and Discussion

Since AI virus and ND virus have an RNA genome, RT-PCR is the starting point for most of the techniques used to detect and differentiate viruses. Using a reverse transcriptase, the RNA genome is transcribed into a DNA copy, which can be used as the template in PCR. Results of simplex RT-PCR amplication of M gene for AI virus and F gen of ND virus respectively, are described by 1,5% agarose gel electrophoresis, as depicted in Figure 1.

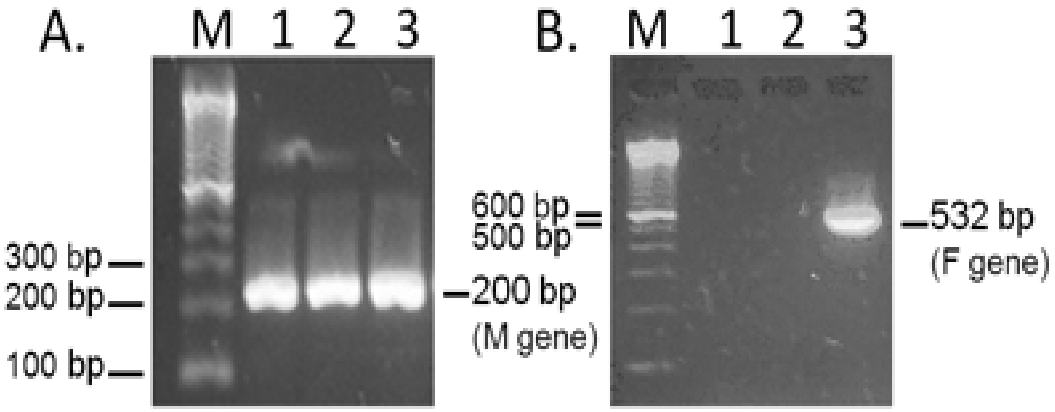

Figure 1. Agarose gel electrophoresis of simplex RT-PCR products for M gene of AI virus and F gene of ND virus. A is RT-PCR amplification of M gene AI virus with expected products in size of 200 bp. B is RT-PCR amplification of F gene ND virus with expected products in size of 532 bp. M = Marker DNA 100 bp ladder; 1, 2, 3 = samples

Figure 1 shown that RT-PCR amplification of M gene AI virus and F gene ND virus in different reaction set can be determined by simplex RT-PCR methods. For AI virus this amplification generated DNA fragment in size of 200 bp, whereas for ND virus generated a 532 bp. It indicated that ampification by simplex RT-PCR for each gene has ability to detect and differentiated AI and ND virus in different reaction set. RT-PCR amplication based on M gene has been reported for the detection of AI virus [7] and F gene of ND virus [8].

Method for detecting and differentiation AI and ND viruses normally utilize the propagation of virus in tissue culture or SPF-embryonated eggs before serological testing by hemagglutination activation (HA), hemagglunation inhibition (HI) and neuraminidase inhibition (NI) tests, which use a monospecific antiserum to each subtype [9, 10]. Although virus propagation in tissue culture or embryonated eggs is sensitive and accurate, it requires several days for a viable virus to cause observable cytopathic effects. These assays are time-consuming and laborious. Other diagnostic tests have also been used, such as immunofluorescence staining and enzyme-linked immunosorbent assay (ELISA). Molecular techniques, such as RT-PCR has enabled major advances in the speed and sensitivity of the laboratory diagnosis of viral infections [11].

Electrophoresis of Amplification product for M gene of AI virus and F gene of ND virus by single step simplex RT-PCR in two different reaction tube is presented more detail in Figure 2.

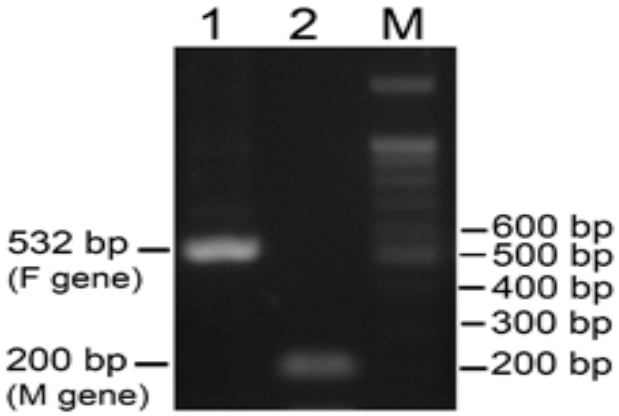

Figure 2. Simplex RT-PCR amplification of M gene AI virus and F gene ND in different canal of agarose gel electrophoresis. M = Marker DNA 100 bp ladder, 1 = AI sample, 2 = ND samples

The amplification was performed simultaneously by single step multiplex RT-PCR on AI virus and ND virus isolates. Reaction of single step multiplex RT-PCR consists of the complementary DNA (cDNA) synthesis was performed for 1 cycle at 48ºC for 30 min followed by denaturation for 1 cycle at 95ºC for 13 min. For PCR amplification, 35 cycles were carried at 94ºC for 1 min, 53ºC for 1 min, and 72ºC for 1 min. For final extension, 1 cycle was performed at 72ºC for 10 min. The amplified products were analyzed by electrophoresis on 1.5% (w/v) agarose gels, stained with ethidium bromide. Agarose gel electrophoresis of RT-PCR products of amplification M gene AI virus and F gene ND virus are more detail presented in Figure 3.

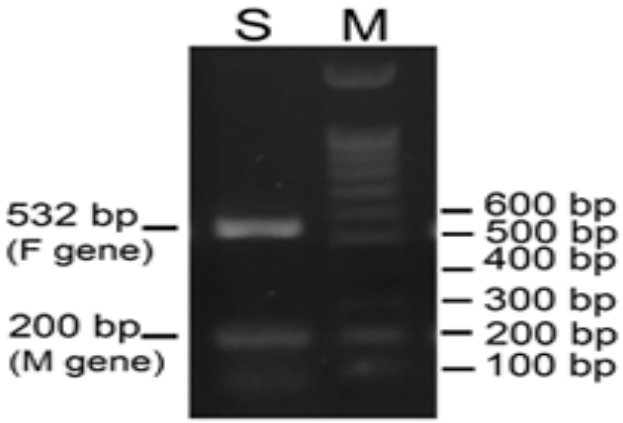

Figure 3. Simultaneous amplification by single multiplex RT-PCR of M gene AI virus and F gene ND virus in agarose gel electrophoresis. S = sampel. M = Marker DNA 100 bp ladder.

Figure 3 showed that single step multiplex RT-PCR method generated a single DNA fragment in size of 200 bp for M gene AI virus and 532 bp for F gene ND virus respectively.

In this work, a single step multiplex RT-PCR method was developed to detect and differentiate two genes (M and F) in two kinds of viruses (AI and ND). Based on the different size of RT-PCR product, Figure 3 showed that single step multiplex RT-PCR could detect and differentiated successfully AI virus and ND virus. For the 24 research samples which tested in this work, the results of single step multiplex RT-PCR in one reaction RT-PCR tube were consistent and in line with the results of separated RT-PCR tube in different reaction.

Conclusion

The single step multiplex RT-PCR is a convenient method for rapid detection and differentiation of AI and ND viruses with the RT-PCR reaction: cDNA synthesis was performed for 1 cycle at 48ºC for 30 min, denaturation in 1 cycle at 95ºC for 13 min. PCR amplification for 35 cycles were carried at 94ºC for 1 min, 53ºC for 1 min, and 72ºC for 1 min, and final extension for 1 cycle was performed at 72ºC for 10 min.

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O-BM07

Overview of Leber's hereditary optic neuropathy and its modifying factors

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ABSTRACT

Leber's hereditary optic neuropathy (LHON) is a maternally inherited disease linked to abnormalities in the mitochondrial DNA (mtDNA). This disease is characterized by acute or subacute central visual loss and lead to blindness due to optic atrophy. However, different age at onset of visual loss among patients and male predominant showed incomplete penetrance and gender bias. Here, the aim of this review was to describe other possible factors involved in the development of the disease either additional genetic or environmental factors. Although respiratory chain deficiency, reactive oxygen species (ROS) and apoptosis are considered having important role in the disease expression, the pathogenesis of this disease remains unclear. Intriguing features of LHON pathogenesis reported by several studies shed light on the complexity. Therefore, further investigations are required to improve not only the development of future therapeutic strategies but also the genetic counseling for the people harboring the mutation of mtDNA and their families.

Keywords: LHON, mitochondrial disease, modifier factors

O-BM08

REFERENCE VALUE OF SOME BLOOD CHEMISTRY PARAMETERS FOR ADULT ETTAWA CROSSBRED GOAT

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ABSTRACT

The study was aimed to determine the reference value for some blood chemistry parameters of indonesian Ettawa crossbred goats. Forty three clinically healthy adult Ettawa crossbred goats (male goats, pregnant goats, lactating goats and non pregnant non lactating female goats) were used in this study. In each animal venous blood samplings were conducted on 08.00-10.00 AM before the morning feeding. Serum was separated immediately for urea nitrogen, creatinine, lipids, glucose and total protein analysis. Chemical analyses were performed using standard methods describd by Kraft and Duerr (1999). The reference value was determined using percentile method described by Kaneko (1989). 2.5^{th} and 97.5^{th} percentile were defined as reference boundaries. The data showed that physiological level of some blood chemistry parameters in this tropical goat were different from those reported for other European and African goats or other ruminant and non ruminant species. It could be concluded that the reference value of some blood chemistry parameters in Ettawa crossbred goats seem to point out some differences from those obtained for other breed of goat or other ruminant species.

Keywords: blood chemistry, ettawa crossbred goat, reference value

INTRODUCTION

In Indonesia, goat are well established in large concentration, over 15.8 million heads and contribute an important proportion (38%) to the total ruminant population (Anonimous, 2008). They play a significant role in protein supply (meet and milk) but there are serious health problems (high mortality and morbidity). According to the statistics for small ruminant in Indonesia, the mortality was at the level of 11-36% (Rangkuti et al., 1984 Gatenby, 1985; Bamu'alim, 1991; Subandrio dan Trisnamurti, 1992) and mostly related to the infection and malnutrition (Boxendell, 1984). In this regards, blood chemistry is very important for health assessment but the blood chemistry reference values for the Indonesian goats are not yet established. It is reported that the physiological value of some blood chemistry parameters in

dog and fish is influenced by breed, physiological status, and geographic location (Kuhl, 1998; Lund, 1998, Mercaldo-Allen et al., 2003). Therefore, the recent research was aimed to determine the reference value for some blood chemistry parameters of adult Indonesian Ettawa crossbred goat. The research should provide basic informations on blood chemistry indices for tropical Indonesian goats.

MATERIALS AND METHODS

Forty three clinically healthy adult Ettawa crossbred goats (lactating, pregnant, non pregnant and non lactating female, and male) were used in this research. The animals were kept in individual cage. Antiparasitic agents were given to control parasites infestation. Before the morning feeding, venous blood was collected and serum was immediately separated for urea nitrogen, creatinine, cholesterol, triglyceride, glucose and total protein analysis. Chemical analyses were performed using the standard methods described by Kraft and Duerr (1999). The data were given as mean and standard deviation. Reference value for the blood chemistry parameter was determined using percentile method described by Kaneko (1989). The 2.5^{th} and 97.5^{th} percentile were chosen as the reference boundaries.

RESULTS AND DISCUSSION

The mean and standard deviation of urea nitrogen, creatinine, cholesterol, tiglyceride, glucose, and total protein concentrations as well as estimated reference value for these blood chemistry parameters in Ettawa crossbred goats were shown in Table 1.

Table 1. Mean and reference range of urea nitrogen, creatinin, cholesterol, triglyceride, glucose, and total protein in serum of adult Ettawa crossbred goats

Blood Parameter	Mean±SD	Reference Range	
		Lower	Upper
Urea nitrogen (mg/dL)	17.27±4.48	10.80	32.39
Creatinine (mg/dL)	0.95±0.21	0.57	1.47
Cholesterol (mg/dL)	82.47±27.26	41.10	142.00
Triglyceride (mg/dL)	35.35±16.41	10.40	91.10
Glucose (mg/dL)	56.63±7.89	31.90	73.80
Total Plasma Protein (g/dL)	7.26±0.79	5.71	9.37

The mean level of urea nitrogen was at the level of 17.27±4.48 mg/dL. It is lower than that reported in West African Dwarf goat, namely 37.9±1.7 mg/dL (Opara *et al.*, 2010). Based on the data from the recent study it could be determined that physiological range of the urea nitrogen concentration for the Ettawa crossbred goats was 10.80-32.39 mg/dL, and therefore, different from the physiological values for european goat, pig, horse (9.00-23.00 mg/dL), for catlle, 9.00-14.00 mg/dL (Kraft and Duerr, 1999), for deer, 28.92-38,19 g/dL (Soppela *et al.*, 2008), and for dairy ewe, 13.56-25.75 mg/dL (Masek *et al.*, 2007). Mean serum creatinine concentration was 0.95±0.21 mg/dL and based on the data it could be determined that the physiological value for this Indonesian goat was 0.57-1.47 mg/dL. It is comparable to the physiological level for sheep (0.60-1.40 mg/dL) and pig (0.45-1.50 mg/dL), but different from the values for horse, 0.80-1.80 mg/dL (Kraft and Duerr, 1999), for deer, 1.19-1.92 mg/dL (Soppela *et al.*, 2008), as well as for goat reported in the previous study, 0.20-2.21 mg/dL (Mitruka and Rawnsley, 1981) and for dairy ewe, 0.77-1.08 mg/dL (Masek *et al.*, 2007). Whereas, mean of the serum cholesterol concentrations (82.47 mg/dL) was in the physiological range reported for local Iraq goats, 76.35-94.6 mg/dL (Juma *et al*., 2009), but higher than that reported for West African Dwarf goat, 47.4±4.5 mg/dL (Opara *et al.*, 2010). Furthermore, the calculated physiological value for serum cholesterol concentration of Ettawa crossedbred goats was 41.10-142.00 mg/dL and showed, therefore, a physiological reference value which is different from the reference value reported before for goat, 55.00-210.00 mg/dL (Mitruka and Rawnsley, 1981) and for dairy ewe, 58.38-90.86 mg/dL (Masek *et al.*, 2007). Serum triglyceride of adult Ettawa crossbred goats ranged at the level of 10.40–73.80 mg/dL. The upper limit of the serum triglyceride concentration for Ettawa crossbred goat was higher than that for cattle (45.00 mg/dL), sheep (30.00 mg/dL), and pig (40.00 mg/dL), but lower than that for horse, 100.00-500.00 mg/dL (Kraft and Duerr, 1999). The mean value of serum glucose concentrations in Ettawa crossbred goat was 56.63±7.89 mg/dL. It is higher than that found in West African Dwarf goat, 32.9±3.8 mg/dL (Opara *et al.*, 2010). Based on the observation results it could be determined that the reference value for glucose concentration of Ettawa crossbred goat is 40.35-65.60 mg/dL.The upper limit of glucose concentration for this Indonesian goat was lower than that reported by Mitruka and Rawnsley (1981) for goat (100.00 mg/dL), but showed a closer similarity to the upper limit of reference value for sheep and cattle, 60 mg/dL (Kraft and Duerr, 1999) and for dairy ewe (68.46 mg/dL) obtained by Masek *et al.*, (2007). Total protein concentrations in adult Ettawa crossbred goats were at the everage level of 7.26±0.79 g/dL or at the range of

5.71-9.37 g/dL. This mean value of total serum protein concentration in Ettawa crossbred goat was higher than that found in West African Dwarf goat, 5.2±0.1 g/dL (Opara *et al.*, 2010). Moreever, the physiological range for Ettawa crossbred goat was different from the physiological level for goat (5.90-7.80 mg/dL) reported by Mitruka and Rawnsley (1981), from that stated by Kraft and Duerr (1999), namely 6.50-7.50 g/dL as well as from that found in Iraqi local goat, 7.08-7.59 g/dL (Juma *et al*., 2009) and in dairy ewe, 6.23-8.27 g/dL (Masek *et al.*, 2007).

CONCLUSION

It could be concluded that the physiological level of some blood chemistry parameters in Ettawa crossbred goats seem to point out some differences from those obtained for other breed of goat or other ruminant species.

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Screening of Indonesia Medicinal Plants Producing Quorum Sensing Inhibitor

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ABSTRACT

Antibiotic resistance of bacteria lead to create different way in the pathogen bacteria handling such us inhibit their quorum sensing mechanism. The goal of this study is to search quorum sensing inhibitor of seven Indonesia medicinal plants. The experiment was conducted by extracting the plants using ethyl acetate subsequently tested on reporter carrying luxR homologous and luxCDABE genes. Reporter luminescence used as indicator of quorum sensing inhibition. The results show that ethyl acetate extracts of buah adas (*Foeniculum vulgare)*, bunga lawang (*Illicium verum*), selasih (*Ocimum basilicum*), temu ireng (*Curcuma aeruginosa)*, temu giring (*Curcuma heyneana)*, dan temu lawak *(Curcuma xanthorriza)* mampu menginhibisi quorum sensing pada *Pseudomonas aeruginosa*. Further analysis was done by observing several metabolites which directly influenced by quorum sensing. The experiment was design by growth *Pseudomonas aeruginosa* at LB medium occurring fennel seeds ethyl acetate extract in the various concentration. Number of biofilms, rhamnolipid and activity of LasA produced by *Pseudomonas aeruginosa* were then measured. The experiment shown LasA activity inhibition reaching 100% was obtained at growth media containing 1.52 mg / ml extract. There was a decrease at inhibition activity when the extract concentration was added above this value. Meanwhile, 19% inhibition of rhamnolipid production occurred at concentrations of ethyl acetate extract of 2.03 mg / ml in growth media. Different results obtained in the production of biofilm which is induced by fennel seeds ethyl acetate extract at the level 123%.

Keywords: Medicinal Plants, Quorum Sensing Inhibitor, Las A, Rhamnolipid, Biofilm

Introduction

Infectious diseases handling, is currently facing many challenges with the emergence of strains of pathogenic bacteria which are resistant to antibiotics. *Pseudomonas aeruginosa* (PA) is one of the pathogen bacteria which have resistance to many antibiotics (multi-drug resistance). Antibiotic resistance arise because the given treatment mechanism try to kill the bacteria. To this treatment, pathogenic bacteria will make a survival mechanism by eliminating antibiotic toxicity and lead the emergence of antibiotic resistance.

Quorum sensing (QS) is bacterial mechanisms which regulate specific proteins expressions by calculating bacterial density in the environment. Genes which are regulated by quorum sensing mechanism will only express when bacteria have reached a high density. Several genes whose expression was regulated by this mechanism i.e. the formation of antibiotics, the formation of flagella, formation bioiflm and genes associated with virulence properties. The emergence of the fact that the virulence factor is influenced by QS raises new hope to overcome bacterial pathogen by utilizing this mechanism.

Several previous studies show some approach in the QS inhibition. Two approaches which are widely used are to destroy the AHL (Acyl Homoserine Lactone) on QS using amidase, asilase, or laktonase[1,2,3] and using AHL analog which is compete with the AHL in interacting with regulatory proteins[4,5]. *Delisia pulchra* known as the most effective substances to inhibit QS[6]. However, at high concentrations this compound is toxic. This

underlies QS inhibitors further exploration. Indonesia medicinal plants, which are traditionally serves in the treatment of infectious diseases, thought to have potential in inhibiting QS of PA.

Research Methods

Preparation of Medicinal Plant Ethyl Acetate Extracts

Medicinal plants are dried and ground up into powder. One gram of finely powdered herbs were weighed and added to 5 ml of ethyl acetate and shaken on a shaker at room temperature for 24 hours. After 24 hours, ethyl acetate is evaporated with a rotary evaporator. Dried extract was weighed and dissolved in 5 ml of ethanol pa[7].

Preparation of AHL PA

24-hour culture of 10 ml of PA O1 centrifuged at 11 000 rpm for 10 minutes. Supernatant was sterilized using 0.2 µm Whatman membrane filters and is called AHL PA (Adonizio, 2007)[8].Penyiapan Kultur Reporter *Escherichia coli* XL1 pSB1075

Preparation of Reporter

A single colony of Escherichia coli pSB1075, inoculated into 10 ml Luria Bertani Broth-amp medium. After incubated in a shaker incubator at 120 rpm at 37 º C for 18 hours, then the culture used as a reporter on a test using a microplate (Lucyana, 2008)[7].

Effectiveness Test of Ethyl Acetate Extracts of Medicinal Plants For QS Inhibitors

Concentration variation of ethyl acetate extract was obtained by performing variations of the volume of the extracts were added to the microplate well, prior adjusted with absolute ethanol up to 210 µL. Solvent is then evaporated by placing microplate in a preheated oven at ± 40-50 0C for 24 hours. Microplate which has been dried, added with 41.7 µL and 100 µL culture AHL reporter PA O1. Each well adjusted with LB media to a final volume of 210 µL per well. Reporter culture as much as 41.7 µL with 100 µL (AHL) O1 PA culture supernatant used as a negative control. The microplate then incubated at 37 º C for 1 hour.

Analysis of QS Inhibitor Effectiveness

QS inhibitor acitivity observed by following reporter luminescence at each different concentration of the extract. The luminescence observed by capturing existing light using X-ray film negatives. The negative films are processed at one of the clinical laboratory located in Surabaya. Interpretation of the results was done with the help of the program MILDA (Digital Automated Microplate Analyzer luminescence). In general, the program will provide a high value on the black color negative film. The black color also shows that there is greater luminescence on micrioplate well in that section. One of the medicinal plants that showed the bestQS inhibitor activity , then tested to see its effect on the formation of protease LasA, rhamnolipid and biofilm.

Las A protease Test

O1 PA that has been incubated on a wide variety of concentrations of ethyl acetate extract of fennel for 12 hours was centrifuged at 11 000 rpm for 20 minutes (4oC). Supernatant obtained subsequently sterilized using 0.02 µm Whatman membrane filter. A total of 750 µL sterile supernatant was added to the microtube which contained 6.75 µL of culture SA (Optical Density / OD 0.6). This mixture was incubated at room temperature and measured value of A600 at 30, 60 and 90 minutes after incubation.

Biofilm Formation Test

PA O1 12-hour culture was transferred into an erlenmeyer in which there has been the ethyl acetate extract of fennel that has evaporated. Cultures were incubated further for 3 days. Biofilms then taken using filter paper. Biofilm number was determined by measuring a constant weight of biofilm on filter paper.

Rhamnolipid Formation Test

Supernatant of 12 hours O1 PA culture was acidified at pH 2 and centrifuged at 4^{o}C, 9000 rpm for 20 minutes. Pellet resulted was dissolved in 750 mL ethyl acetate and centrifuged at a temperature of 20^{o}C with a speed of 9000 rpm for 10 minutes. A total of 500 mL of organic phase then adding into a new microtube, and then heated at 70 °C. After the ethyl acetate evaporated, into the microtube was added 100 µL and 900 µL aquades and orcinol reagent prior heated at a temperature of -80 °C for 30 minutes. The solution obtained is cooled for 15 minutes and measured absorbance at 421 nm.

Results and Discussion

Six medicinal plants selected in this study are: fennel fruit (Foeniculum vulgare), bunga lawang (*Illicium verum*), selasih (*Ocimum basilicum*), temu ireng (*Curcuma aeruginosa)*, temu giring (*Curcuma heyneana)*, temu putih (*Curcuma zeodaria)* dan temu lawak *(Curcuma xanthorriza*. Based on preliminary test was conducted to all the plants, they do not provide antibiotic activities against the reporter used in the research. This result shown that luminescence differences in this study come from different expression at the luminescence gen rather than come from the differences of reporter numbers.

The reporter has lasR gene[9], which is the QS regulator in PA. LasR protein produced by the reporter is able to bind to the auto inducer of C-12-HSL (N-Dodecanoyl-L-Homoserine lactone) thus forming a complex that is able to activate expression of the reporter luminescence. In this study, C-12-HSL, obtained from cultured PA O1 24 hours ethyl acetate extract. AHL extracted from the supernatant culture media using ethyl acetate[8]. Ethyl acetate extracts of medicinal plants expected to have AHL analogues which is compete with C-12-HSL in interacting with LasR. Las -AHL analouge complex will minimize the complex of C-12-HSL-LasR formed, so it will reduce the reporter luminecence[10].

The result of reporter luminescence inhibiton was summarized at Table 1. Almost all medicinal plants provide luminescence inhibiton to the reporter on the selected concentration. Luminescence decreased up to 65.4% compare to the control is the highest luminescence inhibition. It was shown by the ethyl acetate extract of fennel plant at concentration 19 mg / ml. Meanwhile temu giring show a relatively low luminescence inhibition at each concentration tested. Inhibition of reporter luminescence did not appear linear at all concentrations of tested plant extracts. In plants such as fennel inhibition of luminescence increases as the concentration of ethyl acetate extracts were added decreased from 23.8 mg / ml to 19.0 mg / ml. However, the luminescence inhibition decreased when the concentration of ethyl acetate lowered back to 14.3 mg / ml. Something similar happened to temulawak. At the temu putih there was an increase luminiscence inhibition on each decreased concentrations of ethyl acetate extract of the plant. Instead there is a reduction in luminescence inhibition on any reduction in the concentration of ethyl acetate extracts of four other plants.

The phenomenon of luminescence changes in the different of concentration of ethyl acetate extract in accordance with the results obtained by some previous researchers. In general the greater the concentration of QS inhibitors, then the intensity of light produced

will smaller[11,12] . This happens because more and more analog AHL on ethyl acetate extracts, causing a growing number of proteins that will be occupied by the LasR AHL analog and minimize the chance LasR to bind to the C-12-HSL. AHL analog complex - not able to induce expression of LasR luminescence, so it will minimize luminisensi happens.

On the other side of the maximum QS inhibition at a certain concentration which further decreased in the higher concentration also experienced by several other researchers. In general it has been observed the same molecule capable of inducing QS it also can be QS inhiibitor. AHL analog molecules are not purely antagonist, but also has partial agonist properties. Geske et.al[13] using synthetic AHL analogues found that 60% of compounds that are antagonists also have agonist properties at specific concentrations. Two opposite properties in the same molecule is due to disturbance of balance in the hydrogen bonds that occur and that there is steric hindrance between the AHL and the receptor analog (regulatory proteins).

Table 1. Luminescence Inhibition Result

Medical Plants	Concentration (mg/ml)	Luminescence Inhibition (%)	Medical Plants	Concentration (mg/ml)	Luminescence Inhibition (%)
Fennel	23.8	59.2	Temu Giring	38.1	16.3
	19.0	65.4		19.0	3.9
	14.3	53.9		9.5	7.9
Lawang	23.8	34.1	Temu Putih	38.1	-0.3
	19.0	19.2		19.0	4.9
	14.3	-3.2*		9.5	28.8
Selasih	23.8	59.8	Temu lawak	38.1	16.5
	19.2	47.9		19.0	23.5
	14.3	44.0		9.5	6.3
Temu Ireng	38.1	44.1			
	19.0	39.7			
	9.5	7.7			

*** = minus means it was increase at reporter luminescence**

Reporter luminescence inhibition is indirect evidence that the ethyl acetate extracts of medicinal plants have attempted inhibit the activity of PA QS O1. To obtain direct evidence of the existence of barriers QS, we explored further the influence of ethyl acetate extracts of plants to the production of proteases LasA, rhamnolipid and biofilm, which are also influenced by QS. Medicinal plants selected for this testing is the fruit of fennel, since the ethyl acetate extract of the fruit of these plants provide the largest reduction in reporter luminescence test. The test results obtained are summarized in table 2 and table 3.

Tabel 2. LasA Activity Test Result

Concentratin(mg/ml)	**Bacterial Concentration at Certain Time (Minutes)**			**% Decrease of SA Optical Density**	
	30	60	90	60	90
Kontrol	0.12	0.11	0.11	8.33	8.33
0.15	0.12	0.11	0.11	8.33	8.33
0.25	0.11	0.10	0.09	9.09	18.18
0.51	0.11	0.10	0.09	9.09	18.18
1.02	0.11	0.10	0.09	9.09	18.18
1.52	0.11	0.11	0.11	0	0
2.03	0.13	0.13	0.13	0	0

Las A used by PA, when the bacterium infects its host cell. In SA, these enzymes will break the amide bond between D-alanine-D-alanine thus destroying the SA peptidoglycan[14]. SA-LasA mixed, thus going through lysis and SA culture absorbance will decline after some incubation time. In this study the variation between SA and LasA incubation performed in the range of 30, 60 and 90 minutes. LasA activity was calculated by considering the absorbance of the SA at any time of the experiment. Table 2 shown no LasA activity, until the concentration of 0.15 mg / ml of ethyl acetate extract. The decrease of absorbance increase in the concentration of ethyl acetate extract of 0:25 mg / ml to 1:02 mg / ml and no visible decrease in absorbance at the concentration of ethyl acetate extract of 1:52 mg / ml and 2.03/ml.

The greater% decrease in absorbance indicates more active LasA. At concentrations that increased the range of 0:15 mg / ml to 1:02 mg / ml seems the addition of Las activity A. This shows the nature of agonist ethyl acetate extract of fennel. But on the contrary at higher concentrations ie at 1:52 mg / ml and 2:03 mg / ml seen any antagonist properties ethyll acetate extract of fennel. This phenomenon reinforces the effect of ethyl acetate extract of fennel as shown in Table 1.

Judging by the results obtained in this study which showed that the concentration of 1:52 mg / ml of ethyl acetate extract of fennel has been able to stop the decline in OD SA, meaning that production of LasA by PA has been stopped close to 100% at this concentration. It is far more effective than Andonizio[15] research who conduct tests on a variety of spices and get highest QS inhibition to extract B. Buceras (black olive) which is able to reduce LasA production by 96% in concentration 1g/ml crude extract. Further studies on the ethyl acetate extract of fennel plant needs to be done to further confirm these results related to types of compounds contained in the ethyl acetate extract of fennel.

Tabel 3 Result of Rhamnolipid and Biofilm Production Inhibition

Concentration (mg/ml)	Rhamnolipid		Biofilm	
	Absorbance ± Sd	Production Decrease (%)	Weight (gram) ± Sd	Production Decrease (%)
Kontrol	0,42 ± 0,005	0	0,017 ± 0,000529	0
0.15	0,40 ±0,008	4.8	0,015 ± 0,001504	11.8
0.25	0,36 ±0,004	14.3	0,021 ± 0,002762	-17.7*
0.51	0,39 ± 0,007	7.1	0,026 ± 0,000351	-52.9
1.02	0,36±0,004	14.3	0,028 ± 0,000351	-64.7
1.52	0,35 ± 0,006	16.7	0,031 ± 0,001513	-94.1
2.03	0,34 ± 0,003	19.1	0,038 ± 0,001670	-123.5

*** = minus means addition in the production of certain metabolite**

Metabolite which influenced the next QS studied is rhamnolipid and biofilm. As the surfactant, rhamnolipid provide role when bacteria will stick to the surface of the host tissue. It is expected that ethyl acetate extract has an analog AHL molecules will decrease the production of rhamnolipid PA O1. The same is expected in the formation of biofilms produced by the PA as an agent colonizes their place. The results of experiments on both types of metabolites are summarized in Table 3.

Table 3 generally shown opposites thing of plant extract influence to the PA metabolite production. The extract will effect to the reduction of rhamnolipid production (except for the concentration 0:51 mg / ml of the fennel extract). Another thing is that plant extract induce biofilm production with increasing concentrations of ethyl acetate extract of

fennel. This shows that the same molecule has the possibility to give different effects on the mechanism of QS PA.

In general this phenomenon is almost the same as the QS regulation scheme which is proposed by the Christian et.al (1998)[16] who explained that C-12-HSL is able to repress RHL on the PA system, which in turn suppresses the production of rhamnolipid. At the same time the C-12-HSL also trigger the activation of genes involved in biofilm formation. Given the initial screening in this study was conducted using a reporter who has a welding system, then the reporter will be more sensitive to the analog C-12-HSL on the welding system and not the C-4-HSL on RHL systems. This shows also that the AHL analogues compounds contained in the ethyl acetate extract of fennel can interact with the regulator of biofilm production and rhamnolipid.

On the other hand the results of a study similar to the phenomenon in this study are found in the compound indole. Indole class of compounds able to repress virulence factors such as rhamnolipid, pyocyanin and pyoverdin[17]. Yet it is precisely these compounds trigger antibiotic resistance and biofilm formation on the PA. The mechanism of how the biofilm production-induced has not been found to be clear, it's just been proven that these compounds do not trigger the stress on the bacterial culture. The author estimates that there are compounds in the ethyl acetate extract of fennel seeds that have the ability as the indole group.

Conclusion

1. Ethyl acetate extracts of fennel fruit (Foeniculum vulgare), anise (Illicium verum), basil (Ocimum basilicum), Intersection ireng (Curcuma aeruginosa), meeting dribbles (Curcuma heyneana), and temu lawak (Curcuma xanthorriza) have capability to inhibit Pseudomonas aeruginosa quorum sensing.
2. Ethyl acetate extract of fennel 2:03 mg / ml inhibit of Pseudomonas aeruginosa rhamnolipid production up to 19:05% and induces the production of biofilms up to 123.53%. Ethyl acetate extract of fennel 1:52 mg / ml is able inhibit of Pseudomonas aeruginosa Las A production up to 100%PUSTAKA

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O-BM10

ANTI-INFLAMMATORY EFFECT OF PECUT KUDA (*Stachytarpheta jamaicensis* (L.) VAHL) LEAF EXTRACT TO THE TNF-α LEVEL ON WISTAR RATS INFLAMMATORY MODEL

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ABSTRACT

Pecut kuda (*Stachytarpheta jamaicensis (L) Vahl*) has a large flavonoid content. This substance has become potential candidate to supressed the inflammatory process through down regulation the TNF-α level as a potent proinflammatory cytokine in chronic inflammation incident. The research goals was to improve the effect of Pecut kuda leaf extract in vivo to the TNF-α level and considered the optimum dosage of Pecut kuda leaf extract in chronic inflammation model. Twenty five rats was divided into negative control group, positive control group, and treatment groups with Pecut kuda leaf extract dosage 50, 100 and 150 mg/kg BW respectively with 5 rats replication for all of the groups. All of the data was analyzed by computerized using one way Anova with Statistical Product and Service Solution 16 software. The research result showed that the Pecut kuda leaf extract able to inhibit the increasing of TNF-α level significantly ($p < 0,000$). Flavonoid within Pecut kuda leaf extract is predicted able to prevent the oxidation reaction progession of cyclooxygenase-2 (COX-2) to produce prostaglandins. The decreasing of prostaglandin level causes a negative feedback to TNF-α production and iniciating for TNF-α declining level. In conclusion Pecut kuda leaf extract supress the chronic inflammatory process via TNF- α and prostaglandin activity in optimum dosage 50 mg/kg bw.

Keywords : antiinflammation, pecut kuda (*Stachytarpheta jamaicensis (L) Vahl*), TNF-α

INTRODUCTION

Inflammation is a protective response to challenging microorganism or tissue damage that finally leads to tissue repair and restoration of tissue function. Inflammatory reaction involve the secuential release of pro and anti-inflammatory mediators, increase of microvascular permeability and exudation of fluid and plasma proteins into the inflammed tissue. Finally, activated neutrofils undergo apoptosis, a process that placed a central role in the resolution of inflammation (1).

Inflammation is a complex event that is part of the response to all multicellular organisms to indicate on a network that lives and dies due to a foreign agent (2). In recent years the attention focused on the arachidonic acid metabolites as important mediators of inflammation. Arachidonic acid derived from many cell membrane phospholipids that are activated by injury. Arachidonic acid can be metabolized in two different pathways, the

cyclooxygenase pathway produces a number of prostaglandins and thromboxane and lipooxygenase pathway produces leucotrienes (3).

Inflammatory phenomena include microvascular damage, increased capillary permeability and leukocyte migration into inflamed tissue. TNF-α acts as a major mediator of inflammation on the immune response. TNF-α is an acute-phase proteins that initiate a cascade of cytokines and increases vascular permeability, so that macrophages joined neutrophils toward the site of infection. TNF-α produced by macrophages that can aglutinate the blood containing the infection (4).

Previous treatment was known that many devoted to cope with the use of such inflammatory drugs non steroidal anti-inflammatory. Non-steroidal anti-inflammatory drugs (NSAIDs) work by inhibiting the synthesis of prostaglandins (PG) (5). its drugs cause a lot of synthetic basically undesirable side effects. Finally, people tend to use traditional medicine as an anti-inflammatory such, other side of onion bulbs (Eleutherine Meer americana), Wild Ginger rhizomes (Curcuma Roxb xanthorriza), and the roots of Papaya (Carica papaya L.) based on research by Sa'roni (6). One of the other herbs are often exploited by community is pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf. Pecut kuda leaf can treat strep throat predicted by boiling and mixed with some spices, then taken to drink boiled water. These plants usually grow wild on the edge of the road, terrain and other abandoned places.

Utilization of pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) contain compounds anti-inflammatory (anti-inflammatory) were suggested the protective effects of flavanoid (7). Plants have a pecut kuda percentage content of flavonoid compounds are large enough to potentially overcome chronic inflammation by pressing one of the levels of inflammatory mediators is TNF-α. Therefore conducted this research with the title “Anti-inflammatory Effects Pecut Kuda (*Stachytarpheta jamaicensis* (L.) Vahl) Leaf Extract to TNF-α levels on Wistar Rats Inflammatory Model”.

MATERIALS AND METHODS

Animals were fasted for 18 hours before the experiment begins but still given water to drink. Rats were divided into five groups A, B, C, D (K +) and E (K-). The A, B, and C rats groups were fed pecut kuda plants (*Stachytarpheta jamaicensis* (L.) Vahl), extract with variation dosages of 50 mg/kg, 100 mg/kg, and 150 mg/kg. Then volume of the rats’ feet was measured by platysmometer before and after treatment. The animal model were injected with carrageen on their toes between 5^{th} and 4^{th} segment of the right foot. Carrageen (1.5%) 0.1 ml was injected and then waited for 1 week to produce chronic inflammation (8). In this study, the time that used to cause chronic inflammation is 2 weeks. Rats were threated for 1 week with pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract based on the

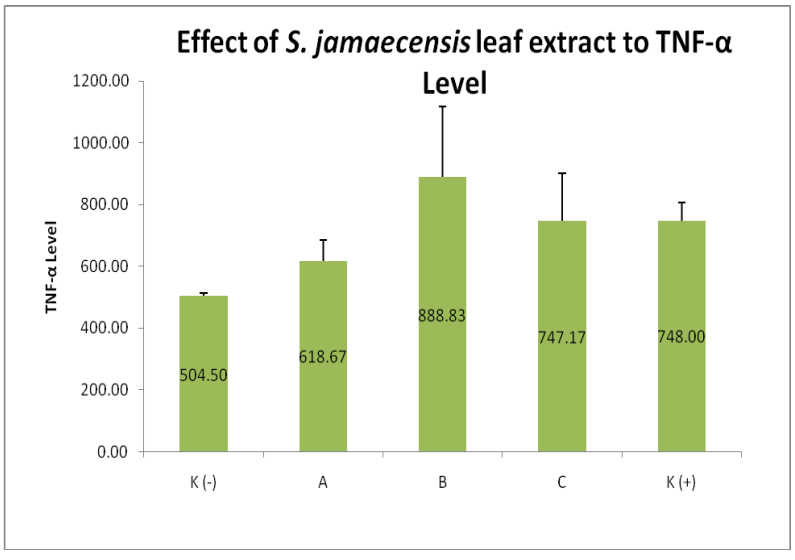
prescribed dosage for each treatment. Threatment of pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract was conducted by gavage. On the 7th day rats were dissected by dislocating the neck to make the rats unconscious so that the blood using spuit 3cc can be acquired from the cardiac (heart). After that TNF-α levels in the blood is measured using enzyme linked immunosorbent assay (ELISA).

RESULT AND DISCUSSION

Result

The average value of TNF-α level of each treatment groups are different. For the negative control group, the middle value/mean was lowest when compared to the means of other groups, valuing at 504.50. This means that between all the groups, the negative control group has the lowest TNF- α level. Meanwhile for group D or the positive control group, the average was 748 that showed a significantly different value with the negative control group but not significant. The same is true got the B group when compared to the negative control group, with their mean being 888.83. The C group is a group with a mean of 747.17, which when statically measured, is not significantly different with the positive control. Group A has the lowest dosage, with average of 618.7, which is the lowest among all treatment groups. Based on the calculation done, it was proven that group A was significantly different and is most significant towards the negative control.

Based on the average levels of TNF-α, which is based on the value of the test results obtained with the ELISA method, a comparison of average values of TNF-α levels between the control group with treatment groups after the treatment of pecut kuda leaf extract by gavage, with dosages of 50mg/kgbw, 100 mg/kgbw, and 150 mg/kgbw, are represented in figure 5.1 as follows:



Description:

1 = negative control groups, i.e. groups of rats are not induced with carrageen and not given the extract of pecut kuda and PBS 1%.

2 = group A, the group of rats are induced by carrageen and given pecut kuda leaf extract dosage of 50 mg / kg bw

3 = group B, the group of rats are induced by carrageen and given pecut kuda leaf extract dosage of 100 mg / kg bw

4 = group C, the group of rats are induced by carrageen and given pecut kuda leaf extract dosage of 150 mg / kg bw

5 = group D or the negative control groups, i.e. groups of rats induced by carrageen but not given the extract of pecut kuda leaf

Test results of One-way ANOVA statistical analysis showed that there is a significant difference or influence of pecut kuda leaf extract at 5% confidence level ($p < 0.05$) between the control groups with treatment groups ($p = 0.000$). Based on these tests, the most significant result lies in the group A (50 mg / kg bw) so it can be said that the dosage of 50 mg / kg bw is the optimum dosage for pecut kuda leaf extract as an anti-inflammatory in this study.

Discussion

In this study, the gavage of pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract on wistar rats induced by carrageen has been known against TNF-α levels in rats that had chronic inflammation. In this study TNF-α was choosen as parameter because TNF-α acts as a major mediator of inflammation, as a form of immune system response (4).

Carrageen is an extract of Chondrus that can cause inflammation if it is induced in rats sub plantar on the soles of their feet (5). The carrageen has several advantages, such as do not leave scars, do not cause tissue damage, and respond more sensitively to anti-inflammatory drugs than any other irritant compounds (5). Based on research by Radhakrishnan (8), carrageen injected with a dosage of 1% for at least 1 week may result in chronic inflammation. This is used as a reference by other researchers to induce carrageen 1.5% for 2 weeks so it can produce a model of chronic inflammation in vivo. Further therapy treatment with variations dosages (50 mg/kg, 100 mg/kg, and 150 mg/kg) of pecut kuda *Stachytarpheta jamaicensis* (L.) Vahl) leaf extract.

Pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract have the potential to be antinociceptive and anti-inflammatory (9). Pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extracts contains an active substance in the form of alkaloids, flavonoids, and glycosides. From all the various contents contained in pecut kuda plants (*Stachytarpheta*

jamaicensis (L.) Vahl) one which is estimated as anti-inflammatory agent is flavonoids. Flavonoids in the form aglycone is nonpolar, whereas in the form of glycosides are polar. Based on the nature of these flavonoids, 70% ethanol can be used for the extraction, because ethanol 70% are semi-polar enabling it to dissolve compounds that are polar and non-polar. In addition, 70% ethanol does not cause swelling of the cell membrane and improve the stability of the drug ingredient dissolved (5).

Pecut kuda plants (*Stachytarpheta jamaicensis* (L.) Vahl) has a percentage content of flavonoid compounds that are large enough, potentially making it able to overcome chronic inflammation. As an anti-inflammatory, flavonoids showed inhibition of cyclooxygenase and lypocsigenase related to the antioxidant activity of flavonoids and may lead to broader impact because it can inhibit the formation of pro-inflammatory arachidonic acid and metabolites (prostaglandins, leukotrienes, and thromboxane) (10).

The content of flavonoids in the ethanol extract of pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf can inhibit inflammation by inhibiting prostaglandin formation of inflammatory mediators. Flavonoids work by inhibiting the release of prostaglandins by inhibiting arachidonic acid metabolism, and non-steroid groups that work through other mechanisms such as inhibition of cyclooxygenase that plays a role in the biosynthesis of prostaglandins (5).

Cyclooxygenase is an enzyme that plays a role in the inflammatory process through eicosanoid biosynthesis. Cyclooxygenase consists of two iso-enzymes with the same molecular weight and enzymatic power, which are COX-1 and COX-2. Cyclo-oxygenase 1 (COX-1) are mostly in tissues such as blood, kidneys, and gastrointestinal tract. Meanwhile, cyclooxygenase 2 (COX-2) in normal circumstances are not present in the network, but is formed during the process of inflammation by inflammatory cells where levels in the cells can increase up to 80 times (11). COX-2 will usually be expressed more due to mitogen stimulation, cytokines and tumor promoters that can be caused by the presence of cell damage or other forms of cell stress (11).

If COX-2 was inhibited, then the synthesis of prostaglandins is also inhibited. Prostaglandins play a role only in the pain associated with tissue damage or inflammation. Prostaglandins cause inflammation to work weakly, yet potentially powerful after joining the mediators or other substances that were released locally, such as histamine, serotonin, leukotrienes and cytokines. Prostaglandins can cause vasodilatation, and increase local blood flow (11). TNF-α is a cytokine that induces inflammation, triggered by prostaglandins that act as initiators of inflammation. Pleiotrophic TNF-α is an inflammatory cytokine that is generally produced by monocytes, macrophages and T cells. It has been known that the expression and synthesis of TNF are not limited to hematopoietic cells, but also applies to

other inflammatory cytokines (13).

When there is inflammation, TNF-α will be mediated by the release of prostaglandins E2 and COX-2 gene expression that occur in human gingival fibroblasts (HGF) (14). The release of TNF-α was induced by PGE2 and COX2 mRNA accumulation in a time where concentration was dependent on HGF; since prostaglandin E2 is not generated then the TNF-α can not be mediated, so its level in the blood will decrease. When prostaglandin is inhibited by the inhibition of cyclooxygenase pathway, then there is negative feedback against TNF-α in order to reduce its production.

In the treatment of pecut kuda leaf extract, there is a significant result at dosages of 50 mg/kg bw. Levels of TNF-α contained in the blood are lower than the positive control rats (group D) and approached the levels of TNF-α negative control group. It proves that the pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract contains flavonoids, and may be anti-inflammatory by inhibiting cyclooxygenase for the formation of prostaglandins. Inhibition of prostaglandin is indicated by a decrease in TNF-α level in the blood of the rat.

Dosages of 100mg/kg bw and 150 mg/kg bw had no significant effect. It is possible that the level of pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract on a certain dosage can be toxic to the body. This can be due to the glycoside content of pecut kuda(*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract that can increase blood pressure in the body so it will increasingly lead to blood flow to parts which become inflamed.

CONCLUSION

Pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) leaf extract affect the decrease of TNF-α with optimum dosage of 50 mg / kg bw. Probably, Pecut kuda (*Stachytarpheta jamaicensis* (L.) Vahl) contains flavonoids that can reduce the amount of TNF-α which acts as an inflammation agent by inhibiting the metabolism of prostaglandins in the cyclooxygenase pathway.

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O-BM11

Importance of delay time for the somite segmentation clock

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ABSTRACT

Repetitive structures in vertebrates including vertebrae, ribs, and skeletal muscles are derived from cell aggregations called somites, which appear transiently during development. Somites are generated by periodic segmentation of the anterior end of the presomitic mesoderm (PSM). It has been reported that several genes such as *Hes7*, *Lfng* and *Nrarp* show oscillatory expressions synchronously with the somite segmentation and a negative feedback loop of *Hes7* plays a key role in the somite segmentation clock. Simulation analyses have proposed that oscillation of gene expression can be sustained by continuous activation of *Hes7* transcription, rapid degradation of Hes7, and delay time, which is a period from *Hes7* transcription to repression of the transcription. Importance of rapid Hes7 degradation has been proved by the experimental data that oscillation of gene expression was dumped in knockin mice carrying the long half-life protein of Hes7. However, it remains unclear whether the delay time is essential for the somite segmentation clock. To test this, we are generating knockin mice of *Hes7* which are extended a period of *Hes7* transcription. We thus insert different size of intron derived from rat *Mapk1* gene (1k, 3k, 7k, or 17k) into a middle of the second intron of *Hes7* gene. We have obtained ES clones carrying the different size (1k, 3k, 7k, or 17k) of *Mapk1* intron within *Hes7* gene, and started to generate chimeric mice using these ES clones. Among them, we have succeeded to obtain a germ-line committed clone from ES cells carrying 17k-intron of *Mapk1* in *Hes7* gene ($Hes7^{17k\text{-}intron/+}$). We will obtain $Hes7^{17k\text{-}intron/17k\text{-}intron}$ mice intercrossing $Hes7^{17k\text{-}intron/+}$ mice and analyze what happens in these embryos. We will also continue to get other germ-line committed clones from the rest of ES clones.

O-BM12

The Effect of *Moringa oleifera* Leaf Powder (MOLP) NTT Variety as New Candidate for Malnutrition Supplement to Albumin Level on Wistar Rats Undercaloric Protein Model

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ABSTRACT

Malnutrition has become main causes from several factor that related to infant mortality in tropical and subtropical area in the worldwide. In the poor country, one from five child was death during development time period. Undernutritional prevalention was increase from 27.5% in 2003 to 28% in 2006 in Indonesia. In other hand, malnutrition number was grow up to 8.2% in 2003 and become 8,5% in 2006. The aim of this study is to produce a new nutritional therapy approach to prevent and offering a novel solution for malnutrition case in Indonesia, especially using local natural nutritional source from *Moringa oleifera* leaf powder green NTT variety. *Moringa oleifeira* leaf powder (MOLP) probably has become nutritional supplement candidate with micro and macronutrient ingredient for malnutrient case management. The research was conducted using randomized groups design (RAK) with treatment doses 180, 360, 720, and 1440 mg/day. The albumin level was measuring using serologic method counting. Experimental groups are divided into five groups, there are negative control groups, positive control and treatment groups with six replication respectively. All of the data was analyzed by computerize using *One way Anova* in *Software Statistical Product and Servive Solution 16 PS (SPSS 16 PS)* with (p < 0,05) significant level to know the difference level of Albumin between all of the experimental groups. The result research showing that based on nutritional measurement of MOLP from green NTT variety consist of 27,01% protein/100 g dried MOLP. From the blood albumin measurement after MOLP treatment, the groups with 720 mg dosage/day has a significant result compare with the other groups (p <0,000). This fact indicate that MOLP green NTT variety can be recommended for further nutritional exploration research to improve how the potention this variety has become a new candidate for combating malnutrition in the future. In conclusion, MOLP green NTT variety able to increase nutritional status of wistar rats undercaloric protein model through stimulate the increasing of albumin level in optimum dose 720 mg/day gavage.

Keywords: *Moringa leaf powder, Albumin level, Undercaloric protein*

Introduction

Malnutrition has become main causes from several factor that related to infant mortality in tropical and subtropical area in the worldwide. In the poorest countries, as many as one child in five will die during infancy. Worldwide it is estimated that seven million people die each year from hunger-related causes, and the vast majority of these deaths are caused by chronic undernutrition ([5]). For malnutrition child, another impact is causing vitamin A deficiency (VAD) whereas it is knowed that vitamin A is key modulator for body immune system, helping against infection and also preventing to several disease like *diarrhea,* tuberculosis and malaria. In other hand, VAD was accelerate to blindness case and xeroftalmia with cornea disruption ([6]).

Undernutritional prevalention was increase from 27.5% in 2003 to 28% in 2006 in Indonesia. Furthermore, malnutrition number was grow up to 8.2% in 2003 and become 8,5% in 2006. NTT province was declared as urgent area for malnutrition incident caused by the increasing of malnutrition cases three time fold in 2004 until 2006 than in one year before (2003). Several data was shows that in April 2008 almost 3.023 under five child /pre school children indicate or vonished has undernutrition status/malnutritional ([16]).

The laboratoric sensitive indicator to measure individual nutritional status for nutritional intake is albumin ([1,2]). Albumin have a long time of half life (14-20 days) and more potential to chronic nutritional marker. One of Indonesian plant that has greater potention for new solution to manage and preventing malnutrition case is *Moringa oleifera* (kelor). Kelor (*Moringa oleifera)* is originally founded in tropical and sub tropical areas. Moringa leaf is consist of nutrient and therapeutic elemen, including anti inflammation, antibiotic, and as immune stimulator ([3,4]). Moringa leaf has greater amount of ferrrum and high protein that very potential for supplementation therapy to underfive children with malnutrition status ([8,9,11]). Moringa leaf powder addition to daily intake for underfive children is predicted able to recovery in short time periode because have 40 nutrient essential ingredient ([12,13]). Moringa leaf is become nutrient sources for combating malnutrition case in tropical and sub tropical climate areas. It is supported by geographical condition in NTT province with tropical dry climate that giving a chance for this plant growing more faster than other area. Eventhough in NTT province Moringa plant is founded dispread in several area (larger scale), there is not found maximal exploration of this leaf plant. The local people are not knowing the potention of this plant and there is no programme from local goverment to using Moringa leaf powder for additional supplementation to pregnant and breastfeeding women, and also for underfive children caused by limited information about nutritional ingredient of Moringa leaf powder. Based on this fact, there is needed further investigation through laboratoric experimental study to explore and proving how the potention on local nutritional source plant especially *Moringa oleifera* leal powder (MOLP) from NTT variety to individual nutrtitional status with undercaloric protein animal model to albumin level as sensitive undernutritional chronic marker.

Matherial and Methods

Moringa oleifera leaf powder (MOLP) Dose

For minimum dose of MOLP from NTT variety that used for animal treatment based on FAO ([15]) standard as follow: MOLP dose treatment for underfive children (3 years old) with 14 kg BW is 25 g/day. In our research the animal BW is 200 g, so the minimal dose is considered = 200/14000 g x 25g = 357 mg. We was establishing that minimal dose

treatment is 360 mg/day and increase two times become 360, 720, and 1440 mg/day based on pharmacologic dose standard.

Undercaloric Protein Animal Model and Experimental Groups

Male *Rattus norvegicus* wistar strain was treated with undercaloric protein daily intake (only 4% protein compound within 30 g daily intake) during 56 days ([10]). After this step, the animal model is claimed UCP (Under Caloric Protein) if the albumin level in peripheral blood under 3,3 mg/dL or on minimum range approximately 2,7 mg/ dL. The experimental groups in divided into five group, there are normal with normal intake (without UCP preparation), UCP without MOLP treatment, and four groups UCP model in 180, 360, 720 and 1440 mg/day dosage MOLP treatment.

Albumin Measurement

The albumin level from all of experimental groups was measured by chemical serologic test with Cobas Mira Plus procedure in Molecular Physiology Laboratory Medical Faculty of Brawijaya University.

Data Analysis

All of experimental data was analyzed with computerized by *One way Anova* using *Software Statistical Product and Servive Solution 16 PS (SPSS 14 PS)* software in ($p < 0,05$) significant level.

Result

The Effect of MOLP Treatment to Albumin Level

The data is showing that after MOLP treatment the albumin level was increase significantly in treatment groups compare with without MOLP daily intake ($p < 0,05$) that describe in table 1 below.

Table 1. Blood Albumin Level (g/dL) on The Experimental Groups

Parameter	Kelompok					
	Normal	UCP	UCP + MOLP 180 mg/day	UCP + MOLP 360 mg/day	UCP + MOLP 720 mg/day	UCP + MOLP 1440 mg/day
Albumin	$3,1 \pm 0,19^{(b}$	$2,5 \pm 0,14^{(a}$	$2,9 \pm 0,12^{(b}$	$2,5 \pm 0,12^{(a}$	$3,6 \pm 0,12^{(c}$	$2,6 \pm 0,16^{(a}$

Different notation shows a significant different between all of the groups by statistical analysis ($p < 0,05$).

Based on serologic albumin level measurement, this indicator is giving a significant different level in UCP animal model on 720 mg /day MOLP dosage. Dose treatment upper 720

mg/day resulting albumin level that not significant compare with UCP groups without MOLP treatment before.

Discussion

Moringa leaf powder from NTT variety especially green type contain nutritional substance that better than red type, including of protein, lipid and also carbohydrate. But, for Ferrum precentage the red type is more potential ([17]). After we compare with nutritional ingredient of Moringa leaf powder from African variety, the protein contain is similar. There are 27,01 % protein / 100 g dried MOLP was founded within MOLP from NTT variety that proved MOLP form this variety have potention to be nutritional supplement from local source. This result research is expected to be early recommendation to further exploration in human therapy for malnutrition cases.

The greater of nutritional value of MOLP NTT variety is stimulate the increasing of nutritional status of UCP rats model that marked with change of albumin level on peripheral blood in our animal model. Based on experimental result from Fuglie ([7]) in Senegal on local community with undernutritional status, the MOLP treatment was showed able to repairing the individual nutritional status of the local people. Tshikaji research is also reporting that one of several solution to prevent and currative action to combat malnutrition is using Moringa leaf powder as additional diet. It is based on nutritional value within this plant leaf with complete protein (contain 9 amino acid), pottasium, ferrum, magnesium, and vitamin A, C, E, B that involve in immune system within our body. Other data from Congo describe that on breastfeeding women and underfive children with Moringa additional diet, the milk production was enough for lactation and showing the increasing of body weight from the child significantly ([15]).

Albumin have long duration for half life (14-20 day), so it is possible to use this kind of serologic protein as a sensitive marker for chronic malnutrition status. The main albumin function is become carrier protein and helping to maintain the oncotic pressure within the body system ([1]). From this research is knowed that the increasing of albumin level is monitored in 80 mg, 360 and 720 mg/day dose groups. In contrast, from 1440 mg/day dose treatment we not found the significant change of albumin level that early condition before treatment. It is suggested that in the high dose MOLP has possibility to suppress hepar activity when this organ make or synthesize the protein (albumin).

On malnutrition case like kwashiorkor, protein deficiency further able to decrease the quality of human life with side effect the decreasing of immune system, body weight, etc. Based on this research, early feature role of MOLP from NTT variety was collected whereas this variety may be has a potent capability to making faster recovery to several potential

indicator of nutritional status. This fact can be found in UCP animal model with MOLP treatment on dose range 180-720 mg/day. Albumin level after MOLP treatment able to limiting the normal range after 3 month peroral therapy.

The increasing of nutritional indicator in this research on UCP animal model is maximum on dose 720 mg/day. This related to one of pharmacological phenomenon that the effect of drug treatment (in this model MOLP peroral therapy) forming sigmoid curve has maximum limit and may be has correlation with toxical dose.

Conclusion

From this research is concluded that MOLP NTT variety treatment during 3 month to UCP animal model able to increase the nutritional status through serologic marker albumin on optimum dose 720 mg/day.

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O-BM13

ACUTE TOXICITY OF PIGEON ORCHID (*Dendrobium crumenatum* Swartz.) *PSEUDOBULB* JUICE ON WISTAR RATS (*Rattus norvegicus* L.)

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ABSTRACT

Orchid is commonly used on medical purposes in China. *Dendrobium nobile* is one of orchid species that is used as anti-microbial, anti-inflammatory agent, and to boost male fertility. Dendrobine, a specific alkaloid isolated from *Dendrobium,* plays the important role in the potency of *Dendrobium* as herbal medicine. As a member of *Dendrobium*, pigeon orchid (*Dendrobium crumenatum* Swartz.) which is found widely in Indonesia, is suggested to have similar potency, however it has not observed scientifically yet. Prior to study the potency of this species for herbal medicine, performing acute toxicity test is a must. This research was aimed to study acute oral toxicity test of pigeon orchid *pseudobulb* juice using Wistar rats as a model, according to OECD protocol. Five animals received a single dose of 1 mL/kg BW pigeon orchid *pseudobulb* juice orally for 14 days. Variables observed including: mortality, behavior, appetite, function test of liver (ALT) and kidney (creatinine), blood glucose level, cholesterol, and routine hematology, which were tested on day 0; 2; 4; 7; 10; and 14. Blood samples were obtained from *sinus orbitalis* of anesthetized animals. It is revealed that according to OECD toxicity test, pigeon orchid is categorized as “practically nontoxic”. No dead animals were found. Behavior investigation demonstrated that all animals exhibited normal activity with raising appetite. Function of liver and kidney were undisturbed, indicated by normal level of ALT and creatinine. In addition, blood glucose level, total cholesterol, HDL, and LDL level, as well as routine hematology were within normal range. Based on this research, experiments on the potency of pigeon orchid as herbal medicine are widely open.

Keywords: oral acute toxicity, pigeon orchid, *Dendrobium crumenatum* Swartz., dendrobine

O-BM14

Biopreservative from Squid Ink (*Loligo* sp.) as Alternative Borax and Formalin Substitution on Food

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ABSTRACT

Many popular foods are still using harmful artificial preservatives. Therefore, a natural and safe compound as a preservative (biopreservatives) for foods is strongly required. Squid ink has an antibacterial activity that can be used as biopreservative. This research aimed to take the advantage of squid ink as a biopreservative to substitute formalin and borax. Antibacterial activity of squid ink was performed to determine the optimal concentration of squid ink as a natural preservative using paper *disc method* to inhibit the growth of pathogenic bacteria (*Escherichia coli, Shigella flexnery,* dan *Salmonella thyposa*). The result showed that the optimal concentration of squid ink as an antibacterial was 120% (v/v). This was used to perform the potential test of squid ink as food preservative in meat balls and noodles. Formalin and borax used as a controls. Noodles and meatballs that supplemented with squid ink were hold on 30 hours, and 20-25 hours without preservatives. Noodles and meatballs that supplemented with formalin and borax were hold on 48-72 hours. Due to the results, squid ink has a potency as a biopreservative.

Keywords: Squid ink, pathogenic bacteria, natural preservative, formalin, borax.

INTRODUCTION

Squids (*Loligo* sp.) are invertebrates that live in the sea. These animals have bags of ink that they may spray to defend themselves. Squids tend to be sold commercially for food purposes, but one of their potentials that ignored by the society is the squid ink[1].

According to research conducted by Naraoka *et al* from University of Hirosaki Japan[2], squid ink has anti-bacterial activity and even anti-cancer. The ability of anti-bacteria showed that the squid ink has other potential as natural food preservatives that are not dangerous.

Squid ink has not been widely exploited for commercial use. In general, they tend to be thrown away or used in a mixture of processed food without knowing its benefits. Meatballs, noodles, and tofu are very popular food in the community. In order to keep the food preserved, traders add preservatives to the food they sold. One example of common preservatives that are used legally in the food industry is sodium benzoate[3]. The price of this particular preservative is still costly, hence many traders opt for harmful preservatives such as formalin or borax that are less expensive[4]. Too much consumption of preserved food is harmful for human health, therefore natural preservatives are needed –

and squid ink is expected to be a natural substitute for formalin and borax, as it is a biopreservative.

MATERIAL AND METHODS

Tools used in this research are glassware includes petri dishes, test tubes, beakers, Erlenmeyer flasks, inoculation needles, drygalsky, pipette pump, measuring pipette, pipette drops, Whatmann paper, cover paper, cotton, tissue paper, plastic wrappers, dishes, pots, stoves, grinders, spoons, forks, paper labels, and hoodsserving. The materials used in this research are, pathogenic bacteria *S. thypii*, *S. flexnery*, *E. Coli*, squid ink, formalin, borax, Nutrient Agar, distilled water, alcohol 70%, and materials for making noodles and meatballs includes wheat flour, starch, cornstarch, eggs, cooking oil, salt, ground beef, garlic, and pepper.

Cultivation of Bacteria – Pure cultures of *Escherichia coli*, *Shigella flexnery*, and *Salmonella thypii* cultivated on Nutrient Agar medium in order to tilt the test tube. Cultivation carried out aseptically to each of the bacteria as a stock culture.

Isolation of Squid Ink – Fresh squid cleaved and taken the ink bag, then the ink bag is inserted into the freezer at a temperature of -20^{o}C.

Anti-Bacterial Activity Test – Antibacterial activity was tested using the method of disc fusion. Pure cultures of *Escherichia coli*, *Shigella flexnery*, and *Salmonella thypii* were grown on Nutrient Agar medium by spread plate. Nutrient agar has melted at a temperature of 55^{O}C is poured into a petri dish and allowed to stand until firm. Each suspension of the bacterium *E.coli*, *S.flexnery*, and *S.thypii* 0,1 ml was inoculated on nutrient agar medium order on different petri dishes and then leveled with the drygalsky aseptically. Filter paper (Whatmann) which had dipped squid ink with various concentrations, namely 30; 60; 120; 250% (v/v), then placed aseptically on the culture that has been flattened in a petri dish. Then incubated at 37^{O}C for 48 hours. Antibacterial activity is known by observing the clear zone formed around the paper disc. Formalin and borax is used as a positive control and untreated Whatmann paper as a negative control.

Potensial Test For Squid Ink Substance Preservatives On Making Noodles and Meatballs – Noodles are made of 300 grams each for treatment using squid ink to the appropriate concentration with an optimal concentration of the anti-bacterial test, formalin as a positive control and untreated as a negative control. After that, the noodles were observed squid ink preservative long time durability and compared with formalin added noodles and noodles without treatment. Meatballs are made of 300 grams each for treatment using squid ink to the appropriate concentration with an optimal concentration of the anti-bacterial test, borax as a positive control and untreated as a negative control. After that, the

meatballs were observed squid ink preservative long time durability and compared with formalin added noodles and noodles without treatment.

Organoleptic test – Organoleptic tests conducted on twenty respondents to determine the flavor and appearance with a rating scale between 1 - 10. Respondents were asked to try two test samples. One sample with the addition of squid ink, and other samples without the addition of squid ink. Then the result is written on the accreditation forms have been provided.

RESULT AND DISCUSSION

Antibacterial power squid ink is known to observe the zones of bacterial growth inhibition test (*S. thypii, S. flexnery,* and *E. coli*) (Figure 1). All three bacteria are used because all three are representatives of the digestion of human pathogenic bacteria and usually grow on food stuffs.

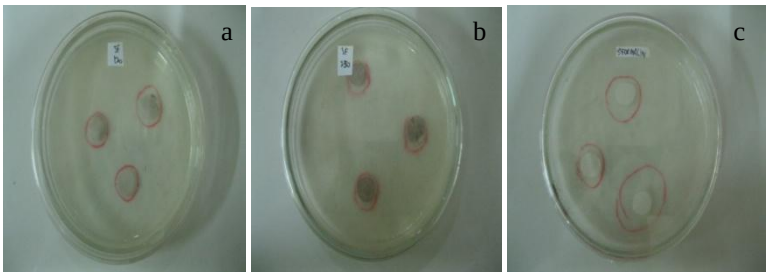

Figure 1. Squid ink antibacterial power against the bacteria *Shigella flexnery* with 120% concentration (a), and 250% (b) with 10% formalin (c) as a control.

Based on test results squid ink as a potential anti-bacterial agents against *S. thypii, S.flexnery, E.coli* with different concentrations of known that the optimum concentration of squid ink in inhibiting bacterial growth in all three trials was 120% (v / v) to form a clear zone of the most extensive, which almost 70% of the petri dish, compared to the clear zone produced by the ink squid at other concentrations. Data analysis of squid ink anti bacterial power of the three test bacteria can be seen in figure 2 below.

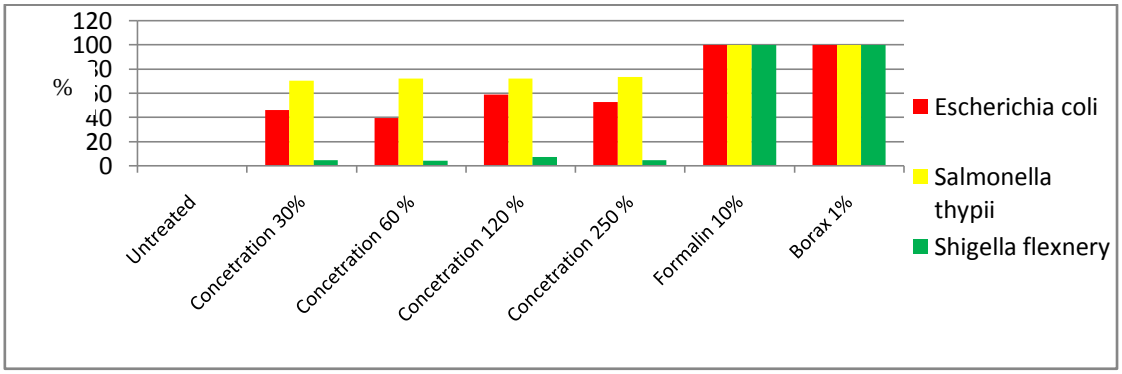

Figure 2. Inhibitory Power Squid Ink, Formalin, and Borax Against Squid Ink

Squid ink with a concentration of 120% (v / v) was then mixed with food (meatballs and noodles) to determine its potential as a natural preservative. The potential of squid ink as a preservatives known based on the length of time food is added resilience squid ink. From figure 3 and 4, can be seen that the noodles and meatballs fed squid ink in open air conditions can last for 30 hours without additional preservatives while only survive for 20-25 hours. Noodles and meatballs with the addition of the preservative formaldehyde and borax can survive for 48-72 hours. Based on this, squid ink has good potential as a natural preservative, but its ability can not exceed the ability of formaldehyde and borax.

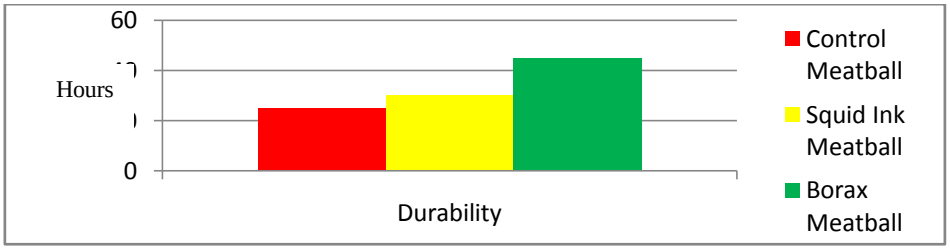

Figure 3. Diagram comparison durability meatballs with squid ink, borax,and without the addition of both

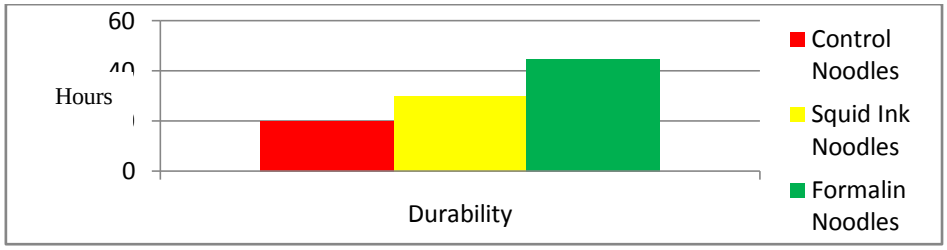

Figure 4. Diagram comparison durability noodles with squid ink, borax,and without the addition of both

Based on the results obtained by organoleptic test results that foods with extra squid ink has a saltier flavor, darker color, and smell more delicious. A more salty flavor that can be addressed by reducing the additional flavor to the food dough, so the taste is more fitting for the squid ink also provides a sense of savory / salty on food (Fig. 5).

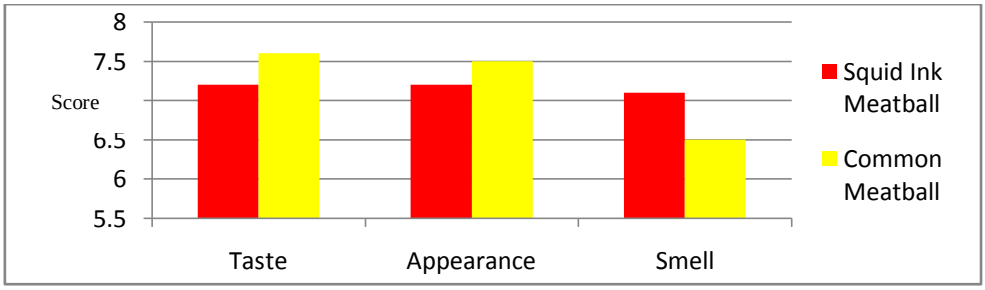

Figure 5. Taste, Appearance, and Smell Test the Meatball

A blackish color in foods (Figure 6) does not become a big problem, because this time the appearance of exotic foods to be a trend in the midst of the community plus a more pleasant aroma, making it more preferable.

Figure 6. A) Comparison of the appearance of meatballs with the addition of borax (left), without the addition of borax or squid ink (middle), and the addition of squid ink (right),
B) Comparison of appearance without the addition of formalin noodles and squid ink(top), with the addition of squid ink (middle), and with the addition of formalin (bottom).

The potential of squid ink as a preservative has not been able to exceed the ability of formalin and borax so it needs to do more research to determine the content contained in squid ink that has potential as a preservative for the safe use of natural preservatives can be further developed.

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O-BM15

Function of hINSR mutant against tyrosine kinase abnormally precedes the clinical onset of diabetes mellitus: *in silico* study[*)]

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ABSTRACT

The pathogenesis of NIDDM has been studied in various ethnic groups. It appears that insulin resistance can precede the clinical onset of NIDDM. Mutations of the human insulin receptor gene have been identified in patients with severe insulin resistance, and studies of these naturally occurring mutants may provide important insights into the relationship between structure and function of the receptor. The aim of our research is to characterize genomic and proteomic insulin receptor (hINSR) of Indonesian diabetes mellitus patients. The bloods were collected from normal and DM patients from some public clinics and Saiful Anwar Hospital, Malang. DNA and RNA were isolated from blood, and then sequenced by ABIPrims Sequencer. To find out the genomic hINSR, DNA sequences were analyzed and characterized by *in silico* analysis, such as alignment by BioEdit & BLAST program from NCBI, and superimposed by Strap JAVA program, 2D- & 3D-structure analysis Swiss Model program. To examine the cytoplasm pathway tyrosine kinase, using docking hINSR-Tyrosine Kinase domain & IRS-1 (PTB domain) analyzed by Hex 5.1. We found specific protein of DM patient from 2D-protein profile and some type mutation of hINSR and can change the INSR 3D-protein structure and the 3D ligand structure of hINSR and insulin completely changed on DM patient. According to our result, we suggested that the hINSR protein mutation of DM patient precede abnormally hINSR function against tyrosine kinase and perhaps correlated with genetic syndrome of insulin resistance. The change function is presumed to inhibit the interaction between hINSR and IRS, makes transduction signals disturbance in the process of absorption of glucose leads to insulin resistance of diabetes mellitus.

Keywords: insulin receptor, diabetes mellitus, genetic syndrome, in silico, tyrosine kinase

Introduction

The human insulin receptor exists in two isoforms, hINSR -A and hINSR-B, which are generated by alternative splicing of a primary gene transcript and differ by a 12-amino acid insertion sequence in the a-subunit. The two receptor isoforms bind insulin with different affinities and are differentially expressed in human tissues. Mutations of human insulin and insulin receptor family can lead autosomal dominant syndrome on diabetes, fasting hyperinsulinemia, and insulin resistant. Activation of the insulin receptor on the plasma membrane of cells by binding of insulin is the initial event that triggers the insulin receptor-signaling cascade, leading to the multiple cellular responses induced by insulin [1, 2]. The

insulin receptor is a tetrameric membrane protein with a α2β2-subunit structure and is encoded by a single gene on chromosome 19 [2, 3]. Processing of the primary α-β gene product yields the mature insulin receptor. Next to insulin receptors, most cells also express IGF-I receptors with similar structure and function [4].

Insulin mediates cell signaling through activation with the insulin receptor (IR), a tyrosine kinase receptor. The activated IRkinase transduces the insulin signal by activating pathways such as the Ras-Raf-MEK-ERK, the PI3K-PDK-AKT, the c-Cbl-Glut4, the PI3K-Rab4-Glut4 and the PI3K-Rac-MEKK1-MKK4-JNK pathways. These pathways are modulated by complex networks of signaling inputs. The balancing of signals that transit the pathways stimulated by insulin provide the specific cell response to insulin signaling. Insulin signaling is mediated by cascades of phosphorylation/ dephosphorylation events, guanine nucleotide exchange events and spatial positioning of signaling, scaffolding and adaptor molecules. Molecules that bind directly to the IR receptor such as the IRS family, SHC, PI3K and GRB10 transducer the insulin signal into the appropriate pathways [5].

A few patients with homozygous mutations in the *INSR* gene have been described, which causes Donohue syndrome or Leprechaunism. This autosomal recessive disorder results in a totally non-functional insulin receptor. These patients have low set, often protuberant, ears, flared nostrils, thickened lips, and severe growth retardation. In most cases, the outlook for these patients is extremely poor with death occurring within the first year of life. Other mutations of the same gene cause the less severe Rabson-Mendenhall syndrome, in which patients have characteristically abnormal teeth, hypertrophic gingiva (gums) and enlargement of the pineal gland. Both diseases present with fluctuations of the glucose level: after a meal the glucose is initially very high, and then falls rapidly to abnormally low levels [6].

Studying multiple patients with the same mutation, insight can be obtained into what extent the genetic background is an important modulator of phenotypic expression of insulin receptor gene mutations. Studies of the signaling properties of natural mutants are also important, not only because of unique insights into structure/function that may emerge, but also because it is possible that the diverse phenotypes associated with severe insulin resistance may in part be due to the ability of some mutations to differentially affect insulin-regulated cellular events [7]. The aim of our research is to characterize genomic and proteomic insulin receptor (hINSR) of Indonesian diabetes mellitus patients.

Materials and Methods

Place

This research was analyzed at Bioinformatics Laboratory of Central Laboratory of Life Science, Brawijaya University, Malang.

Ethical clearance certificate

This research has been certified by Brawijaya University Ethical Clearance Committee (143/KEPK-FKUB/EC/VI/2008), June 16th 2008.

Research Works Procedure

Sample took from blood gene sequence DM type-2 patients of Saiful Anwar hospital, Malang. Blood DNA isolation has been done by Fatchiyah et al. [7] at Central Laboratory of Life Science, Brawijaya University, Malang. Blood DNAs were amplified with six pairs of primer that specific for hINSR gene with GeneAmp PCR Systems 9700 (Applied Biosystems). Then, the DNA-amplified were sequenced by ABI Prism Sequencer (Applied Biosystems).

To find out the genomic hINSR, DNA sequences were analyzed and characterized by *in silico* analysis, such as alignment by BioEdit & BLAST program from NCBI, and superimposed by Strap JAVA program, 2D- & 3D-structure analysis Swiss Model program. To examine the cytoplasm pathway tyrosine kinase, using docking hINSR-Tyrosine Kinase domain & IRS-1 (PTB domain) analyzed by Hex 5.1.

Result and discussion

Recently, our research group reported that the mutation types of hINS gene exon 22 of some DM patients are point mutation with single base deletion and substitution. We found mutation of single deletion at Met^{1295}→Cys^{1295} and $Glut^{1300}$→Gly^{1300}, and also point mutation at Met^{1296} →Ser^{1296} and Trp^{1299}→Ala^{1299} and Met^{1389} →Iso^{1389}. These series mutations are made the polypeptides sequence changed as frame shift mutation, actually normal INSR has six amino acids -Met Arg Met Cys Trp Glut- and DM patient has sequence of the five amino acids - Cys Ala Ser Ala Gly, as properly [7]. This domain is suggested as the ATP-binding site of tyrosine kinase of INSR, according to Kodawaki research group were identified two point mutations in the insulin receptor tyrosine kinase domain in subjects with the Type A syndrome of insulin resistance: Trp^{1200}→ Ser^{1200} and Ala^{1134}→ Thr^{1134} [8]) To examine the cytoplasm pathway tyrosine kinase, we anlyzed the amino acid sequences of DM patients. We found that some other mutation on DMT1 and DMT2 as Table 1 (below).

Table 1. Mutation Type of Nucleotide of INSR gene and amino acid residue of INSR protein at DM Type1 and DMType2 Patients

No	DNA Sequence Mutation		Protein Sequence Mutation		Mutation Type	DM Type	Remarks
	Normal	DM	Normal	DM			
1.	AAG	AGG	Lysine (K_{1266})	Arginine (R_{1266})	Missense	DMT1	
2.	ATG	-	Methionine (M_{1267})	None	Deletion	DMT1	
3.	GAG	GTG	Glutamic Acid (E_{1288})	Valine (V_{1287})	Missense	DMT1	
4.	GAG	AAG	Glutamic Acid (E_{1344})	Lysine (K_{1345})	Missense	DMT1	
5.	ATG	ATA	Methionine (M_{1352})	Isoleusine (I_{1351})	Missense	DMT1	
6.	G_{3800} AC	Deletion	Aspartic Acid (D_{1255})	None	Deletion	DMT2	
7.	CT	Deletion	Leucine (L_{1256})	None	Deletion	DMT2	
8.	ATG	CTG	Methionine (M_{1257})	Leucine (Q_{1255})	Missense	DMT2	Frameshift Mutation was produced deletion of G_{3800} to T_{3805} and A_{3824}
9.	CGC	GCG	Arginine (R_{1258})	Alanine (A_{1256})	Missense	DMT2	
10.	ATG	CTG	Methionine (M_{1259})	Glutamine (Q_{1257})	Missense	DMT2	
11.	TGC	GTG	Cysteine (C_{1260})	Valine (V_{1258})	Missense	DMT2	
12.	TGG	CAG	Tryptophan (W_{1261})	Glutamine (Q_{1259})	Missense	DMT2	
13.	CA$\underline{A}_{3824}$	GCA	Glutamine (Q_{1262})	Alanine (A_{1260})	Missense	DMT2	

Interestingly, the mutations of amino acids of hINSR of DMT2 patients is changed differently as normal hINSR structure (fig 1), the mutation of amino acid in red area when the normal structure is dark blue mesh, this abonormal structure was affected to change the neighboring-site structure of hINSR (green solid), when the normal is blue mesh. Meanwhile, the DMT1 of hINSR structure was no change significantly as hINRS structure on DMT2 patients.

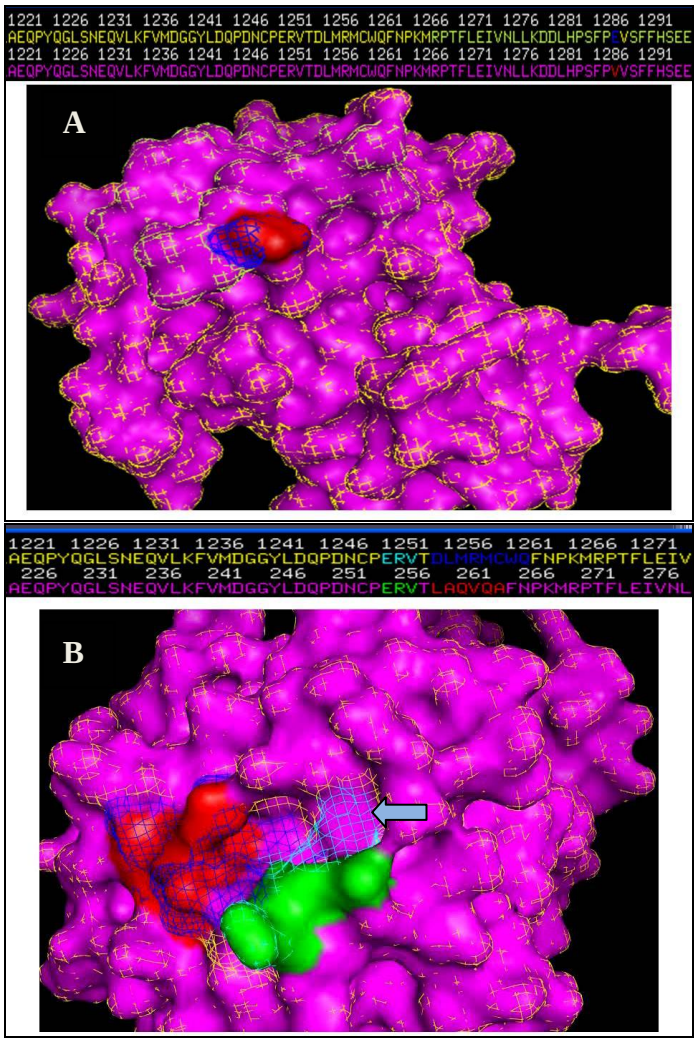

Figure1 3D-Strucrure of hINSR between normal (mesh) and DM patients (solid) by Superimpose Analysis. A. Superimpose hINSR structure of Normal (yellow-blue mesh) dan DMT1 patient (Purple Solid), the mutation amino acid of DMT1 on red solid. B. Superimpose hINSR structure of Normal (yellow mesh) and DMT2 patients (purple solid). Interestingly the mutation of amino acid in red area (dark blue mesh is normal) affected to change the neighboring-site structure of hINSR (green solid), when the normal is blue mesh (arrow).

We suggested that the abornomality of hINSR structure on DMT2 patients precede abnormally hINSR function against tyrosine kinase and perhaps correlated with genetic syndrome of insulin resistance. The change function is presumed to inhibit the interaction between hINSR and IRS, makes transduction signals disturbance in the process of absorption of glucose leads to insulin resistance of diabetes mellitus. Insulin binds to its receptor which in turn starts many protein activation cascades. The main activity of activation of the insulin receptor is inducing glucose uptake. For this reason *insulin insensitivity*, or a decrease in insulin receptor signaling, leads to diabetes mellitus type 2 - the cells are unable to take up glucose, and the result is hyperglycemia -an increase in circulating glucose-, and all of the squealer which result from diabetes [6]. Tyrosine kinase receptors, including the insulin receptor, mediate their activity by causing the addition of a phosphate group to particular tyrosines on certain proteins within a cell.

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O-BM16

In Silico Prediction of Insulin Receptor Diabetes Melitus Type-2 Patient Capability on Insulin Receptor Substrate-1 (IRS-1) Activation

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ABSTRACT

Human Insulin receptor (hINSR) is one of insulin receptor family protein that has an intracellular kinase activity on glucose uptake control. The research aim is to predict tyrosine kinase hINSR DM tipe-2 patient's capability on IRS-1 activation as in silico. The methods comprised; aligning gene of tyrosine kinase hINSR from blood DNA of DM type 2 patients (DMK9 and 8-3F) by using Bioedit *version 5.0.6*, obtaining three dimension protein from *Swiss model server*, viewing structure alteration by using *Pymol 0.99rc6* and *Hex 5.0*, and then docking by using *Hex 5.0*. The results showed that one substitution and one deletion of 8-3F mutant's ekson 22 tyrosine kinase hINSR gene cause lost of four helixes and three coils structures on tyrosine kinase hINSR protein, whereas six deletions and six substitutions on DMK9 mutant changed the two helixes became coil structure. The alterations of structures were changed not only on mutation area, but also on whole structure and surface protein. These alterations could be able to influence tyrosine kinase hINSR and PTB domain IRS-1interaction. Based on docking analysis, binding energy between tyrosine kinase hINSR with IRS-1 showed that normal is E= -494,67 kJ/mol, DMK9 mutant is E= -458,4 kJ/mol), and 8-3F mutant is E=-544,20 kJ/mol. Interaction between 8-3F mutant's tyrosine kinase hINSR and PTB domain IRS-1 is more spontaneous than DMK9, but both of them were reduced on IRS-1 activation respectively. This defect induced the intracellular signaling inhibition as well as on patients' medical record. We also suspected that DMK9 patient's prognosis has a better physiological condition than 8-3F patient.

Keywords: *Diabetes Melitus Tipe-2, hINSR, In Silico, IRS-1*

INTRODUCTION

Background

Insulin is one of hormones that has a metabolism regulation function inside cells [1]. This action is mediated by *Human Insulin Receptor* (hINSR), one of heterotetramer ($\alpha_2\beta_2$) *Insulin Receptor family* protein. Two receptor that have sequences and structural homolog similarities are *Insulin-Like Growth Factor 1* (IGF-1R) and *Insulin-Receptor-Related Receptor* (IRR) [2]. hINSR has a tyrosine kinase intrinsic activities by catalyzing transfer *γ-phosphate* from *Adenosine Triphosphate* (ATP). Insulin will bind with extracellular part of hINSR protein [3]. These activities will induce intracellular responses of Insulin Receptor Substrate-1 (IRS-1) [4]. These protein have glucose uptake role in skeletal muscle and adipose cells [3], or mitogenic induction [5].

Defect Insulin signaling from hINSR to IRS-1 is one kind of type-2 diabetes mellitus (DM type-2) insulin resistance [6]. In vivo study of signaling tyrosine kinase hINSR to

second messenger case showed reducing sensitivities in the present of insulin up to 50% in DM type-2. The case assessed caused by insulin intrinsic signaling defect. The defect also detected as in vitro (adipose cells), in which there is reduction of glucose transport and hINSR responses [7]. These case caused by mutation of hINSR coded gene [8, 9]. Fatchiyah [10], showed that there is a mutation of tyrosine kinase hINSR coded gene that contributed in DM type-2 pathogenesis [11].

Mutations effect of tyrosine kinase hINSR showed that the varied on physiologies effect either *in vivo* or *in vitro*. The mutations assessed influence protein functions in receptor-ligand interaction. *in silico* analysis required to clear the mechanism understanding. This analyzing could give both physics-chemistry visual information contribution and also molecules within the process. The method advantage, will help to clear the tyrosine kinase hINSR mutations effect in insulin intracellular signaling mechanism to IRS-1 knowledge.

Objection

This research aims to predict tyrosine kinase INSR DM tipe-2 patients' capabilities on insulin receptor Substratee-1 (IRS-1) activation as *in silico*.

Materials and Methods

Time and Place

This research was analyzed from October 2010 until May 2011, at Bioinformatics Laboratory of Central Laboratory of Life Science, Brawijaya University, Malang.

Ethical clearance certificate

This research has been certified by Brawijaya University Ethical Clearance Committee (143/KEPK-FKUB/EC/VI/2008), June 16th 2008.

Research Works Procedure

Sample took from blood gene sequence DM type-2 patients of Saiful Anwar hospital, Malang. Blood DNA isolation have been done by Fatchiyah et.al (2009) at Central Laboratory of Life Science, Brawijaya University, Malang, then continued by *sequencing.*

Data Analysis

Sequencing data is done as *in silico*. Normal tyrosine kinase hINSR DNA sequence took from NCBI (*National Center for Biotechnology Information*) (source code: CCDS12176.1), and PTB domain *Insulin Reseptor Substratee*-1 (IRS-1) protein (source code: CCDS2463.1). Mutants tyrosine kinase hINSR gene sequences took from patients

(code 8-3F and DMK9-3K). The first test was aligning mutant's gene sequences to know the position of gene mutation and amino acid that formed. The effect of amino acid changing could check on three dimension structure of protein. Signaling intracellular function checked by *docking* analysis, by interacted IRS-1 PTB domain against tyrosine kinase hINSR.

DNA and amino acid Sequence alignment

Alignment is done on DNA and amino acid both normal and mutant sequences by *BioEdit version 5.0.6.*

Three dimension Of Protein Structure Analysis

Three dimensions structure of protein both normal and mutants, IRS-1 PTB domain have been analyzed by *web service Swiss Model.* Protein was visualisazed by using *Pymol*.

Superimpose

Superimpose is done by using *pymol* and *Hex 5.0*. Protein rotation degrees are measured by *virtual refractor Picpick.*

Docking

Here there are several docking Setting that used:

1. *Refinement* is done by *energy minimization* at *post processing*
2. The Algorithms was using FFT (*Fourier Fast Transforms*)
3. *Docking scan* process was using on both protein surface topology and also amino acid electrostatics interactions
4. Ligand and receptor rotation set up with 180^0

Results and Discussions

Tyrosine Kinase hINSR coded Exon 22 Gene of DM type-2 patients Mutations Analysis

The research is focused on exon 22 tyrosine kinase hINSR gene. Normal tyrosine kinase hINSR gene is aligned with tyrosine kinase hINSR gene's patients. There are one substitution and one deletion in 8-3F patient. Substitution T^{3812}→A caused amino acid change Met^{1271}→Lys. One deletion, A^{3822} caused *frameshift mutation*. This mutation caused amino acid changing from Gln^{1274}, Phe^{1275}, Asn^{1276}, Pro^{1277}, Lys^{1278}, Asn^{1278}, and Met^{1279}→ His, Ser, Thr, Pro, Arg, and stop codon ($T^{3836}G^{3837}A^{3838}$). The Stop codon that appears caused earlier termination so that amino acids are become shorten than normal. On DMK9-3F mutant, there are six deletions and six substitutions. The deletions are:

(1) $G^{3799}A^{3800}C^{3801}C^{3802}T^{3803}$→(- - - - -) caused Asp^{1268} and Leu^{1269} have not synthesized on mutant. The deletion could made *frameshift mutation*: Met^{1270} Arg^{1271} Met^{1272} Cys^{1273} Trp^{1274} Gln^{1275} Glu^{1356}→Leu, Ala, Gln, Val, Gln, dan Ala.

(2) Deletion A^{3822} bring back the amino acid reading frame to be normal again.

The six substitutions are;

(1) Four substitutions: A^{3805}→T, T^{3806}→G, T^{3812}→A, T^{3817}→A (in the same position in which *frameshift mutation occured*).

(2) Two substitutions later (G^{4071}→A) caused changing of Glu^{1356}→lys, meanwhile substitution (G^{4097}→A) caused changing of Met^{1364}→Ile

Three dimension of Protein Structure alterations Analysis

The alteration structure of tyrosine kinase hINSR protein 8-3F mutant is shown by lost of four helixes and three coils. Granner et al. [12] showed that early termination at transcription process caused incomplete form protein. Mutation on DMK 9-3F patient caused structure alteration from helixes to become coil. Branden and Tooze *in* Putri et.al [13] showed that insertion and deletion in a small part amino acid sequence caused structure become coil.

Superimpose result shown that the alterations of protein structure were also occurred not only on mutation area, but also on whole structure and surface protein. The alteration of structure showed by two lines (green and red) that closed each other. The fact was shown that exon 22 tyrosine kinase hINSR able to caused alteration structure of tyrosine kinase hINSR wholly. The alteration structure of protein 8-3F mutant shown more significantly change than DMK9-3F mutant (shown by red and green lines that farther separated). Early termination suspected losing some amino acid that reducing the three dimension protein stability. Compared with 8-3F mutant, DMK9-3F mutant have a mild alteration, showed by 2 lines still on same directions.

Alteration of three dimension structure protein analyses shown that surface topology on protein become reduce both 8-3F and also DMK9-3F. Putri et al. [13] suspected that these alteration could inhibited interaction between tyrosine kinase hINSR with IRS-1 PTB domain. These defect suspected also could disrupted insulin intracellular signaling [14] showed that reducing insulin action effectiveness so that insulin resistance occurred [3].

Tyrosine kinase hINSR mutant DMK9-3F against PTB domain IRS-1 *docking* Analysis

Docking result shown that the mutation have no inhibited interaction between tyrosine kinase hINSR with IRS-1 PTB domain, but at these interaction, there is no tyrosine residue of mutant tyrosine kinase hINSR DMK9-3F. Wholly alteration of protein structure caused by

mutation suspected disrupting the function of Tyr^{972} residue of tyrosine kinase hINSR, so that insulin intracellular signaling become defect although energy binding have negative value. Fort et al. [3] showed thar Tyr^{972} have important role in phosphorylation mechanism on IRS-1.

Our *in silico* result different with Takata et.al [15], showed that mutation of Tyr^{1316}→Phe and Tyr^{1322}→Phe at C-terminal part of tyrosine kinase INSR rat fibroblast-1 cells have no effect in metabolic signaling. Yamamoto-Honda et al. i*n* Krook at al. [8] showed that tyrosine kinase hINSR without 86 amino acid residue at C-terminal part tyrosine kinase hINSR *Chinese hamster ovary* cells have no effect in intracellular metabolism signaling activities against IRS-1 PTB domain but have defect of autophosphorylation process. Murakami and Olsen [16] showed that C-terminal tyrosine kinase hINSR mutation in CHO cells have no effect on metabolic function.

Tyrosine kinase hINSR mutant 8-3F against PTB domain IRS-1 *docking* Analysis

Docking result shown that tyrosine kinase hINSR mutant 8-3F with IRS-1 PTB domain still could interact with IRS-1 PTB domain. exon 22 Mutation-coded tyrosine kinase hINSR carboxyl terminal have no effect on IRS-1 interaction, nevertheless have effect on alteration of three dimension structure of these protein. We suspected, these alteration inhibiting Tyr^{972} role, so that the activation become reduce respectively. *Docking* result shown that there are no tyrosine residue role. Seems like tyrosine kinase hINSR DMK9-3F mutant, there is reducing activation of tyrosine kinase hINSR with IRS-1 PTB domain.

This result have also shown the role of Ser^{1062} and Ser^{1064} residues of tyrosine kinase hINSR 8-3F mutant and Ser^{189} residue of IRS-1 PTB domain occured. We also suspected that significant of protein alteration of tyrosine kinase hINSR 8-3F mutant have great role on interaction between these proteins inhibiting. Serin have similar role like a tyrosine residue in transfer gamma phosphate, but suspected have a different physiology effect. Krauss [4], shown that serine residue have role in intracellular signaling by gamma phosphate ATP transferring. This fact was also strengthening by more spontaneous energy interaction than DMK9-3F mutant and normal. Taniguchi et al. [17] shown that serine phosphorylation have negative regulation at IRS-1 downstream intracellular signaling, by increasing pathogenesis insulin resistance[18]. Yi et.al [19] also showed that serine residue (ser^{312}) phosphorylation related with insulin resistance.

Our *in silico* result as physics-chemistry way shown that tyrosine kinase hINSR still can interact with IRS-1 PTB domain, but there is reducing activation of IRS-2 PTB domain. Structure reduction, wholly alteration of surface topology protein caused by mutation suspected disrupts interaction between tyrosine kinase hINSR mutants with IRS-1 PTB

domain in DM tipe-2 pathomechanism. Reduce of IRS-1 activation also causing inhibiting on substrate proteins downstream activation related insulin metabolic pathway. These were inhibitions disrupting in GLUT-4 protein releasing from *GLUT-4 vesicle* on *glucose uptake*. This mechanism could state that mutation of exon 22 coded tyrosine kinase hINSR C-terminal region disrupt metabolic signaling pathway.

Comparing with DM9K-3F mutant protein, 8-3F mutant protein has a significant alteration on three dimension structure of protein. Binding energy interaction of tyrosine kinase 8-3F have more spontaneous condition than DMK9-3F on PTB domain IRS-1 interaction. Moreover, docking result tyrosine kinase hINSR 8-3F mutant showed role of serine residue. We suspected that this condition would abnormality pathogenesis insulin resistance condition in 8-3F patient. This statement is also supported by medical report of patient, that blood glucose rate and LDL rate 8-3F is higher than DM9-3F. This above profile showed that there is difference prognosis occurred between DM tipe-2 patients, in which 8-3F patient have worse prognosis than DMK9-3F patient. We strong recommended giving different treatment and curing, although these patients have same disease.

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POSTER PRESENTATION

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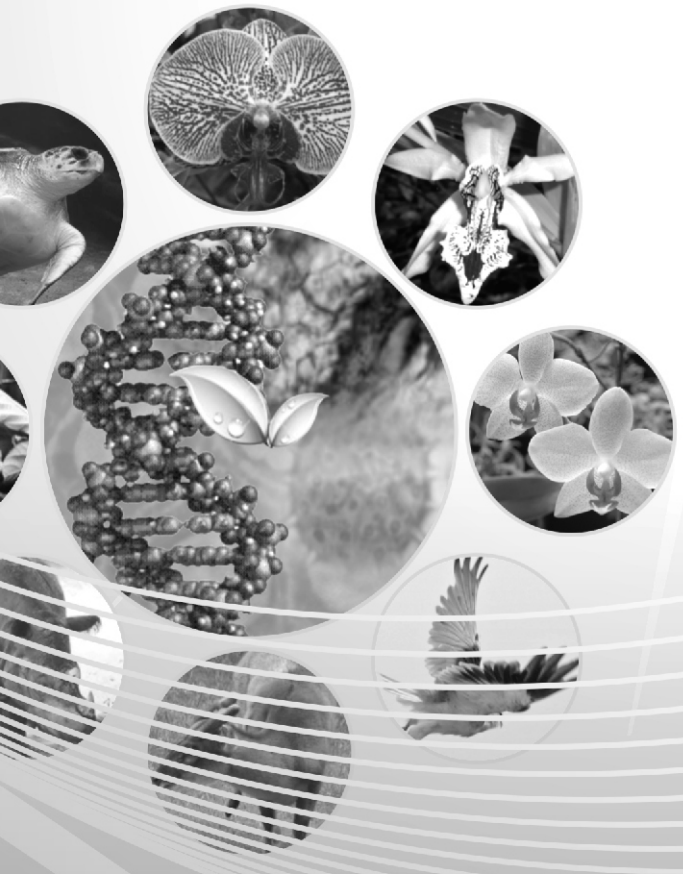

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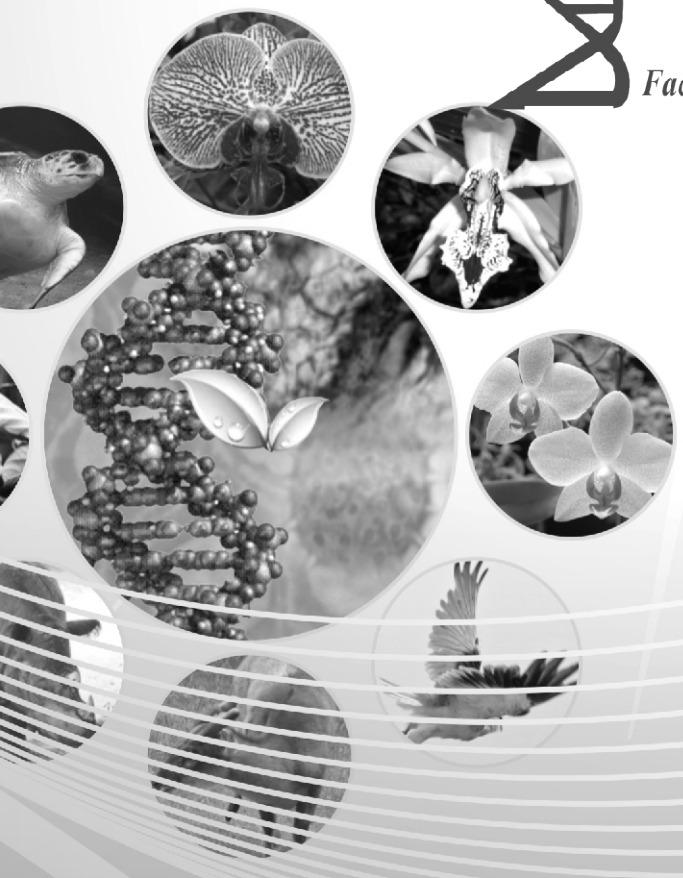

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P-MB01

Identification of Growth Hormone Gene Polymorphism among Madura Cattle and Madura Crossed Cattle

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ABSTRACT

Madura cattle was one of Indonesian local cattle and more than ten years was crossed with Limousin cattle (Madura crossed) to improve its productivities. The aim of this study was to identify polymorphism at growth hormone gene in Madura cattle and Madura crossed cattle by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). A 211 bp fragment of growth hormone gene spanning over fourth intron (49 bp) to fifth exon (162 bp) was amplified and digested with *Alu*I restriction enzyme. The result indicated that Madura cattle was monomorphic for this locus producing only one genotype LL and one allele L. Polymorphism was found in Madura crossed cattle producing two alleles L and V. Two genotypes LL and LV were identified with higher allelic frequency for L allele. The frequencies of L and V alleles were 0.96 and 0.04, respectively. This polymorphism may be caused the lost of restriction *Alu*I site, since this enzyme does not recognize its target sequence when a G is present instead of a C at position 53 of this fragment.

Keywords: Polymorphism, growth hormone gene, Madura cattle, Madura crossed cattle

INTRODUCTION

Indonesia is well known as a rich country in biodiversity (megabiodiversity), one of them is cattle especially beef cattle. Indonesia has two type of beef cattle, these are: domestic cattle and local beef cattle also it crossed. In Madura island there was one of local beef cattle that famous in Indonesia called Madura cattle. Two bovine species contribute to the Madura cattle, zebu (Bos indicus) and banteng (Bos javanicus), respectively (Mohamad et al., 2009). This cattle has a good reproductive performance than crossed cattle also adaptable to Indonesia environment and feeding management (Huitema, 1982). Madura cattle also prepared to their performance in bull race (Karapan Sapi) for and sonok contest (Kontes sapi Sonok) for cows, but more than ten years there was crossbreeding program by the government. The government claimed that the purpose of that program are for increasing economic fund of the farmer and accomplishing meet necessity in Indonesia also improving Madura cattle productivity. Madura cattle has been crossed with Limousin cattle by AI (Artificial Insemination) program. Limousin was an exotic cattle that originally from French (Ngadiyono, 2007 and Mohamad, 2009), the offspring of this croseed program is known as Limura (Madura crossed) cattle. Gluckman et al. (1987) said that growth hormone helps in body growth and metabolism through protein synthesis, protein deposition in tissues and organs. Growth hormone is a polypeptide hormone with 191 amino acid sequences.

Growth hormone gene has been assigned to 19q26q-ter position of bovine chromosome (Hediger et al., 1990), with five exons and four introns. Fifth exon of the growth hormone gene at 127 amino acid position was found to be polymorphic with two allele L and V corresponding to Leucine and Valine variant of growth hormone polypeptide, respectively. This variation was due to C to G substitution at growth hormone gene, which was detected by *Alu*I RFLP. Hence, the present investigation was carried out to find polymorphism of growth hormone gene.

MATERIALS AND METHODS

Animals and frozen semen

The present study was carried out in twenty eight Madura and Madura crossed cattle, fourteen respectively, age five months which is maintained by traditional farmer in Pamekasan district, Madura island. About ± 3 ml venous blood was collected under sterile conditions from the jugular vein of the cattle into a sterile 3 ml polypropylene vial containing K_3EDTA as anticoagulant.

DNA analysis

Genomic DNA was isolated from blood samples following direction by *PrimePrep*™ Genomic DNA Isolation KIT from GeNet Bio, Korea. A 211 bp fragment of growth hormone gene spanning 4th intron and 5th exon was amplified with the GHF (forward) primer: 5'GCTGCTCCTGAGGGCCCTTC-3', and GHR (reverse): 5'CATGACCCTCAGGTACGTCTCCG-3' primers. PCR was carried out in a final volume of 20 µl reaction mixture containing 4 µl double destilated water, 2 µl forward primer (10 pmol/ µl), 2 µl reverse primer (10 pmol/ µl), and 10 µl *FastStart PCR Master* Kit.

Two stage of PCR programmes were followed to obtain the optimum PCR yield. In the first stage, the cycling conditions were at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 65°C for 30 sec, extension at 72°C for 30 sec followed by final extension at 72°C for 5 min. The PCR products were separated on 1% agarose gel at 100 volt for 30 min. The gel was stained with ethidium bromine.

The 211 bp amplicon was treated with *Alu*I enzyme to indentify polymorphism at growth hormone gene. A volume of 10 µl PCR product was digested with 0,5 µl *Alu*I enzyme (10 U/µl), 2 µl 10xbuffer, and aquabidest until 20 µl total volume at 37°C for 2 h. The digestion product was separated on 12% polyacrylamide gel at 50 V for 2 h. The gel was stained with ethidium bromide. The length of fragment 211 bp and each fragment generated by *Alu*I restriction enzyme digestion were compared with the markers 72 bp - 1353 bp DNA ladder, loaded in a separate lane in the same gel.

RESULTS AND DISCUSSION

The PCR amplification generated a 211 bp segment of growth hormone gene in Madura, Madura crossed are shown in Figure 1. Two different restriction patterns were obtained corresponding to two different genotypes, LL and LV. Two fragments of 159 bp, 52 bp, and 211 bp were found in individual with LV genotype whereas none restriction fragment with 211 bp were observed in LL genotype. The results of the digestion product was described in sketch on Figure 2.

In Madura crossed cattle, the genotype frequency of LL homozygotes was found to be 91,67, whereas frequency of LV heterozygotes was 8,33. Allelic frequency for V allele was 0,04, whereas that of L allele was 0,96. Thus, the frequency of L allele was found to be more than that of V allele. In Madura cattle was totally has LL allel. Two types of allels differ only in terms of restriction site of *Alu*I endonuclease enzyme (5'-AG│CT-3'). The L indicated the presence of restriction site while its absence was assigned as allele V that was in the 4^{th} position of five exon. L allele has restriction site that contained the nucleotide C while a transition with G at the same site indicated the absence of *Alu*I restriction site. The total length of amino acid in growth hormone is 191. The presence of nucleotide C at triplet codon encodes the amino acid leucine while the nucleotide; G encodes the amino acid valin. In Madura crossed cattle, the frequency of L allele was found to be higher than V allele, correspondingly frequency of LL genotype was more than that of LV genotype. Thus the present result was in agreement to the report by Mu'in (2008), which reported the similar higher gene frequency of L allele in local cattle, PO (Peranakan Ongole), and crosbreed cattle, SIMPO (Simmental-PO). Mu'in (2008) also reported that monomorphism of growth hormone gene with predominance of LL genotype in Indigenous cattle (Bali cattle). Although there were some reports in allelic variability in different cattle breeds, but there is a quite less chances to have nucleotide variability in exon region of growth hormone gene. Growth hormone as a vital endocrine secretion is mostly conserved in nature as far as polypeptide sequence is concerned. It indirectly reflects the conservation of nucleotide sequence of the gene more specifically coding region (Aruna, 2004). However, nucleotide sequence of growth hormone gene in cattle was reported by Gordon et al. (1983). Nucleotide alignment of cattle and human growth hormone gene showed enormous conservation between them (Gordon et al., 1983).

The present study was the first report on growth hormone genotyping in Madura crossed cattle and has to be considered as a preliminary study. A larger number of observation are needed to establish or deny the exsistence of an association between growth hormone genotypes and quantitative traits in those cattle also to evaluate crossbreeding program of Madura cattle in Indonesia.

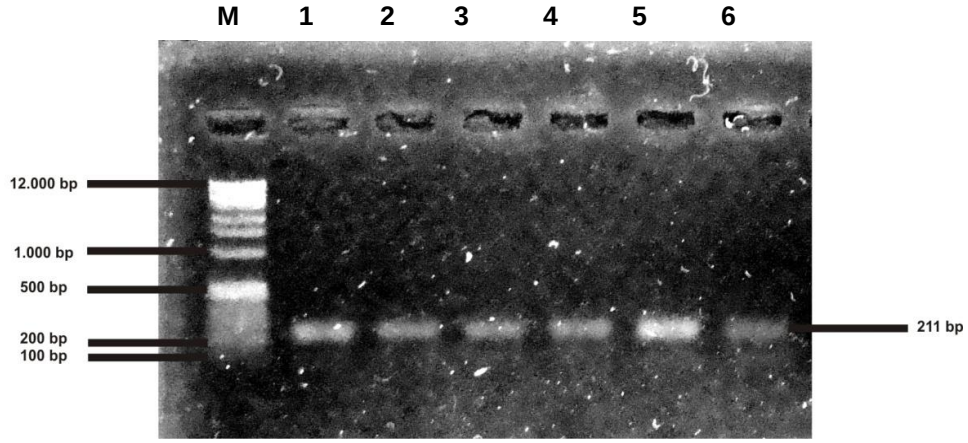

Fig. 1: A 211 bp fragment. M: 100 bp ladder, lane 1-6: PCR product (211 bp)

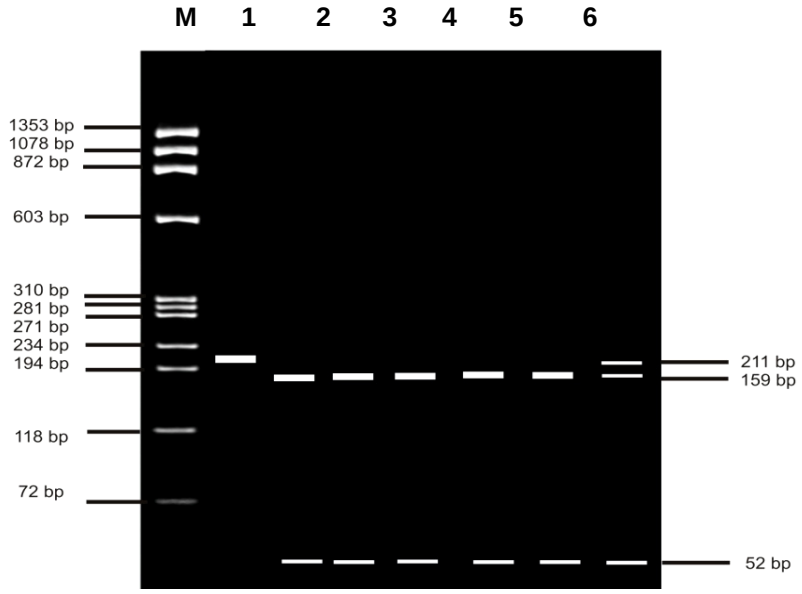

Fig. 2: Sketch analysis of *Alu*I polymorphism at 211 bp fragmen of GH gene. M: Marker, 1: PCR product as a control, 2-6: LL genotype, 7: LV genotype

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P-MB02

RAPDAnalysis of Genetic Similarity in Teak (*Tectonagrandis* Lf.)

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ABSTRACT

In tree improvement program, genetic analysis take many roles as selection or verification tools. In vegetative propagation, genetic analysis can be used as quality control to check genetic similarity between the parent tree and its progeny. The purpose of this study was to analyze genetic similarity of 8 clonesof teak plus treeswith their progenyat Cepu Clone Bank using RAPD (Random Amplified Polymorphic DNA).Six primers used in the PCR process were OPA-03, OPG-19, OPT-20, OPM-20, OPO-06 and OPE-14. Result of DNA analysis shown that rametsof clone D, F and H were 100% identic with their parent tree. Percentage of indentic ramets of other clones are clone A (60%), clone B (80%), clone C (88.9%), clone E (90%) and clone G (85.7%). Tested ramets can be used for further research and development in tree improvement program.

Keywords : RAPD, genetic similarity, teak

Introduction

Teak (Tectona grandis Lf) is one of the most important tropical tree with international reputation for itsexcellence for properties and decorative use. Teak can be foundat Southeast Asia, in Indonesia there are natural teaks, especially in Java, Kagean, Bali, Muna, Buton, Maluku, Sumbawa and Lampung.Java island have large area of potential teak forestsand PerumPerhutani have responsibility to manage them. Teak Plus Tree of Perum Perhutani (*Pohon Plus Perhutani*) isaterm for selected individual trees which have the best phenotype compared to the surrounding trees in a forest and has met the assessment criteria of plus tree. The purpose of Teak Plus Tree selection was to obtain basic population for tree improvement programs.

In tree improvement program, genetic analysis take many roles as selection or verification tools. In vegetative propagation, genetic analysis can be used as quality control to check genetic similarity between the parent tree and its progeny. The purpose of this study was to analyze genetic similarity of 8clone numbersofTeakPlusTreeswith their progenyat Cepu Clone Bank using RAPD (Random Amplified Polymorphic DNA).Tested ramets can be used for further research and development in tree improvement program such as micropropagation, control polination etc.

Material and Methods

Plant Material

The leaves of 8 clones of Teak Plus Trees and their progeny collected from Cepu Clone Bank. The leaves used as the raw material for DNA isolation.

DNA Isolation

A modified CTAB (CationicHexaecylTrymethyl Ammonium Bromide) procedure (Roger and Bendich, 1994)was usedfor the DNA extraction.The DNA was isolated from young leaves for each clon number and its progeny. The tissue was ground to fine powder in liquid nitrogen using a mortar and pestle and transfered to a 1.5 ml sterile extraction tube. The samplethen added with500ul of 2X CTAB and5ul of β-mercaptoethanol as the extraction buffer. The mixture was stirred by vortex and incubated in 65° C for 1-2 hours and stirred occasionally every 15 minutes.The suspension was extracted with500ul of chloroform($CHCl_3$):isoamylalcohol (IAA) (24:1) to denature proteins and facilitate the phase separation. The mixture was stirred then centrifuged at 12,000 rpm for 10 minutes. The upper aqueous phase was carefully taken out and transfered into new sterile tube and added with 400ul CHCl3: IAA.The mixture was stirred then centrifuged at 12,000 rpm for 10 minutes. The upper aqueous phase was carefully taken out and transfered into new sterile tube and added with 270ul (2/3 vol)of cold isopropanolthen mix slowly and restore in -20°C chamber for 2 hours or over night for DNA precipitation. The mixture then centrifuged at 10,000 rpm for 5 minutes to obtain DNA pellet. The DNA pellet then addedwith 200ul of TE 50/10 and mixed gently until completely dissolved and mixed(formed wringkled yarn like shape), then added with 20ul Na-Acetate and 500ul absolute ethanol, mixed gently then precipitated at -20°C for 2 hours. After precipitation, the mixture were centrifuged at 10,000 rpm for 5 minutes. Pellet DNA was added with 720 ulof 70%cold ethanol then centrifuged at11,000 rpmfor 5 minutes. The remaining DNA pellet were dried in decikator for 20-30 minutes until all water evaporates and added with 100 ul of TE 10/1. Ready to use DNAthen stored in -20°C freezer.

DNA Purification

The isolated DNA was purified using the Wizard DNA Clean Up with the Vacuum Manifold.For DNA purification, 50 ul of isolatedDNA added with 1000ulBuffer and mixed until homogeneous.The mixture of DNA and Buffer placed in the syringe barrel and vacuumed until the solution is on the syringe dried out. The Minicoloum containing DNA was washed with 1ml of 80% isopropanol, then vacuumed again until exhausted, this phase was done twice.Minicoloum was transferred into 1.5 ml tubes and centrifuged at 1000 rpm for 2 minutes to remove the rest of isopropanol.Minicoloum then moved to a new tube and added with 50 ul dH2O or TE 10/1, then centrifugedat 1000 rpm for 20 seconds. Purified DNA can be stored in -20°C freezer.

Calculation of DNA concentration

Calculation of DNA concentration used a spectrophotometer, this calculation is very important to know the ratio and concentration of isolated or purifiedDNA.

The fine DNA (free of phenol and RNA protein) should have1.8ratio. If the ratio is more than 1.8 means that the isolated DNA contained high RNA and if the ratio is less than 1.8 means that the isolated DNA contained high protein (Pancoro, 1997).All samples then diluted to make DNA stock for PCR. All samples were diluted to get 20ng/µl DNAconcentration and then diluted again until they reach 2 ng/µl concentration.

PCR (Polymerase Chain Reaction)

PCR is a technique to amplify specific DNA sequences using the primers.Componentsfor PCR mix as follow:

No	KomponenReaksi	KonsentrasiAkhir
1	Deionized Water	10 ul
2	PCR Buffer	1 x
3	$MgCl_2$	1,5 – 2 mM
4	NTPs	
	dATP, dTTP, dCTP, dGTP	50 – 200uM setiap dNTP
5	Taq Polymerase	1 unit
6	Primer	25 Pmoles
7	DNA Template	20 g/µl

PCR performed usingPCR machine, consist of several process includingDenaturation (the separation of double-strain DNA into single-strainDNA, Annealing (the attachment of single-strain DNA with the primers) and Extension (elongation or polymerization of double-strainsDNA). In all 45 cycles, each cycle consisting ofDenaturation at 95 ° C for 1 minute, Annealing at 94 ° C for 30 seconds, 37 ° C for 30 seconds and 72 ° C for 1 min 30 sec, Extension at 72 ° C for 7 minutes.

Electrophoresis

Electrophoresis is a method to separate DNA in the form of lines or bands according to its density. Electrophoresisused 1% -2% concentration of agarose gel dissolved in 0.5 X TBE buffer containing 625ug/ml EtBr. Before running into electrophoresis tray, sample DNA added with loading buffer (25% volume), 25 ul DNAthen inserted into gel's wells alongwith the lambda marker (standarized DNA size). The voltage used for electrophoresis ranges from 60 to 80 Volts for a medium-size tray.Gel electrophoresis results seen above the UV rays with 312 nm wave lenght. The luminesence patterned gels then photographed using a Polaroid camera as material for data analysis.

Data Analysis

DNA amplification resultcan be demonstrated as the presence or absence of lines/bands. This result can be read as the qualitative data and valued 1 for the presence of theband and valued 0 for the absence of the band.Dataanalysis tocalculate similarities index and dendogram accomplished using the NTSYS program (Numerical Taxonomy System). Similarity index ranges from 0-1, thenearer value to 1 means the closer genetic relationship, but the nearer value to 0 meansthe farther genetic relationship among the samples(Pancoro, 1997).

Results

Six different decamer primers were used to evaluate the geneticsimilarity of 8 Teak Plus Trees and their progenies (ramets). Primers used in the PCR process were: OPA-03, OPG-19, OPT-20, OPM-20, OPO-06 and OPE-14.Result of DNA analysis shown that there are several progenies wich have different DNA patterns with their mother tree (Fig. 1)

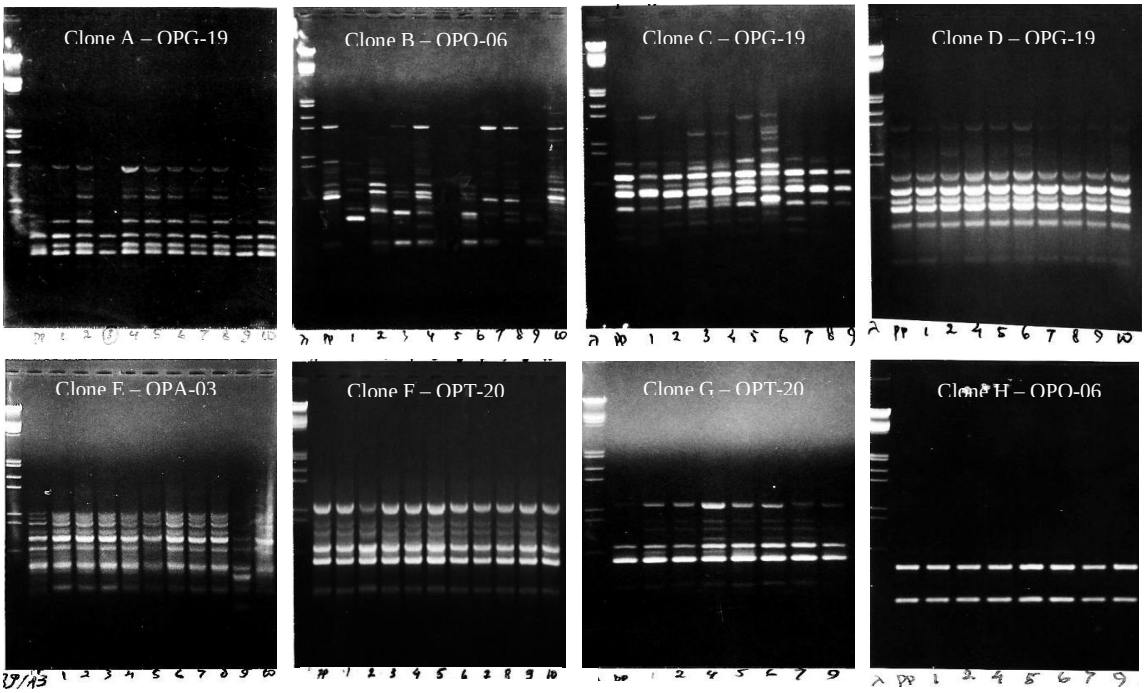

Fig 1. Band patterns for eight clones using certain RAPD primer.

All band patterns then scored and analized using NTSYS program to get similarity data. Similarity indexportrayed in dendogram graphic in the form of genetic relationship tree(Fig. 2).

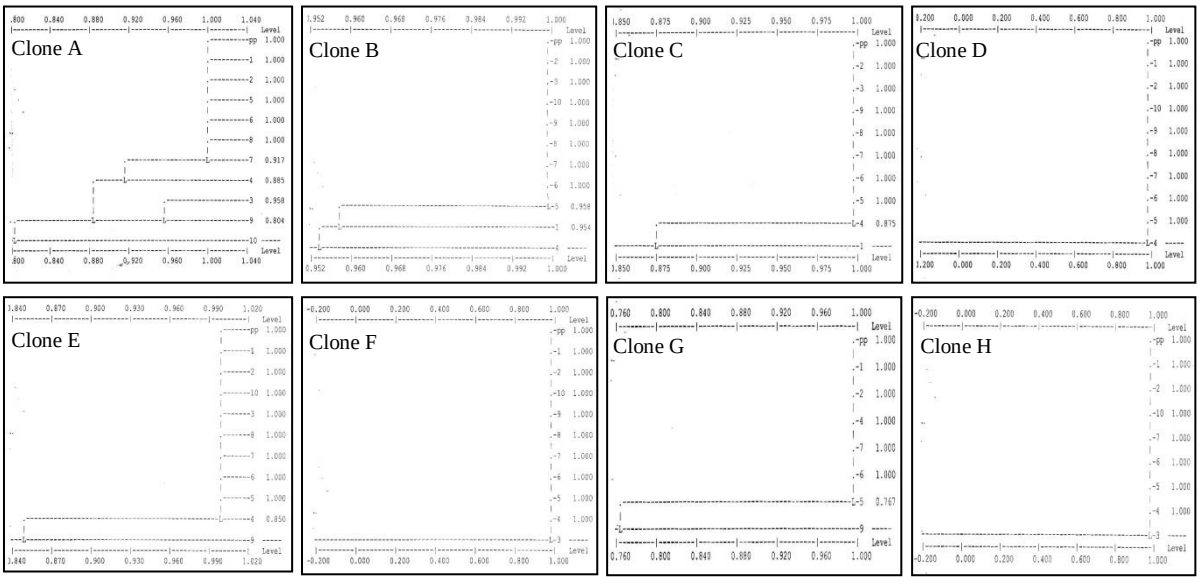

Fig 2. Dendogram graphics based on band patterns scoring resulted from PCR process using six primers for each clone number

Seventy three (73) ramets from eight clone numberswere tested in this research. DNA analysis shown that all ramets/progenies of 3 clones (Clone D, F and H) have 100% similarity with their parrent tree, while other clone's similaritiesrange from 60 – 90%(Table 1).

Table 1.Number of identical ramets foreach clones.

No	Clone	Location	Number of ramets	Number of identic ramets	Percentage of identical ramets (%)
1	A	Cepu Bank Clone	10	6	60
2	B	Cepu Bank Clone	10	8	80
3	C	Cepu Bank Clone	9	8	88.9
4	D	Cepu Bank Clone	9	9	100
5	E	Cepu Bank Clone	10	9	90
6	F	Cepu Bank Clone	10	10	100
7	G	Cepu Bank Clone	7	6	85.7
8	H	Cepu Bank Clone	8	8	100
		Total ramets	73	64	87.7

Results Application for PerumPerhutani

Genetically tested ramets were marked, especially identical ramets. Identically tested ramets can be used for further research and development in tree improvement program such as seedling production using macro or micropropagation, control polination for generative progeny test etc.

Aknowledgement

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P-MB03

The Effect of Accession and Media on Callus Induction of Jatropha (*Jatropha curcas L.*)

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ABSTRACT

Jatropha curcas L. is a tropical plant which has potential to be developed as an alternative energy feedstock. The research to develop this commodity is still far, but step by step development should be conducted there. Propagation of superior provenances in this commodity is essential to support plant breeding program. Propagation can be done with conventional or unconventional techniques (biotechnology). Biotechnology approach uses multiplication by tissue culture. The research aims to evaluate the accession and media on jatropha callus induction. Thee research was conducted in October 2008 - January 2009 at the Tissue Culture Laboratory of IPB. Materials used are jatropha accessions from Lampung, Cigawir, Dompu-NTB, and Cibedug-Sukabumi, and media consisting of NAA and BAP as plant growth regulators. Results showed that the accession and media gave a significantly different effect on the success of jatropha callus induction. Accession that showed has best callus development is Dompu, whereas precise media is MS + BAP 2.6 mg/l + NAA 0.6 mg/l. Best treatment is obtained from the interaction between Dompu accession with media C (MS + BAP 2.6 mg/ l + NAA 0.6 mg/ l).

Keywords: callus induction, accession, *Jatropha curcas*

INTRODUCTION

Indonesia need to look for alternative energy sources as a substitute fuel (diesel) due to energy based fossil oil crisis. Jatropha curcas as bioenergy feedstock is alternative to this problem. Jatropha has its own advantages, including relatively easily cultivated by small farmers, can be grown on marginal lands, and the highly efficient processing of castor oil (Mahmud, 2006).

In order to supply plant materials, it's necessary to develop a rapid method of plant propagation to meet production needs. Propagation of plants themselves can be either conventional or non-conventional technique. In-vitro culture through callus induction is one way of non-conventional plant propagation. Uniformity, large numbers production in narrow field in a short time, and health and qualified seeds and seedlings can be obtained in in-vitro culture. Plant growth regulator that play a role in the formation of callus including auxin (NAA, IAA, 2,4-D) and cytokine (BAP). However, each plant and each accession of plant has a different response to callus induction media.

The research aimed to obtain accession and media on callus induction success of Jatropha plant (Jatropha curcas L.).

MATERIALS AND METHODS

Thee research was conducted in October 2008 - January 2009 at the Tissue Culture Laboratory of IPB. Explants used are multiplicities from stem shoot aged a month. The experimental design used was factorial Randomized Design Group (RGD). Accession as the first factor consisting of Lampung (Lg), Cigawir (Cr), Dompu-NTB (Du) and Cibedung-Sukabumi (Cg). The second factor is media, namely: A = MS + BAP 0 mg / l NAA + 0 mg / l; B = MS + BAP 1.3 mg / l NAA + 0.3 mg / l; C = MS + BAP 2.6 mg / l NAA + 0.6 mg / l, and D = MS + BAP 5.2 mg / l NAA + 1.2 mg / l.

Observations made on:

1. Development of callus

   Observations carried out every week and began at 2 weeks after planting (2WAP) to 5 WAP based on score.

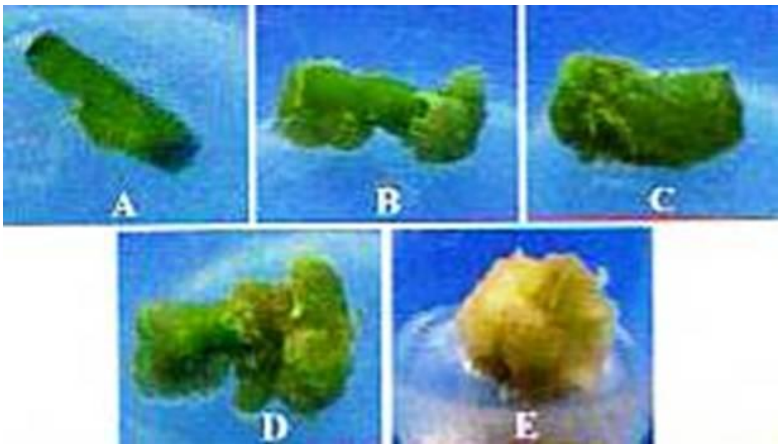

Figure 1. Scoring of callus development on explant (A) explants swell (score 1). (B) 1-25% callus (score 2), (C). 26-50% callus covered explant (score 3), (D) 51-75% callus covered explant (score 4), (E) 76 - 100% callus covered explant (score 5)

2. Callus diameter (cm)

   Observations were made at 5 WAP by measuring the diameter of the callus.

3. The weight of callus (g)

   Observations were made at 5 WAP by weighing callus.

To see the effect of treatment of the observed parameters tested F. Furthermore, if the F tests real test is carried further by Duncan's test Multiple Random Test (DMRT) 5%.

RESULTS AND DISCUSSION

The development of explants forming callus reaches 1% - 100%, whereas in the media A media control callus formation does not occur. In the media A explants developed swell but was unable to grow to form a callus. Callus growth differences caused by different viability of each accession. According to Fitch (1993), the factors that influence the

successful regeneration of callus among other plant species, origin, type and concentration of plant growth regulators.

Table 1. Effect of Accession and Media on Callus Development

Accession	Media			
	A	B	C	D
----------1 WAP----------				
Cigawir	1c	3.2ab	4.2a	4.6a
Cibedug	1c	1.8bc	1.2c	1c
Dompu	1c	4.6a	4.2a	4.6a
Lampung	1c	2.4bc	3ab	2.4bc
----------2 WAP----------				
Cigawir	1d	2.4c	5a	4.6a
Cibedug	1d	1d	1d	1d
Dompu	1d	5a	4.4a	4.6a
Lampung	1d	2.8bc	3.8ab	3.8ab
----------3 WAP----------				
Cigawir	1e	2.6d	5a	4.6ab
Cibedug	0e	0e	0e	0e
Dompu	1e	5a	5a	5a
Lampung	1e	3.6bcd	3.8abc	2.8cd
----------4 WAP----------				
Cigawir	1d	2.8c	4.6ab	4.8ab
Cibedug	0.2d	0d	0d	0d
Dompu	1d	5a	5a	5a
Lampung	1d	4ab	3.8bc	4.4ab

Means followed by the same letter do not differ significantly at the 0.05 level

Table 2 below showed that each accession and growing media used affect the variable diameter of the callus. The formation of callus tissue explants involves the development of cell division randomly and evenly distributed, which is still not specific cells and loss of organized cell structures (Gamborg 1988). Dompu is the best accession in callus development, which is showed in callus diameter in all media, but media A. Furthermore, precise media generated from C media containing MS + BAP 2.6 mg / l NAA + 0.6 mg / l.

Table 2. Effect of Accession and Media on Callus Diameter (cm)

Accession	Media			
	A	B	C	D
Cigawir	0d	1.36bc	2.46a	1.96ab
Cibedug	0d	0d	0d	0d
Dompu	0d	2.5a	2.38a	2.26a
Lampung	0d	1.42bc	1.84abc	1.3bc

Means followed by the same letter do not differ significantly at the 0.05 level

Table 3 showed that the accession and the media used affect the weight of callus. Similarly, the callus diameter, good callus weight obtained in media C containing MS + BAP 2.6 mg / l NAA + 0.6 mg / l is a good media. Highest callus weight (Dompu) obtained in media B containing MS + BAP 1.3 mg / l NAA + 0.3 mg / l, it's differ significantly to media C.

Table 3. Effect of Accession and Media on Callus Weight (g)

Accession	Media			
	A	B	C	D
Cigawir	0d	2.518bc	4.728a	2.866bc
Cibedug	0d	0d	0d	0d
Dompu	0d	4.694a	3.658ab	2.764bc
Lampung	0d	1.474cd	1.778cd	1.848cd

Means followed by the same letter do not differ significantly at the 0.05 level

Based on callus diameter (Table 2) and callus weight (Table 3) obtained in each accession, media C is the most appropriate media for callus induction of Jatropha plants. Best treatment is obtained from the interaction between the accession of Dompu and media C (MS + BAP 2.6 mg/ l + NAA 0.6 mg/ l).

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P-MB04

Growth Hormone Gen as A Genetic Marker for Identifying Local Cattle (Madura) in Indonesia

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not presented

P-MB05

Exploring Arbuscular Vesicular Mycorrhizal (AVM) On the Rhizofer of Cassava in Order to Construct the AVM Tablets

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ABSTRACT

AVM isolates scarcity is one factor limiting widespread use of AVM. Any attempts to obtain isolates from a particular ecosystem must begin with the exploration of AVM on the ecosystem. Our descriptive research objective is to find AVM contained cassava rhizofer of maniok cassava as well as cece udang cassava in the age of 1, 5. and 10 months growing on mediteran and latosol soil type. The identification results are expected to be raw materials in order to construct any AVM tablet. We had identified ten types of AVM spores and most of the spores are of *Gigaspora gigantea, Glomus claroideum* as well as *Glomus etunicatum*. The three types of spores were then propagated in order to multiplicate the AVM isolates using corn as its host plant before constructing the AVM tablets.

Keywords: Arbuscular vesicular mycorrhizal, cassava rhizofer

INTRODUCTION

AVM isolates scarcity is one factor limiting widespread use of AVM. Any attempts to obtain isolates from a particular ecosystem must begin with the exploration of AVM on the ecosystem, continued with purification, propagation and effectiveness testing of isolates obtained. The research results of Soedradjad (No Year) showing *G. manihotis* external hyphae able to transfer N in an sufficient amount to enhance significant growth of peanuts. *G. manihotis* fungus that colonizes in the soil medium was obtained from the land planted with cassava.

According to Mosse and Hayman (1980) all soils contain the AVM but their densities and types are different. Sieverding (1991) said that not all types of plants could provide a positive response to each of AVM fungus, because each of plant had different levels of dependence and response to AVM. We are interested to identify the AVM on cassava rhizofer of maniok cassava (bitter taste) as well as on cece udang cassava (sweet taste), in the age of 1, 5 and 10 months, growing on mediteran and latosol soil type and then finally we are interested too make the AVM tablet

MATERIALS AND METHODS

Materials used for collecting, isolating and identifying AVM was soil sample, glucose, and water. Materials used for the multiplication cassava AVM fungal spore isolates were sterile soil, sterile water, and corn seeds.

The soil samples arround and in the rhizofers of maniok cassava as well as Cece Udang cassava growing on mediteran and latosol soil type in the age of 1, 5 and 10 months were obtained with a diagonal method to a depth of 00-20 cm. The extracting process of the spores from the soil, were carried out using wet sieving method with the procedure of Gedermann and Nicholson (1963). Spora identifications were carried out with a binocular microscope according to the procedure Brundrett et al (1996), based on the shape, color, size as well as on wall thickness of spores. Each spores were separated and grouped according to their type, after their identification.

AVM isolates propagation using corn plants as their host and sterile soil as the medium (Abdelhafez and Abdel-Monsief, 2006). All the activities were conducted in a greenhouse using plastic pots.The corn seeds had been planted before and after four days old, all the corn plantlets were inoculated with AVM spores and 10 spores were grown in each pot. After two months, cassava AVM spores were harvested by cutting the roots of the host plants and mix the spore together with the medium used. The mixture of soil containing pieces of infected roots, spores and hyphae was used to prepare tablets based on the procedure as carried out by Sastrahidayat ((1995).

RESULTS AND DISCUSSION.

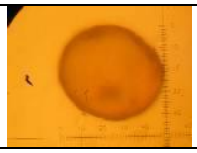
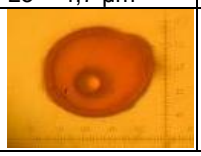
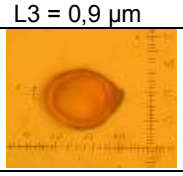
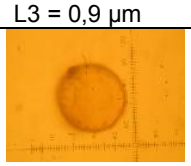
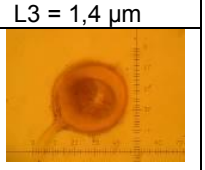
We had found 10 types of AVM spores on the soil samples around and in the rhizofers of Maniok cassava as well as Cece Udang cassava growing on mediteran and latosol soil type in the age of 1, 5 and 10 months. Based on the InAVM identification and clarification instructions, the ten types of AVM spores and their characteristics can be seen in Table 1. The number of AVM spores in the rhizofer of maniok cassava as well as of cece udang cassava in the age of 1, 5 and 10 months, growing on mediteran and latosol soil type is presented in Figure 1 to 4.

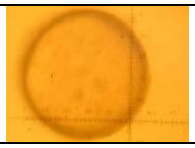
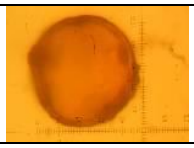
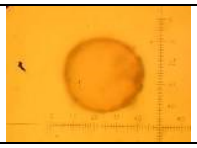
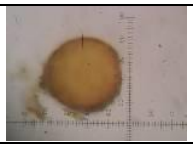
Sieverding (1991) said that not all types of plants can provide a positive response to each of AVM fungus, because each plant has different levels of dependence and response to AVM. Cassava has a positive response to the ten types of AVM spores (Table 1). Among the ten types of AVM spores found, the most abundant type were *Gigaspora gigantea*, *Glomus claroideum* and *Glomus etunicatum* (Figure 3 and 4). The cece Udang cassava has better responce to the *Gigaspora gigantea, Glomus etunicatum , Glomus fistulosum, Glomus coronatum, Glomus mosseae,* and *Acaulospora koskey.* While the maniok cassava hasbetter responce to the *Glomus claroideum, Glomus fasciculatum, Acaulospora rugosa*

and *Acaulospora morrowiae* (Figure 4).

The majority of AVM spore types found in the cassava rhizofer are the genus of Glomus. Due to their better growing and adaptation to cassava, compared to the genus of Gigaspora as well as Acaulospora. Shamdas (2007) explained that Glomus has greater capability to grow and adapt to a wider range compared to other AVM genus, especially in agriculture. This is consistent with the results of research conducted Hasbi (2005) on pineapple, mustard, papaya, kale, eggplant, spinach plant that the Glomus genus has the greater capability of adaptation to the type of cultivation crop compared to the Acaulospora genus.

Table 1. The type and characteristics of each of spores on the rhizofer of Maniok cassava as well as Cece Udang cassava in the age of 1, 5 and 10 months, growing on mediteran and latosol soil type

Featur	SPECIES MVA				
	Glomus coronatum	*Glomus mosseae*	*Acaulospora rugosa*	*Acaulospora morrowiae*	*Acaulospora koskey*
Color	Pale orange-brown to dark orange-brown	Straw to dark orange-brown	pale yellow brown	pale yellow brown	Pale yellow-brown to dark orange-brown most pale orange-brown
Shape	Globose, subglobose, some irregular.	Globose to subglobose, some irregular	Mostly globose, subglobose, occasionally irregular.	Mostly globose, subglobose, occasionally irregular.	Globose, subglobose, some oblong to irregular.
Spore size	154 µm	196 µm	80 µm	75,6 µm	187 µm
Wall Structure	Two layers	Three layers	Three layers	Three layers	three layers
Wall Thickness	L1 = 3µm, L2 = 5,9 µm	L1 = 2,1 µm, L2 = 1,2 µm, L3 = 4,7 µm	L1 =< 0,75 µm, L2 = 2,2 µm, L3 = 0,9 µm	L1 = < 0,5 µm, L2 = 2,2 µm, L3 = 0,9 µm	L1 = 1,75 µm, L2 = 1,9 µm, L3 = 1,4 µm
Spore image					

Featur	SPECIES OF AVM				
	Gigaspora gigantea	*Glomus claroideum*	*Glomus etunicatum*	*Glomus fistulosum*	*Glomus fasciculatum*
Color	Bright greenish yellow – to bright yellow-green	Cream to light yellow	Orange to red brown	Cream to light yellow	Pale yellow to pale yellow-brown
Shape	globose - subglobose	Globose to subglobose	Globose, subglobose.	Globose to subglobose	Globose, subglobose.
Spore size	324 µm	115 µm	129 µm	102 µm	60-110 µ m
Wall Structure	Three layers	Four layers	Two layers	Four layers	three layers
Wall Thickness	L1 = 3,2 µm, L2 = 16,9 µm L3 = 2,5 µm	L1 = 0,6 -1,8 µm, L2 = L1, L3 = 3,8 µm, L4 = 0,5 µm	L1 = 1,7 µm, L2 = 5,3 µm	L1 = 1,2 µm, L2 = 1,8 µm, L3 = 3,8 µm, L4 = < 0,5 µm	L1 = 1,2 µm, L2 = 6,5 µm, L3 = <1,0 µm
Spore image					

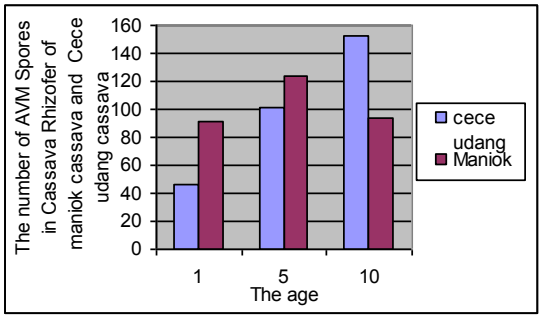


Figure 1 The number of AVM spores In the Cassava rhizofer in the Age of 1, 5, and 10 months

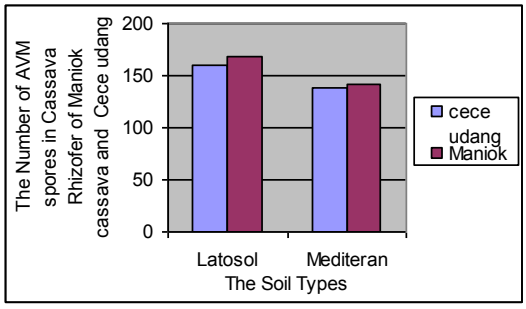


Figure 2 The number of AVM spores in the cassava rhizofer growing on mediteran and latosol soil type

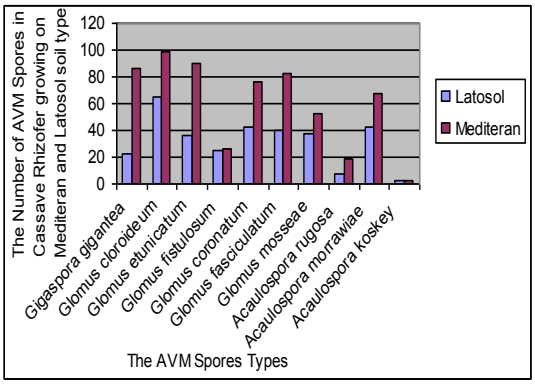


Figure 3 The Number of each of AVM spores type in the Cassava Rhizofer growing on mediteran and latosol soil type

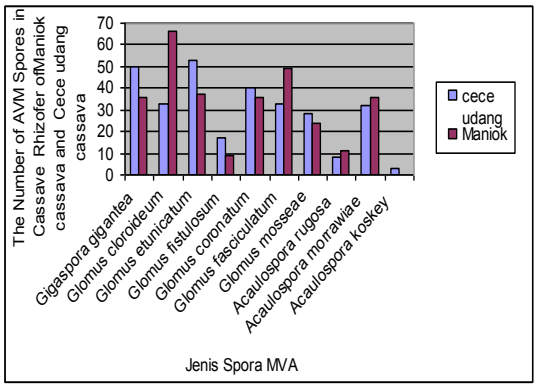


Figure 4 The Number of each of AVM spores type in the Cassava Rhizofer of maniok cassava and cece udang cassava

From the above discussion it can be concluded that in rhizofers of maniok cassava and cece udang cassava in the age of 1, 5 and 10 months growing on soil type of mediteran and latosol there are 10 speciies of AVM spores, consisted of: 1) *Gigaspora gigantean*, 2) *Glomus claroideum,* 3) *Glomus etunicatum*, 4) *Glomus fiistulosum*, 5) *Glomus fasciculatum*, 6) *Glomus coronatum*, 7) *Glomus mosseae*, 8) *Acaulospora rogusa*, 9) *Acaulospora morrowiae*, and 10) *Acaulosporakoskey*. The largest number of spores of the AVM species are of *Gigaspora gigantea*, *Glomus claroideum* as well as *Glomus etunicatum*. The three types of spores were then propagated in order to multiplicate the AVM isolates using corn as its host plant before constructing the AVM tablets.AVM tablets based on the procedures performed by sastrahidayat (1995),

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P-MB06

POPULATION GENETIC OF *Hoya multiflora* at SUKAMANTRI GUNUNG SALAK, WEST JAVA, INDONESIA BASED ON ISOZYME ANALYSIS

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Not presented

P-MB07

PHYLOGENETIC ANALYSIS OF THE *Anguilla bicolor bicolor* BASED ON THE CYTOCHROME OXIDASE SUBUNIT I (CO1) MITOCHONDRIAL

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ABSTRACT

The phylogenetic of the *Anguilla bicolor bicolor* were analyzed based on cytochrome oxidase subunit I (COI) mitochondrial gene sequence data. This study was conducted to examine *Anguilla bicolor bicolor* from Citandui Rivers, Cilacap. DNA sequences data from two samples *Anguilla bicolor bicolor* along with those of 18 species/subspecies Genus of *Anguilla* obtained from GenBank. The phylogenetic tree were constructed by Neighbor-Joining (NJ) method by using two species as outgroup. DNA sequences data from two samples *Anguilla bicolor bicolor* have been obtained and analyse to examine for phylogenetic status of the spesies.

Keywords : *Anguilla bicolor bicolor*, Phylogenetic, Cilacap, CO1

P-MB08

GENOMIC DNA ISOLATION OF *Gracilaria verrucosa* (Huds) Papenfuss MACROALGAE BY USING DELLAPORTA METHOD

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ABSTRACT

Gracilaria verrucosa (Huds) Papenfuss is a macroalgae species which has economic value as raw material for agar production. Isolation in order to obtain high-quality DNA is a basic rule that must be fulfilled in molecular studies. Isolation of macroalgae genomic DNA, particularly members of agarophyte group such as *Gracilaria verrucosa* (Huds) Papenfuss proved difficult. The problem occurs because the content of polysaccharides, in the form of agar, which is a viscous component and often cause difficulty in the process of DNA isolation. The purpose of this study is to learn a high quality DNA isolation technique from vegetative organ of *Gracilaria verrucosa* (Huds) Papenfuss. DNA isolation by using Dellaporta method. The result of agarose gel electrophoresis showed less optimal quality bands, there are appear smear or gradation at the band patterns. It shows that the result of DNA isolation by using Dellaporta method has a lot of contamination. Conclusion of this study is modification of standard DNA isolation method is needed, especially in plant such as *Gracilaria verrucosa* (Huds) Papenfuss macroalgae.

Keywords :DNA Isolation of Dellaporta method, *Gracilaria verrucosa* (Huds) Papenfuss, contamination

Introduction

Macrolagae species *Gracillaria verrucosa* (Huds) Papenfuss is one of water natural resources that are founded abundant in East Java, and it is needed attention because of many demands both locally and abroad. *Gracillaria verrucosa* (Huds) Papenfuss is macroalgae species which has economic value as .a raw material for agar production. According to Lee (1980) agar is a phycocholoid component that is available in the wall of cell. The use of agar has a wide spectrum; for instance in the food industry field especially for food stabilizer and food gelling agent.

According to Joubert, Y & Fleurence (2005), DNA isolation from macroalgae; especially agarophyte group such as *Gracillaria verrucosa* (Huds) proved difficult. It occurs because of this group has cell wall that contains cellulose and abudance of polysacharides content; such as hydrocoloid (agar). Hydrocoloid is viscous component and it is often to be main source of DNA contamination. So, based n this reason, the purpose of the study is to learn DNA isolation technique by using Delaporta method from vegetative organ *Gracillaria verucossa* (Huds) Papenfuss.

Materials and Methods

The study has been conducted in moleculer Biology laboratory, Malang State University and Molecular Biology laboratory, Biology Department, The Faculty of Mathematics and Science, Brawijaya University in January-March 2010. The material that used is a tip of vegetative organ *Gracillaria verrucosa* (Huds) Papenfuss which is obtained from fishermen's dyke in Tegalsari village, Sidoarjo. Other material that used are liquid nitrogen, composition PCl for 50 ml is liquid phenol 25 ml, chloroform 24 ml, and alcohol isoamil 1 ml, placed in a dark bottle and wrapped tightly, TE Buffer pH 7,6 composition for 50 ml is 10 mM Tris-Cl pH 7,6 0,06057 g and 1 mM Na2EDTA 0,0186 g is stored in the freezer, TBE Buffer pH 8, loading dye. The tools are micropipette, tip, centrifuge, vortex, mortar and pistle, scissors, autoclave, tube, gloves, digital analitic scale, timer, beaker glass, water bath with shaking, tube shelves, vaccum decicator, freezer, spectrophotometer, electrophoresis, PCR machine, PCR tube, UV lamp, microwave. DNA isolation is conducted by using Delaporta method (1983).

Result and Discussion

Isolation of DNA from macroalgae is difficult because of their cellulosic walls and abundant polysaccharide content, which differs among species. The liberation of such compounds during cell lysis leads to highly viscous supernatants, the main source of DNA contamination (Joubert, Y & Fleurence, J. 2005). The result of electrophoresis runing agrosa gel is shown on figure 1, that revealed the quality of bands is less optimal. It shows the smear or graduation from the band pattern result. This means that the result of isolation by using Delaporta method still has a lot of contamination. The plant sample that is used is *Gracillaria verrucosa* (Huds) Papenfus which contains polysaccarides; such as agar that is phycocoid component inside cell wall (Graham & Wilcox, 2000).

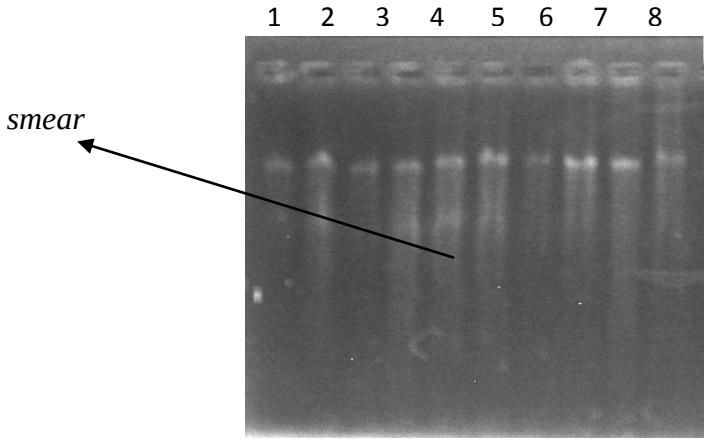

Figure 1. Agarose gel electrophoresis results of the *G. verrucosa* (Huds)Papenfus

According to Wattier, et al (2000) in the process of DNA isolation, genom macroalgae species that contains a lot of polysaccaride often pursues the process of DNA isolation (nucleat acid). Polysaccaride structure resembles with nucleat acid, it will cause this polysaccaride sedimented with nucleat acid. This metabolism result is also able to obstruct enzim work. The polysaccarides existence in the plant is marked by the viscousity of the DNA isolation result, that cause difficulty in the PCR reaction as the result of obstruction in taq polimerase activity. For this reason, it is needed a technique of DNA isolation macroalgae genom which is suitable so the good quality DNA will be obtained for the PCR amplification process.

Generally, the plant DNA extract by using buffer extract CTAB. For the plants which contain a lot of polysaccarida, needed a modification in DNA isolation. DNA isolation by Delaporta method et al (1983) is a modification method by using extract buffer CTAB. In the process of DNA isolation after adding extract buffer CTAB to macrolagae that is smoothened, will form viscous emulsion that shows the high value of of polysaccarides content. Modification in DNA isolation is to clean out polysaccarides by using phenol, chloroform and isoamil alcohol (PCl 25:24:1). In Bao Guo Xue opininon (1996), usually plant DNA extraction bases on CTAB, but in the plants that has high polysaccaride content is needed a modification in DNA extraction. To clean out polysaccaride, we need chloroform more suggested rather than alcohol isoamil, because of the efficiency in DNA isolation. While phenol is an organic emulting that dissolve lipid, polysaccaride and protein. But, based on the runing result of electrophoresis agarose gell by using Delaporta method seem less optimal. That is why, needed a reexperiment to obtain a good quality in the process of PCR amplification.

Conclusions

Although there are many methods in plant DNA isolation, the three main factors that should be fulfilled are : (1) the way in homogenizing plant compund, especially cell wall, (2) buffer emulsion compotition that added, (3) contaminant disappearant. Modification in standard method DNA isolation is needed; especially in the plant such as macroalgae species *Gracillaria verrocosa* (Huds) Papenfuss.

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P-MB09

The Utilization of Local's Beji Soybean For Improving The Welfare of The Community And Microspore Culture Research

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ABSTRACT

Soybean is an important food crop. 70% of the national soybean is imported from the United States, China and Brazil. Local soybean productivity needs to be improved to support community food security. Research in the framework of Education for sustainable development since April 2011 until now (August 2011) is still done in the village of Beji, District Ngawen, Gunung Kidul Regency, Yogyakarta Special Region. Counseling and training of soybean processing is done to educate the public. Soybeans seed are processed into 'soybean milk' and added to the manufacture of 'tiwul' to supplement protein in these traditional foods. Communities are trained to process tempe and tofu into nuggets to create a variety of flavors. After the training they are expected to have increased nutritional intake, especially for the Beji village children who will be given soybean milk and rural communities are hoped to be more healthy. If there are entrepreneurs making soybean milk and nuggets the earnings of Beji village communities will increase resulting the increase of the welfare. The microspore culture studies are conducted in laboratory Plant Tissue Culture of soybean flower bud material. Microspore culture can shorten the duration of the study to obtain a pure strain of soybean that has many superior properties as the seed. Initial studies with the determination of microspore developmental stage use medium-B and temperature shock treatment of cold and heat. After 4 and 8 days of microspore culture with the temperature of 4 ^{0}C and 33 ^{0}C subcultured into MS medium plus 2.4 D 2 mg per liter with a temperature of 25 ^{0}C. The success of microspore culture of Beji local soybean will benefit the germplasm
preserve local rerources.

Keywords: soybean, welfare, microspore culture

P-MB10

Formulation of Nanoparticles from Short Chain Chitosan and Short Chain Chitosan-TPP as Non Viral Gene Delivery System and Transfection Against T47D Cell Line

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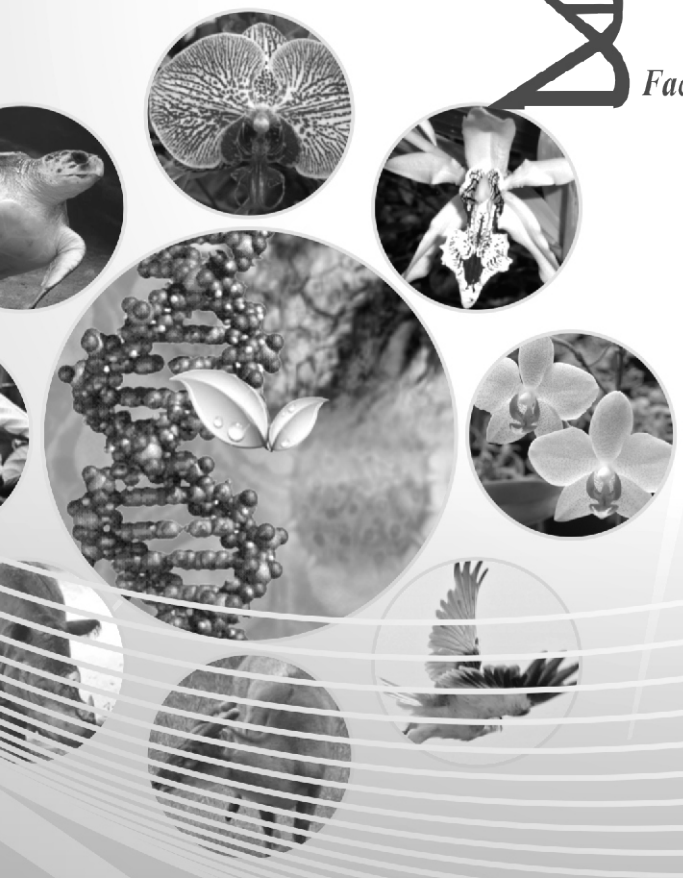

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

P-EC01

Ecological Aspects and Socio-economic Preferences of Local Communities into Species Selection for Water Spring Habitat Rehabilitation: Case Study in Purwodadi, Pasuruan

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ABSTRACT

This study is aimed to select plant species for water spring habitat rehabilitation in Sub District of Purwodadi, District of Pasuruan that satisfies ecological and socio-economic aspects. Ecological aspect was assessed based on vegetation analysis at three sites of natural water spring using parameters on species richness and diversity, and Important Value Index. Socio-economic aspect was evaluated based on the level of community preference using interview method to 60 respondents of local communities nearby the water springs. The result of vegetation analysis showed that there were 120 species of tree found at three study sites with species from Moraceae (figs) and Poaceae (bamboos) families dominated the sites. In accordance, socio-economic analysis also showed that some species from Moraceae family was also the most preferred species for habitat rehabilitation program by local communities. *Ficus benjamina* had the highest acceptance which was preferred by 75% respondents, followed by *Artocarpus elasticus* (61,7% respondents), *Artocarpus altilis* 'seedless' (53,3% respondents), *Durio zibethinus* (45 % respondents) and *Ficus drupacea* (43,3%). The *t-student* test showed that older respondents (> 50 yr) preferred more number of species than younger respondents (< 49 yr) (*t-student* = 2.515; df = 58; *P* < 0,05). The result of this study can be used as an alternative reference for selecting tree species on habitat rehabilitation program of water spring which is not only ecologically sustainable but also socially acceptable.

Keywords: plants species selection, ecological aspects, socio-economic preference, water spring habitat rehabilitation.

INTRODUCTION

Water is one of the products of ecosystem services which is important for humans. Without water, humans cannot live. As consequence of population growth, economic development and changes in consumption patterns demand for water continues to rise. It is estimated, for 10 years (2000-2010) the use of fresh water by the world population increased by 10% [1]. On the other hand, the environmental carrying capacity in the water supply decreased, primarily due to changes in vegetation cover for agricultural activities, forestry, plantations and settlements. As a result, there is a change in the pattern of evapotranspiration, infiltration rate, and the quality and quantity of surface flow (run-off), resulting in the decrease of water flow in dry season while in the rainy season it causes flooding and landslides [2]. In Indonesia, the availability of water will become a major problem in the future caused by the high rate of forest destruction. The average of deforestation rate in Indonesia is 1.6 to 2.4 million ha per year [3].

Various efforts of land and forest rehabilitation in Indonesia have been conducted by many parties, however, these were often limited in species diversity. Yet, not all rehabilitation sites are ecologically, socially and economically suitable for being planted using selected species. Even, the wrong choice of species, can lead to counterproductive results. Experience showed that in the areas successfully reforested with pine, residents complained that the water was shrinking [4]. Recently the government also recommends for planting trembesi (*Albizia saman*) on a large scale in degraded lands especially for the purpose of sequestration of carbon emissions [5].

In an ecologically important habitat, such as water spring, the biodiversity reason becomes important consideration in rehabilition programs. Therefore, in restoring vegetation cover around the springs required scientific studies that can be possibly received from various viewpoints. Therefore, this study is aimed to select plant species for water spring habitat rehabilitation in Sub Purwodadi, Pasuruan that satisfies ecological and socio-economic aspects.

MATERIALS AND METHODS

The research was conducted at three locations within the District of Purwodadi Pasuruan East Java, which were Cowek, Gajahrejo and Parerejo. Ecological aspect was assessed based on vegetation analysis at three sites of natural water spring respectively using Mueller-Dombois's method with parameters on species richness, diversity and Important Value Index. The list of species richness of each location was compiled into a new combined list as a basis for making questions list for respondents.

Purposive sampling using questionnaire method was conducted to get socio-economic data. The respondents were the people who were living around and frequently using the water spring. The questionnaire was used to get information on what is the preferred trees to be planted around the springs. In more detail, the respondents were asked for the main reason underlying their choice (such as benefit from flowers, fruit, wood, roots and canopy). Questionnaire results were then tabulated and *t-student* test were used to examine the difference between older and younger respondents.

RESULTS AND DISCUSSION

Ecological Aspect

The result of vegetation analysis at three study sites showed that there were total of 120 tree species around the water springs with the number of tree species at Cowek site

ranked first (72 species), followed by Gajahrejo (69 species) and Parerejo (54 species) (Table 1). Species from Moraceae family were dominant at Cowek site with *Ficus racemosa* as the most important species, while bamboos were dominant at two other sites with *Bambusa blumeana* as the most important species.

Table 1. Result of vegetation analysis around the water springs at three sites in Purwodadi, Pasuruan.

Location	Cowek	Gajahrejo	Parerejo	Combined three sites
Family number	30	28	23	
Genus number	55	37	42	
Species number	72	69	54	120
Diversity index	5,08	5,06	4,5	-
Codominance of species	*Ficus racemosa*. *Ceiba pentandra Artocarpus elasticus Swietenia macrophylla Ficus virens*	*Bambusa blumeana Dendrocalamus asper Ceiba pentandra Gigantochloa atter Ficus benjamina*	*Bambusa blumeana. Syzygium javanicum Ceiba pentandra Ficus virens Dendrocalamus asper*	
Density/hectare	64	110	80	

Despite the high diversity index, site at Cowek has the lowest tree density with only 64 individual/hectare, while Gajahrejo site ranked first with 110 individual/hectare. It implies that there still needs enrichment planting at Cowek site in order to increase the density especially with species that ecologically important but only found in a small number.

Socio-economic aspects

The result showed that *Ficus benjamina* was the most preferred tree species for water spring rehabilitation with 75% of respondents chose this, followed by *Artocarpus elasticus* with 61.7% of respondents and *Artocarpus altilis* “Seedless” with 53.3% of respondents (Figure 1). Six of ten most important species were species belong to Moraceae family, showing that this family was not only important ecologically on water spring habitat but also accepted socially. Non Moraceae species preferred by respondents, such as *Durio zibethinus*, *Pangium edule*, *Michelia champaca* and *Aleurites moluccana* have various benefits so that they can be used as enrichment plants in rehabilitating water spring. The result of *t-student* test showed that there was a significant difference on the number of species chosen by younger and older generation (*t-student* = 2.515; df = 58; $P < 0,05$). Older respondents with ages more than 50 year tended to choose more species (on average of 22

species) than younger respondents with ages less than 50 year (on average of 13.45 species). This result indicated that there is a reduction of local knowledge in traditional botany.

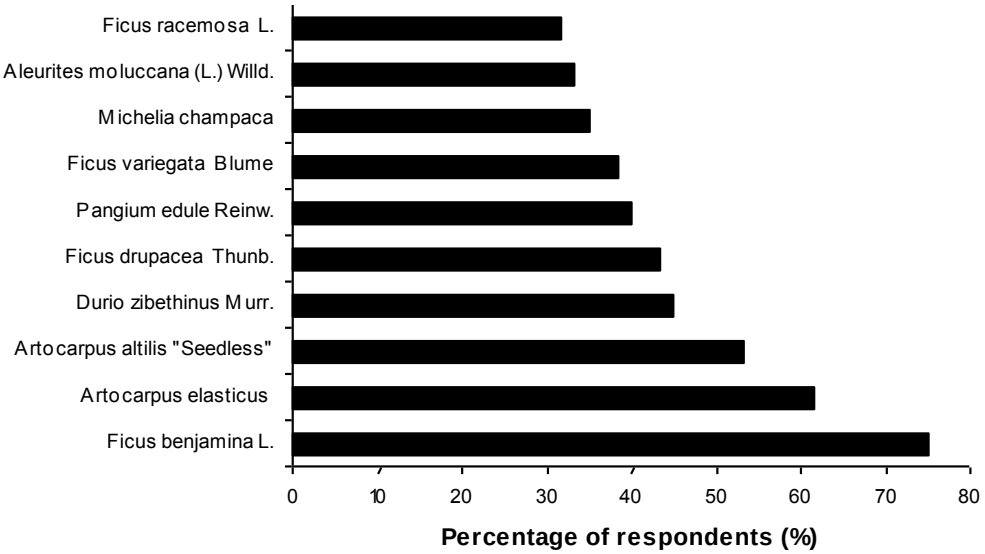

Figure 1. The most preferred tree species for water spring rehabilitation.

Further analysis on the reason of tree species selected by the respondents showed t that respondents tended to consider multi purposes trees rather than merely to get benefit from one particular. This was more apparent in species selection due to economic benefits, such as the species with high potential value on its flower, fruit and timber.

The most preferred tree species because of the flower was *Michelia champaca* with 25% of respondents. *Cananga odorata* ranked second with 23.3% of respondents, followed by *Gnetum gnemon* with 6.67% of respondents. Both *M. champaca* and *C. odorata* flower is usually extracted to produce volatile oil for perfume industry need.

Artocarpus elasticus was the most preferred tree species because of its fruit with 50% of respondents, followed by *Artocarpus altilis* 'Seedless' with 46.67%. The popular fruit *Durio zibethinus* ranked third with 43.3% of respondents, followed by *Pangium edule* and *Aleurites moluccana* with 36.67% and 33.33% of respondents respectively. The large portion of respondents that chose tree species due to fruit reason indicates that the potential aspect of fruit become important consideration in selecting trees for water spring rehabilitation.

In term of timber potential, *Artocarpus elasticus* was also the most preferred tree species due to of its timber with 48.3% of respondents. *Calophyllum inophyllum*, *Durio zibethinus*, *Michelia champaca*, *Pangium edule*, *Terminalia microcarpa* and *Terminalia bellerica* ranked second and shared similar portion of the respondents with 25%. Again, the high proportion of respondent that select tree species in regard to its timber suggests that

timber production should also be considered in selecting species for water spring rehabilitation. However, some major timber tree species such as *Tectona grandis*, *Pinus merkusii* and *Swietenia macrophylla* were less preferred by the respondents, indicating that ecological aspect still outweighs economical aspect in context of water spring rehabilitation.

Viewed from ecological aspect, *Ficus benjamina* was the most considered tree species for water spring rehabilitation in the aspect of rooting system with 55% of respondents. Four species from Moraceae family following were *Ficus drupacea* (with 31.67% of respondents), *Ficus variegate* (with 21.67% of respondents), *Artocarpus altilis* 'Seedless' (with 18.33% of respondents) and *Ficus kurzii* (with 18.33% of respondents). The common belief that fig rooting systems affect the occurrence of water spring probably influences the respondents to select the Moraceae species especially *F. benjamina*.

Ficus benjamina was also the most preferred tree species in term of its crown structure with 25% of respondents, followed by *Artocarpus altilis* with 15% of respondents and *Ficus kurzii* with 13.33% of respondents.

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P-EC02

POPULATION DISTRIBUTION OF THE FRUITFLY *Bactrocera carambolae* (DIPTERA: TEPHRITIDAE) ON SOME FRUIT PLANTS

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P-EC03

Behaviour Curik Bali (*Leucopsar rothschildi* Stresemann, 1912) at Bali Barat National Park

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Abstract

Since 1966 Curik Bali (*Leucopsar rothschildi* Stresemann, 1912) was grouped as threatened with critical category by IUCN and was protected by Indonesian both International law.

The behaviour Curik Bali at Bali Barat National Park was observed using look down method, focal animal sampling method, and terestrial navigation technique.

The behaviour Curik Bali occurred in the afternoon (52,06%). The highest behaviour was roosting (85,84%) which was composed of singing (59,54%), look around (21,48%), preening (13,61%), feeding (3,12%) and bobbing (2,24%). The most roosting tree was Pilang (Acacia leucophloea)(45,48%) and Walikukun (Schoutenia ovata)(17,3%).

Key words: Curik Bali, Leucopsar rothschildi, Bali Barat National Park, behaviour.

Introduction

Curik Bali *(Leucopsar rothschildi* Stresemann, 1912) is a member of the familia Sturnidae and including Passeriformes order. Curik Bali sized about 25 cm long, white fur, except on the wing tip and tail are black, open the skin around the eyes are bright blue. Crest plumage very long, especially in the male. Iris and beak is gray, while the legs bluish gray (1). At a national seminar on IPB dated December 23, 2006 agreed that the indigenous species *Leucopsar rothschildi* as Curik Bali and non indigenous species as Jalak Bali.

Curik Bali including a bird in a critically category and endangered (2,3,4,5). Curik Bali in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) is included into Appendix I, that protected species are endangered if it continues to be trade thus not given permission trading (6).

Curik Bali is an endemic bird on Bali Island, in 1911 Erwin Stresemann found Curik Bali at Bubunan Buleleng. Distribution of Curik Bali at Bali Barat National Park (BBNP) until at Village Bubunan Buleleng (7,8), but in 1950's Curik Bali was not seen again in Seririt District (9), 1960s in Tabanan Curik Bali distribution reach Selemadeg District and the 1990s are still visible flew Pupuan District. In 1980's Curik Bali distribution reach Melaya district at Jembrana regency (9). Curik Bali allegedly been found on the island of Nusa Penida (10), but according to IUCN (3) P. Nusa Penida is not a natural distribution area of Curik Bali.

Materials and Methods

Research material is curik bali which is occur in in the Peninsula Prapat Agung Bali Barat National Park. To observe the behaviour of curik bali at Bali Barat National Park using ad libitum, and focal animal sampling method (11,12).

Results and discussion

Habitat

The trees are widely used by the Curik Bali to roost and sleep is pilang (*Acacialeucophloea*)(48.1%) and walikukun (*Schoutenia ovata*) (17%). Other trees are: talok (*Grewia koordersiana*), tekik (*Albizia lebbeckioides*), kemloko (*Phyllantus emblica*), kesambi (*Schleira oleosa*) and intaran (*Azadiracta indica*). In pilang tree, walikukun and talok Curik Bali perched besides also getting caterpillars, ants and termites for food.

While in P. Nusa Penida there are 27 trees are used by Curik Bali for perching and sleeping are palm trees (*Cocos nucifera*), mango (*Mangifera indica*), tamarind (*Tamarindus indica*), singapur (*Muntingia calabura*), angih (*Ficus* sp.), ancak (*Ficus rumphii*), bunut (*Ficus glabella*), pungak-pungak (*Ficus* sp.), api-api (*Avicennia marina*), buni (*Antidesma bunius*), kluwih (*Arthocarpus altilis*), sugar palm (*Arenga pinnata*), pule (*Alstonia scholaris*), cashew (*Anacardium occidentale*), teak (*Tectona grandis*), kayu urip (*Euphorbia tirucali*), tuwi (*Sesbania grandifolia*), santan (*Lannea grandis*), duwet (*Syzgium cumini*), kampuak (*Psidium* sp.) krasi (*Lantana camara*), banana (*Musa paradisiaca*), cassava (*Manihot utillisima*), hibiscus (*Hibiscus sinensis*), jackfruit (*Artocarpus heterophylla*), gamal (*Gliricidia sepium*), lamtara (*Leucaena glauca*) and frangipani (*Plumeria acuminata*) (13).

Behaviour

Daily Behavior Curik Bali in Bali Barat National Park is: perching 85% and moving 15%. While perching consist of singing 60%, look around 20%, body care (15%) , feed 3% and 2% enforce crest. While the daily behavior Jalak Bali in P. Nusa Penida (13) were: perching 45% and 55% moving. While perching consist of singing 45%, feed 40% and 10% body care and enforce crest of 5%. (Figure 1).

Curik Bali's food in Bali Barat National Park is the caterpillars, ants and termites. Curik Bali in Bali Barat National Park also eat caterpillars (Familia Geometridae and Familia Pieridae), ants (Familia Neridae) and locusts (*Ducetia thymifolia*)(14). While Jalak Bali food in Nusa Penida (*Ficus glabela*), angih fruit (*Ficus* sp.), rack fruit (*Ficus rumphii*), mantis sword (*Acradium ornatum*), praying mantis (*Creoboter* spp.), Termites (Order Isoptera), juvenile ant (*Oecophylla smaradigna*), caterpillars (Geometridae Familia), lizard (*Hemidactylus frenatus*) and earthworms (*Pheretima* sp.) (13).

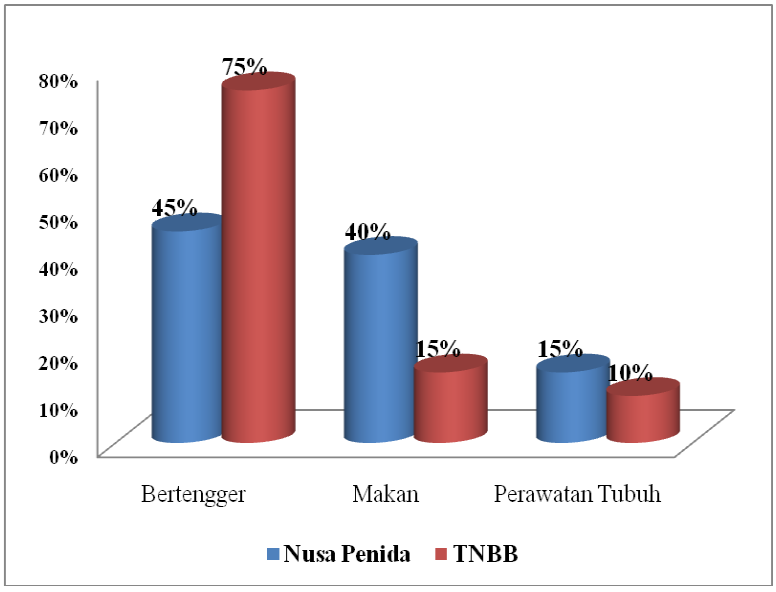

Figure 1. Daily Behavior Curik Bali

Curik Bali in Bali Barat National Park make nests in Pilang trees (*Acacia leucophloea*). According to Cahyadin in Bali Barat National Park Curik Bali also make nests in trees klumprit (*Terminalia edulis*), walikukun (*Schoutenia ovate*), kaliombo (*Terminalia microcarpa*), kemloko (*Phyllanthus emblica*) and talok (*Grewia koordensis*)(14). Noerdjito also said that Curik Bali in Bali Barat National Park make nests in trees talok (*Grewia koordensis*) (15). In Batu Madeg village and Ped village at Nusa Penida island Jalak Bali make nests on 11 tree species, ie coconut trees, Bunut, pungak-pungak, angih, ancak, tamarind, api-api, kluwih, kampuak, lamtara and palm (13).

Conclusion

The behaviour Curik Bali occurred in the afternoon (52,06%). The highest behaviour was roosting (85,84%) which was composed of singing (59,54%), look around (21,48%), preening (13,61%), feeding (3,12%) and bobbing (2,24%). The most roosting tree was Pilang (*Acacia leucophloea*)(45,48%) and Walikukun (*Schoutenia ovata*)(17,3%).

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P-EC04

Behaviour of the Juvenile Komodo Dragons (*Varanus komodoensis* Ouwens, 1912) at Komodo National Park

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ABSTRACT

Komodo dragons were first recorded by scientists in 1910. In the wild their range has contracted due to human activities and they are listed as vulnerable by the IUCN. They are protected under Indonesian authority, and a national park, Komodo National Park, was founded to aid protection efforts.

Behaviour of the dragons at Komodo National Park was observed using focal animal sampling method.

The result of study showed that the hatching rate was 100% (n=24). The sex ratio of the hatchlings was observed to be skewed toward males (X^2=3,68; 0,025<P,0,05). Hacthlings spent most (86,1%) of their time on the trees to search for food and to avoid that danger of canibalism from the bigger ones. Behavioural activities observed during the study showed that 27,3% was used for basking, 30,6% for moving, 36,7% stay under the shade, 2,9% of its activity has shown the agonistic activity, 1,6% for eating, 0,2% drinking, 0,4% defication and 0,4% rubbing.

Key words: hatching, behaviour, komodo dragon, Komodo National Park

Introduction

Komodo dragon (*Varanus komodoensis* Ouwens, 1912) is an endemic species at Komodo, Rinca, Gilimotang, and Wae Wuul (Flores) islands. Komodo was first discovered by Van Steyn in 1911, then be described and published first by the PA Ouwens in 1912 [1]. Komodo is an endangered species included in Appendix I of CITES. In 1992 komodo dragons designated as Indonesia National Wildlife [2].

Comparison of the number of dragons with their prey is pretty good, but there are people hunt dragons prey such as deer and buffalo, so it worried can disrupt dragons conservation in Komodo National Park [3]. As in Padar island no longer found the komodo dragons, but there are still found dragons prey such deer.In 1997, deers population at padar island are 2000 head with density 93 head/km2

Vegetation that arrange padar island dominated by *Zyziphus rotundifolia* tree and *Themeda frondosa* grass so called Zyziphus-Themeda facies formation [4].

Komodo dragons are carnivores, and obtain their prey by attacking animal when sleep or ambush their prey. If the dragon can not directly kill its prey, by attacking animal when sleep or or leg, then the dragons will follow and wait for its prey to weaken or die. Komodo dragon prey that has been bitten may experience death due to bacteria in Komodo dragon saliva.

There were found 62 types bacteria in komodos saliva such as: *Pseudomonas aeruginosa, Proteus mirabilis, Prividencia stuartii, Pseudomonas cepacea, Staphylococcus xylosus, S. aureus, Escherichia coli, Enterobacter* sp., *Bacillus* sp., *Kliebsiela* sp. Bacteria were found in komodos cloaca there are 8 species ie: *Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, Proteus vulgaris, Enterobacter* sp., *Citrobacter freundii*, Bacillus sp., *Kliebsiela* sp. [3].

Materials and Methods

Materials research is the juvenile dragons where found on Rinca and Komodo Island at Komodo National Park. On Rinca island research conducted at Loh Baru, Loh Tongker and Loh Buaya, and on Komodo island conducted at Loh Liang and Loh Kima. This research used focal animal sampling methods [7].

Results and discussion

Komodo dragons female lay eggs and hatch as many as 24 grains of all, but who lived only 22 dragons. Komodo dragons females during reproductive period is more aggressive, but most of its time spent in the nest. Daily behavior komodo dragons female during reproductive period are : sunbathing 36.9%, moving 16%, shade 25.4%, resting in the hole 17.7% and agonistic 4% [5].

Juvenile Komodo dragons after hatching, out from ground nest, ran and climbed into a tree. Juveniles dragon habitat until 2 years reach 1 m long, live in arboreal on tree 86.1 % and on the ground 13.9%. In Loh Liang Komodo island there are 29 species of plants used for habitat, such as: tamarind (*Tamarindus indica*) 20%, kesambi (*Schleichera oleosa*) 12%, paci (*Cordia* sp.) 19%. High utilization at the three plants by juveniles dragon because there are dominant plant in these habitat. Juveniles dragon live in arboreal in order to avoid cannibalism behavior from older dragons and also to find prey.

Daily behavior of juvenile dragons are: sunbathing 27.3%, moving30.6%, shade36.7%, agonistic 2.9%, eating1.6%, drinking 0.2%, defikasi 0.4% and rubbing 0, 4% [3.5]. Juvenile Komodo dragons most active at 10-11 o'clock at 11.61% and at 15-16 o'clock for 9.74% [6]. Juveniles komodo dragons (n=22) prey are 46% insects consist of grasshoppers (*Locusta* sp.), Locust leaf (*Microcentrum* sp.), And the praying mantis (*Stagmomantis* sp.), Reptiles 40% consist of the lizard (*Platyurus platyurus*), skink (*Sphenomorphus* sp.), gecko (*Gekko gekko*) and the flying lizard (*Draco volans*), mammals, 6.7% concist of mice (*Mus musculus*), and Aves 6.7% consist of timor sunbird (*Nectarinia solaris*).

Conclusion

The result from this study showed that the hatching rate was 100% (n=24). The sex ratio of the hatchlings was observed to be skewed toward males (X^2=3,68; 0,025<P,0,05). Hacthlings spent most (86,1%) of their time on the trees to search for food and to avoid that danger of canibalism from the bigger ones. Behavioural activities observed during the study showed that 27,3% was used for basking, 30,6% for moving, 36,7% stay under the shade, 2,9% of its activity has shown the agonistic activity, 1,6% for eating, 0,2% drinking, 0,4% defication and 0,4% rubbing. The juvenile komodo dragons prey are insects 46.6%, reptiles 40%, aves 6.7%, mammals 6.7%.

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P-EC05

ORCHID CONSERVATION OF *Paraphalaenopsis serpentilingua* BY IN VITRO CULTURE

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ABSTRACT

Paraphalaenopsis serpentilingua is a rare orchid with restricted habitat endemic to Kalimantan. Furthermore, this presticious species also has commercial value, as a parent for breeding. Research on the growth of *P. serpentilingua* by *in vitro* culture was done to increase the quality of explants to support conservation purpose. Combination between foliar fertilizer with organic matter in media was treated on the growth of protocorm like bodies (plb) of *P. serpentilingua*.

The result showed that combination of foliar fertilizer Hyponex 25-5-20 + peptone 2g/l gave better effect to the first root emerged, number of roots, and height explant at 32 weeks after subculture. Combination of foliar fertilizer Hyponex 6.5-6-19 + peptone 2g/l provide better effect to the first leaf initiation, number of leaf, number of root, and leaf area at 32nd week after subculture. Addition of peptone 2 g/l provide positive effect to the growth of explants *P. serpentilingua***.** However, addition of banana 20 g/l and sweet potato 15 g/l inhibited growth of the protocorm.

Keywords: *Paraphalaenopsis serpentilingua*, foliar fertilizer, organic matter, *in vitro*

INTRODUCTION

Orchid *Paraphalaenopsis serpentilingua* is one type of wild orchids from West Kalimantan. Based on Government Regulation No. 7 in 1999 this species is included in protected orchid plants. To prevent extinction, it must be achieved with the appropriate techniques culture as a means of providing rapid orchid seeds with good quality and quantity. It is more mainstream is to conserve rare orchid species from the threat of extinction.

Basically, the culture medium contains not only the macro and micro nutrients, but also carbohydrates as a source of carbon [3]. Widiastoety [12] informs that the addition of complex organic material such as, water coconut, banana, peptone, tripton and casein hydrolysates in the culture medium can increase the growth of orchid plantlets. Murdad *et al*. [7] states that the addition of coconut water and activated charcoal in the medium XER can enhance proliferation the protocorm of *Phalaenopsis gigantea*. Peptone is often also referred to as organic nitrogen. Peptone included in the complex additive containing pyridoxine (2.5 μg/g), biotin (0.32 μg/g), thiamine (0.5 μg/g), nicotinic acid (35 μg/g) and riboflavin (4 μg/g) [1].

Knudson C media can be replaced with a simpler media foliar fertilizer [4]. Lakshmi [5] proved that Hyponex foliar fertilizer gave the highest number of leaves on orchid *V. tricolor* than half concentration of MS medium, Vacin & Went and Knudson C. Bety [2] reported that media foliar fertilizer with high nitrogen give the same effect as good as the media Vacin & Went to the growth of leaf length, leaf number, root length, root number, plant height, number of shoots, plantlets weight of *Vanda*. Park *et al*. [8] found that the regeneration protocorm (PLB) Doritaenopsis grown on MS medium with modifications, can grow better into plantlets after subculture on media Hyponex.

Based on preliminary research that protocorm (PLB) orchid *P. serpentilingua* can grow better in the media Hyponex foliar fertilizer than the media KC, VW and MS [10], then further research. This research aims to study the effect of foliar fertilizer Hyponex media combinations with the addition of organic matter on the growth of orchid PLB *P. serpentilingua*.

MATERIALS AND METHODS

The materials used in this study consisted of Hyponex foliar fertilizer with their respective levels of NPK 25-5-20 and 6.5-6-19, peptone, banana, sweet potato, distilled water, alcohol 70% and agar-agar powder. While the plant material used was PLB orchid *P. serpentilingua* that has been available in the Tissue Culture Laboratory of the Bogor Botanical Gardens.

This research using completely randomized design with one factor. Consisting of eight standard treatments with ten replications. Each treatment consisted of 5 explants, totally there are 80 units of the experiment. Standard treatment used in this study are as follows:

M1 = Hyponex 25-5-20
M2 = Hyponex 25-5-20 + pepton 2 g/l
M3 = Hyponex 25-5-20 + 2 g/l + banana 20 g/l + sweet potato 15 g/l
M4 = Hyponex 25-5-20 + banana 20 g/l + sweet potato 15 g/l
M5 = Hyponex 6.5-6-19
M6 = Hyponex 6.5-6-19 + pepton 2 g/l
M7 = Hyponex 6.5-6-19 + pepton 2g/l + banana 20g/l + sweet potato 15g/l
M8 = Hyponex 6.5-6-19 + banana 20 g/l + sweet potato 15 g/l

The data will be tested by F test, and if it shows a real effect, the test will be followed by contrast test.

RESULTS AND DISCUSSION

Explant growth is variable, size of explants height ranging from 0-5 cm. Leaf area explant was less than 0.5 cm^2. Rate of growth looked differences in each media used until week 26^{th} (Fig 1), M2 and M6 give better growth protocorm of *P. serpentilingua* compare to the series row media.

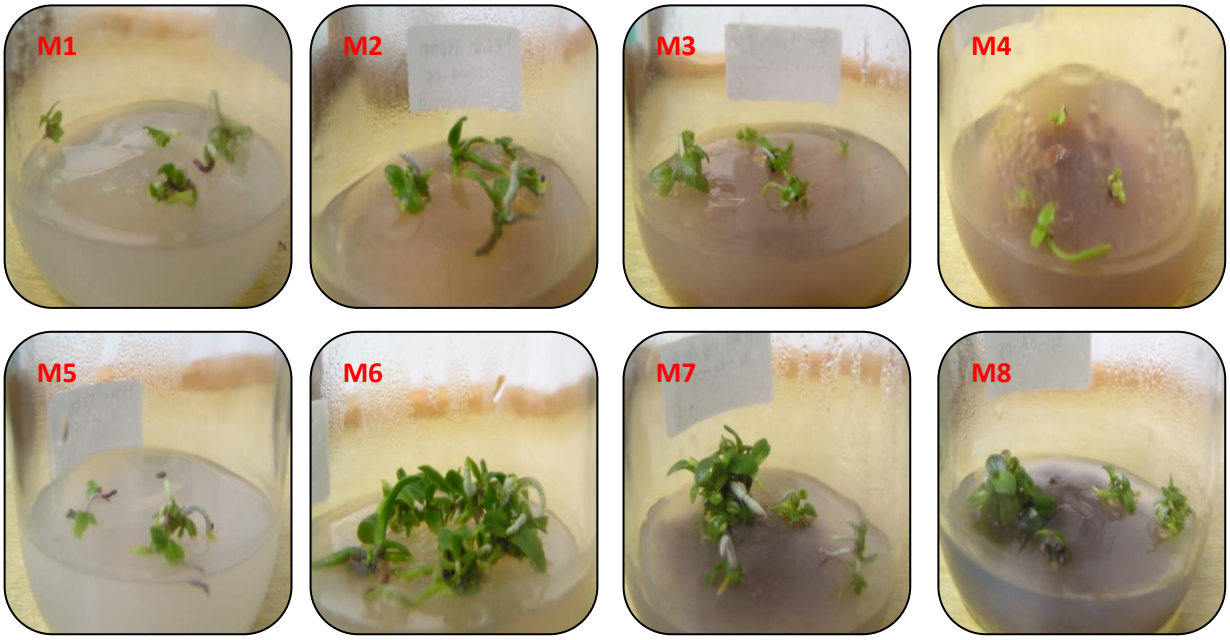

Fig 1. Protocorm growth of *Paraphalaenopsis serpentilingua* on week 26th after planting

Leaf Growth

Fig 2 illustrates time of first leaf initiation on each treatment. Based on Contrast test showed that treatment M2 was significantly different with treatment M1, while treatments M7 and M8 were significantly different with treatment M5.

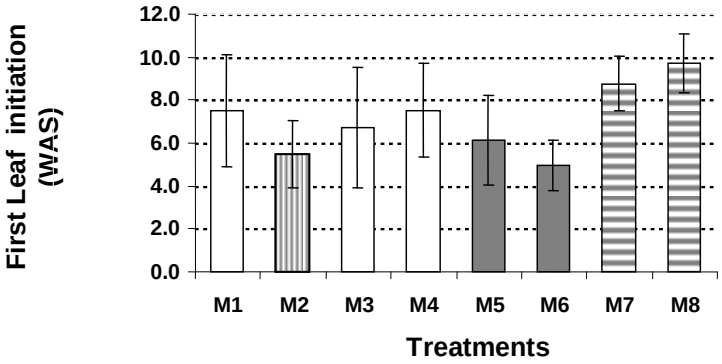

Fig 2. The effect of media on the leaf initiation of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%
WAS = Weekly Period After Subculture

Treatment both M6 (Hyponex 6.5-6-19 + peptone 2 g/l) and M2 (Hyponex 25-5-20 + peptone 2 g/l) were able to speed up time of first leaf initiation earlier than other media ±5 weeks after subculture. M2 and M6 also can increase the number of leaves (Fig 3) as well as the leaf area (Fig 4). This means that different concentration of Hyponex did not affect to the early leaf initiation, leaf number, as well as the leaf area, but the addition of peptone 2 g/l provide a positive effect on the leaf initiation, leaf number as well as the leaf area. M2 and M6 were believed to have a better nitrogen content to stimulate the first leaf initiation of *P. serpentilingua*. Sources of nitrogen from both treatments were derived from peptone. Peptone often used as a source of nitrogen [9]. Nitrogen is the main nutrient for plant growth, especially the growth of vegetative parts [6].

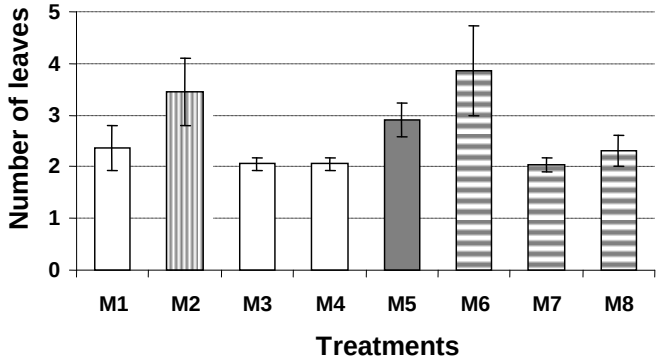

Fig 3. The effect of media on the leaf number of *Paraphalaenopsis serpentilingua*
note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%

The addition of organic matter banana 20 g/l + sweet potato 15 g/l inhibited number of leaves as well as leaf area. Inhibition was due to the concentration of sugar (carbohydrate) in the culture medium is higher than the concentration of liquid in the plant cells cause increasing osmotic pressure. So that the liquid in the cell diffuse out resulting in metabolic disorders. signs of plants that suffered because of the influence of osmotic pressure can be seen visually from the inhibition of growth in leaf size. This result supported with the research of Widiastoety and Purbadi [13] that the addition of sweet potato inhibit the number of leaves *Dendrobium*,

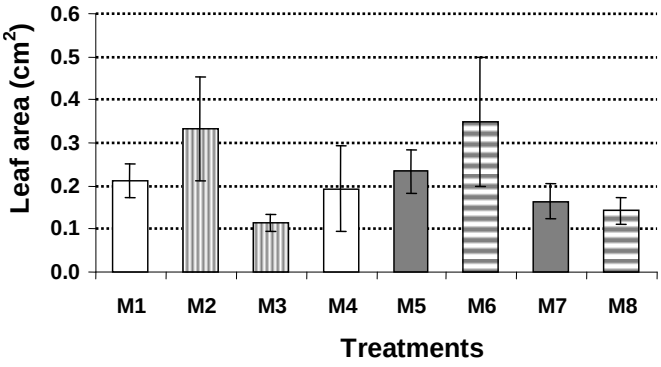

Fig 4. The effect of media on the leaf area of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%

Explants Height

High orchid explants *P. serpentilingua* at 32 MST for each treatment can be seen in Figure 5. Treatment of M2, M3 and M4 significantly different to the treatment of M1 while the treatment M6 was significantly different with treatment M5. Treatment of Hyponex 25-5-20 + peptone 2 g/l (M2) gave better results on the growth of plant height; (3 cm) compared with other media and the results are significantly different to the treatment with Hyponex 6.5-6-19 + peptone 2 g/l (M6) with a height of 2.4 cm explants. This means that the high nitrogen concentration giving a positive response to the explants height of *P. serpentilingua*. The results are consistent with the parameters of leaf area that the addition of organic matter banana 20 g/l + sweet potato 15 g/l also tends to inhibit the growth of explants height of *P. serpentilingua* (M3, M4, M7 and M8). This was allegedly because of carbohydrate derived from banana and sweet potato in culture media increase

medium viscosity and osmotic pressure so that it can interfere with absorption of nutrients and result in inhibiting the growth of explants. Widistoety and Bahar [11] also found that the high carbohydrate content have led to the inhibition of plant growth.

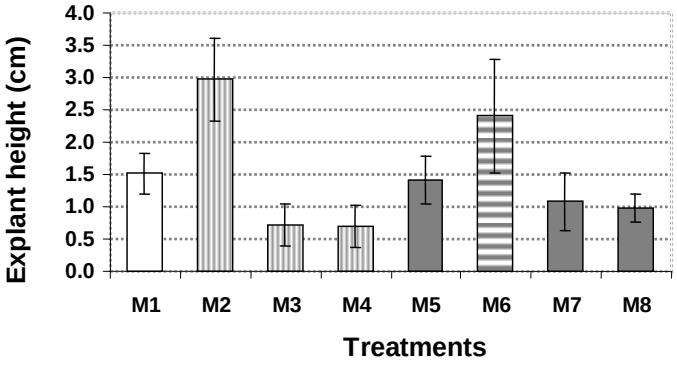

Fig 5. The effect of media on the explants height of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%

Root growth

Contrast test resulted that treatment of M2 and M4 significantly different to the treatment of M1 while the treatment of M5 and M6 was significantly different with treatment M7 and M8 (Fig 6). Treatment M2 and M6 emerged first root initiation earlier (8.8 WAS and 9.3 WAS respectively) and the two treatments were not significantly different. While M7 (Hyponex 6.5-6-19 + peptone 2 g/l, banana 20 g/l and sweet potato 15 g/l) emerged the longest first root iniation (13.7 WAS).

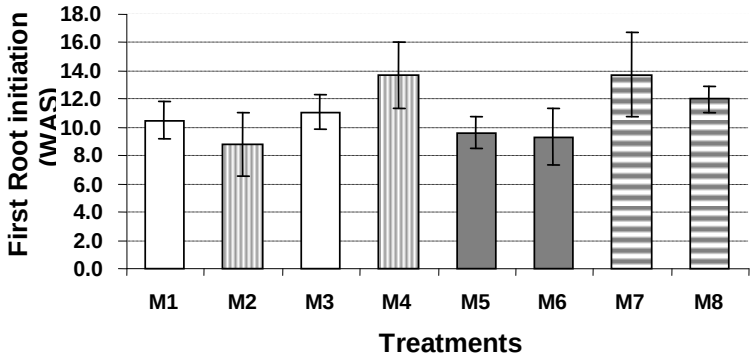

Fig 6. The effect of media on the early root initiation of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%
WAS = Weekly Period After Subculture

Figure 7 shows that Hyponex treatments with the addition of peptone 6.5-6-19 2g/l (M6) shows the number of root best and the results are significantly different from M2 (Hyponex 25-5-20 + peptone 2 g/l). This suggests that differences in the composition of N, P and K on Hyponex used in each treatment produced significantly different responses to the number of orchid root *P. serpentilingua* at 32 WAS. P content is higher in M6 treatment allegedly able to increase the number of roots and root length.

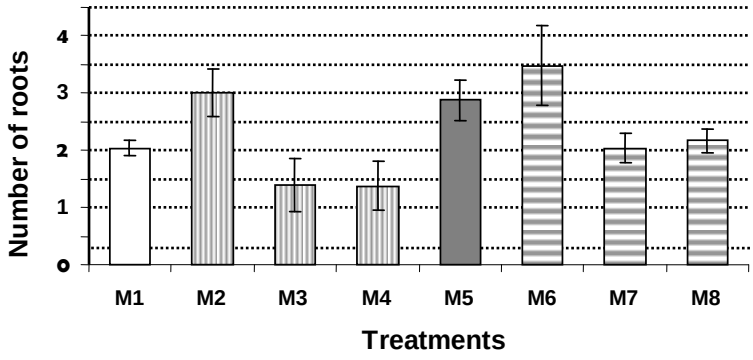

Fig 7. The effect of media on the root number of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%

Fig 8 shows that Hyponex 25-5-20 + peptone 2 g/l (M2) produced the longest root length of 1.9 cm and the results are not significantly different from Hyponex 6.5-6-19 + peptone 2 g/l (M6). While the addition of organic matter (banana and sweet potato) on M3, M4, M7, M8 produced shorter root length than control (M1 and M5).

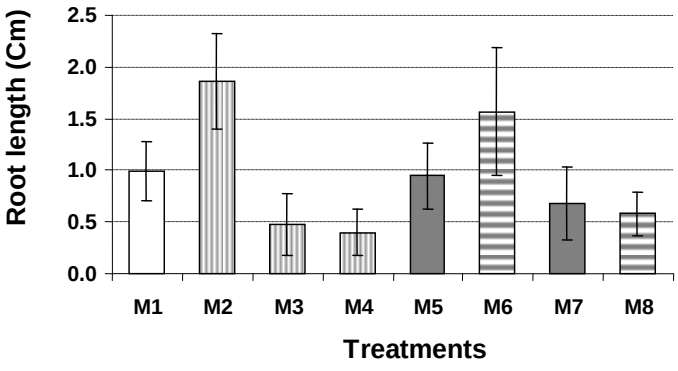

Fig 8. The effect of media on the root length of *Paraphalaenopsis serpentilingua*

note:
= Significantly different to M1 at level 5%
= Significantly different to M5 at level 5%

Hyponex 25-5-20 + peptone 2 g/l (M2) and Hyponex 6.5-6-19 + peptone 2 g/l (M6) give better results on the early growth of root initiation, number of roots and root length, compare to control and other media addited with organic matter (banana and sweet potato). The addition of organic matter banana 20 g/l + sweet potato 15 g/l (M3, M4, M7 and M8) inhibit the root initiation, number of roots and root length of *P. serpentilingua*.

The content of nitrogen in the treatment of M2 and M6, give positive effect to accelerate the emergence of root initiation early, number of roots and root length. Nitrogen is the main nutrient for plant growth, in general is necessary for the formation or growth of vegetative parts [6]. Sources of nitrogen from both treatments were derived from Hyponex and peptone. Peptone often used as a source of nitrogen [9].

According to Gunawan [4] The main function P was to assist root growth and plant maturation. Nitrogen is a nutrient essential for vegetative growth, but the right amount for plant growth depends on the type and species [6]. Expected content of nitrogen and other nutrients in the treatment of M6 is an appropriate composition to increase the number of roots of plantlets *P. serpentilingua*. The addition of organic matter banana 20 g/l + sweet potato 15 g/l inhibit the root growth of *P. serpentilingua* (M3, M4, M7 and M8). Root length was very inhibited on Hyponex 25-5-20 + banana 20 g/l and sweet potato 15 g/l (M4) with a size of 0.4 cm. This is consistent with the results of research Widiastoety and Purbadi [13] that the addition of sweet potato inhibits the number of roots of *Dendrobium* orchid.

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P-EC06

A METAPOPULATION STUDY ON THE MIGRATION ECOSYSTEM OF *Ardeola speciosa* AS THE RESULT OF ABRASION IN BEDONO DEMAK

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ABSTRACT

From 2160 hectares of mangroves in the coastal areas of Demak, about 713 hectares are in critical condition. Abrasion destroyed the mangrove areas and hundreds of hectares of fish ponds. About 500 hectares of damaged ponds are in Bedono, a village in Demak. This caused the fishermen were forced to switch their profession. To repair the damage, reforestation were conducted.20 thousands mangroves had been planted. The mangroves are now grown big and lush; especially those are located in the hamlet of Tambaksari, Bedono. The area which has been submerged by seawater now becomes a new habitat for *Ardeola speciosa* or called belekok bird. This research has been conducted on May 14 until June 6, 2011 with the aim to find out the metapopulation of the *Ardeola speciosa* (of Ardeidae family) birdnesting in Avecinnia trees or called brayo. The change of the ecosystem of the village that becomes wetland (as the result of abrasion) has caused many *Ardeola speciosa* which are suspected from Srondol Semarang migrate to Bedono. The study was conducted using exploratory survey covering the scope of research in Bedono. It was conducted in three hamlets of Bedono, namely: Tambaksari, Senik,and Mandaliko. The data of bird population were collected since in the process of licensing survey of this research. The data were recorded in standardized form on a regular basis and then the calculations of data from each hamlet were collected to be analyzed. The results showed that the most bird populations are sequentially located in the hamlet Senik, Mandaliko, and Tambaksari. The metapopulation was not affected by the distance of place because it can still be affordable.

Keywords: Metapopulation, Migration Ecosystem, *Ardeola speciosa*, Abrasion

INTRODUCTION

The birds egrets have settled in Srondol for Decades. They Make the place as a stopover the the between foraging activity. First, Pls Semarang still have a lot of rice acreage, swamps, ponds, and mangrove forests, the number of egrets in Srondol Relatively large. Along the rampant land use, many are choosing Egret migration. They seek new haven Closer to food sources. Keep in mind, tailless bird food is fish, marine animals, snails, frogs, and snakes.

As an illustration, a professor of Biology Department Undip, Karyadi Baskoro SSi MSi explained, until the end of the 1990s its population ranged 1.000ekor. The birds that inhabit the 24 trees, consisting of geese, acids, and mango. But now, based on observations, the population is shrinking dramatically, to 200's. Trees are also habitable living four rods. "Most egrets migrate to the area of mangroves in the area Sayung, Demak. Certain occupied more comfortable, Because it is Relatively close to the food source, says Karyadi four-Type noise reduction does not affect the population. The reason, as a species of water birds, egrets are

not too sensitive to noise. If noise Influential factors, Karyadi said, would have gone from Srondol long ago.

From about 2160 hectares of mangroves in coastal areas of Demak, about 713 acres of Them in critical condition. Abrasion resulted in mangrove areas and Hundreds of hectares of fish ponds destroyed. About 500 hectares of ponds That are Damaged in the village Bedono, so many were the resource persons Fishermen forced to switch Professions. Such damage to repair, do replanting of mangroves by 20 thousand sticks. Mangroves are now grown big and lush, especially those located in the hamlet Tambaksari, Bedono. That Hamlet has been submerged by sea water is now a new habitat for birds *Ardeola speciosa*. Blekok quiet life here is also protected to people, hunting whoever warned and fined if caught Sanctions Rp.100.000, - / bird. The money to building. This is supported by the district authorities and NGOs Sayung. "The money for development." He explained. according to him, in 2009, there is an NGO researching bird Populations in the village. At That time, the number blekok 10 thousand heads. Now he estimates, the population to 20 thousand heads. "Because, one tree can be used for 4-5 Nests. are thought to have come from Srondol. Transfer of new habitat or migration Because of the same type and Interact on Several levels known to metapopulasi or population to population. Metapopulation That is comprised of groups are spatially separated Populations of the same type and Interact on Several levels. Metapopulation terms selected by Richard Levins in 1970 to describe a model of population dynamics of insect pests on agricultural land, but the idea was widely developed and applied in a fragmented habitat is naturally or artificially. By the term Lavin explained metapopulation That is the population of the population.

A metapopulation generally considered several distinct populations consists of the which together occupy an area with suitable habitat That is now no longer occupied. In the classical theory metapopulation, each cycle of the population is relatively free from other populations Will Become Extinct as a consequence of demographic stokhastik (fluctuations in population size depends on the random demographic events); Smaller population would be more prone to Become Extinct.

Although individual Populations have a limited life span, usually stable metapopulation overalls Because of immigration from a population (for example, may be due to the explosion of population). They also drain to the small population and save the population from extinction (Called the rescue effect).

Metapopulation theory was first developed for Terrestrial Ecosystems, and then applied to the ocean realm. In fisheries science, the notion of "subpopulations" with metapopulation scientific term "local population. Metapopulation developmental theory, associated with the development of the theory of the dynamics of" source-sink ", giving more

attention to the importance of the relationship the between the Separate Populations. Although no population. http://aadrean.wordpress.com/tag/metapopulasi/ as an example of bird populations in Bedono blekok surroundings.The classification of birds Blekok are as follows:

Scientific classification

Kingdom : Animalia, Phylum : Cordata, Class: Aves, Upkelas : Neomithes, Infrakelas: Neomagnathae, Superordo : Neoaves, Order : Ciconiiformes, Family : Ardeidae, Species: intermedia Ardeidae.

Description

The herons are long-legged freshwater and coastal birds in the Ardeidae family. There are 64 recognised species in this family. Some are called egrets or bitterns instead of herons. Within the family, all members of the genera Botaurus and Ixobrychus are referred to as bitterns, and including the Zigzag Heron or Zigzag Bittern are a monophyletic group within the Ardeidae. However, egrets are not a biologically distinct group from the herons, and tend to be named differently because they are mainly white and/or have decorative plumes. Although egrets have the same build as the larger herons, they tend to be smaller.

The classification of the individual heron/egret species is fraught with difficulty, and there is still no clear consensus about the correct placement of many species into either of the two major genera, Ardea and Egretta. Similarly, the relationship of the genera in the family is not completely resolved. However, one species formerly considered to constitute a separate monotypic family Cochlearidae, the Boat-billed Heron, is now regarded as a member of the Ardeidae.

METHODOLOGY

Materials and Methods (Technical Analysis)

The study was conducted with exploratory survey method that includes scope of the study all birds blekok ranging from small to adult was observed during the third time the survey began week I (May 14, 2011), Week II (May 21, 2011), and week III (28 June 2011) in the three hamlets in the village Bedono. Starting at 6 am to 6 pm special birds that live in trees blekok Avesinnea sp. The study was conducted in three hamlets, namely: Tambaksari, Senik, and Mandaliko. Population data collected from the initial licensing survey research. Blekok bird count data recorded in a standardized form on a regular basis and then calculating each location information is collected for analysis.

Generally stable overall since the immigration of a population (for example, may be due to the explosion of population). They also drain to the small population and save the population from extinction (Called the rescue effect). Metapopulation theory was first developed for Terrestrial Ecosystems, and then applied to the ocean realm. In fisheries science, the notion of "subpopulations" with metapopulasi scientific term "local population. Metapopulation theory was first developed for Terrestrial Ecosystems, and later applied to the ocean realm. In fisheries science, the notion of" subpopulations "with metapopulation "/ local population. Metapopulation developmental theory, associated with the development of the theory of the dynamics of "source-sink", giving more attention to the importance of the relationship the between the separate Populations. Although there is no population as an example of bird Populations in Bedono blekok surroundings.The classification of birds are as follows Blekok:

Results and Discussion

Observations showed that bird populations blekok in three hamletswere observed for 3 weeks starting May 14 until June 6, 2011 from 6 AM until 6 PM to get the following results; shown in table 1, table 2 and table 3.

Table 1: Results of Observations Number of Birds blekok (*Ardeola speciosa*) in the morning

No	Observasi	Tambaksari			Senik			Mandoliko			Time
		Three	Nest	Birds	Three	Nest	Birds	Three	Nest	Birds	
1.	I (14 Mei 2011)	245	1225	4025	296	1480	5480	173	863	2865	06 .00 – 08.00
2.	II (21 Mei 2011)	245	1214	4019	296	1480	5487	173	865	2869	06 .00 – 08.00
3.	III (28 Mei 2011)	245	1126	4011	296	1480	5489	173	867	2868	06 .00 – 08.00
	Average	**245**	**1169,5**	**4018,3**	**296**	**1480**	**5483**	**173**	**865**	**867**	

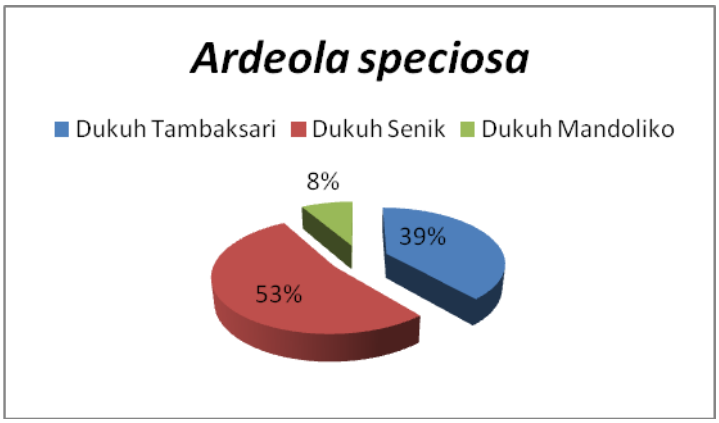
Table 2: Results of Observations Number of Birds Blekok (*Ardeola speciosa*) in the afternoon

No	Observasi	Tambaksari			Senik			Mandoliko			Time
		Three	Nest	Birds	Three	Nest	Birds	Three	Nest	Birds	
1.	I (14 Mei 2011)	245	1008	4025	296	1480	5480	173	763	865	16 .00 – 18.00
2.	II (21 Mei 2011)	245	1007	4019	296	1482	5482	173	690	869	16 .00 – 18.00
3.	III (28 Mei 2011)	245	1003	4011	296	1478	5481	170	680	768	16 .00 – 18.00
	Average	**245**	**1006**	**4005**	**296**	**1480**	**5481**	**172**	**711**	**767**	

Table 3: The Number of Bird Observation difference Blekok (*Ardeola speciosa*) in the morning and afternoon Elam three weeks (May 14 to 28)

No	Observation	Tambaksari			Senik			Mandoliko			Time
		Three	Nest	Birds	Three	Nest	Birds	Three	Nest	Birds	
1.	Average	**245**	**1169,5**	**4018,3**	**296**	**1480**	**5483**	**173**	**865**	**867**	06.00-08.00
	Average	**245**	**1006**	**4005**	**296**	**1480**	**5481**	**172**	**711**	**764**	16.00-18.00
	Defference	**0**	**63,5**	**13,3**	**0**	**0**	**2**	**1**	**154**	**103**	

Most birds are sequentially blekok in hamlet Senik, Mandaliko thenTambaksari. Metapopulasi not affected by the distance separating the place to another because it can still be affordable



Graphic 1 : : The Number of Bird *Ardeola speciosa*

Conclusion

Indicate that most bird populations are sequentially blekok inhamlet Senik, Mandaliko then Tambaksari. Metapopulasi not affected by the distance separating the place to another because itcan still be affordable.

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P-EC07

Distribution and abundance of ground vegetation in traditional gold mining area of Sekotong, West Lombok, West Nusa Tenggara

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Abstract

Gold mining activity often destroys tropical rain forest ecosystem. The area of Sekotong, West Lombok is one of the gold-rich region in Indonesia. Lately, an illegal traditional gold mining called PETI has appeared in this region. This mining uses mercury in its process. The Purposes of this research were to examine the distribution and abundance of ground vegetation in traditional gold mining of Sekotong, and study responses of ground vegetation communities to their environment. This research was conducted in 3 location of Sekotong; Simba reserved Forest, Selodong, and Pelangan. Ground vegetation communities were taken by using the random quadrat methods with unit 1 m x 1 m. Sixty nine of quadrat plots were placed in several regional studies with randomly stratified structure. In addition, it was also performed morphometric measurements of *Eupatorium inulifolium*, mercury content in the quarry of sluice box and also in *Eupatorium inulifolium* leaves. Measurement of several physico-chemical factors were also performed. The results of this research showed that grasses life form, species *Cynodon dactylon* dominated the quarry of gold's sluice box (*gelondong*) in Selodong (874 individu / 24 m^2), and species *Ischaemum* sp.2 dominated quarry in Pelangan (402 individu / 16 m^2). In otherwise, shrub life form, species *Eupatorium inulifolium* dominated in Simba reserved forest (706 individu / 22 m^2). The existances of mercury pollutant gives a sublethal effect for spesies which have mercury hyperaccumulator character.

Keywords : ground vegetation, life form, quarry, PETI, Sekotong

Introduction

Gold mining activity often destroys tropical rain forest ecosystem. The area of Sekotong, West Lombok is one of the gold-rich region in Indonesia. Lately, an illegal traditional gold mining called PETI has appeared in this region. This mining uses mercury in its process. According to Butler (2010), for extracting the gold from the big stone, it is used sluice box (*gelondong*) which contains mercury to collect the gold. In Indonesia, the exploration for gold mining are taking place by many companies such as PT Newmont in Sumbawa Barat, PT Freeport in West Papua, PT East Asia Mineral Corporation in Aceh and PT Indotan Inc. in West Lombok. The traditional gold mining is also being conducted in some areas of Indonesia such as Kalimantan, Aceh, and Lombok**.**

Sekotong rural area is an area that has been opened for the benefit of traditional gold mining. The process of succession is happening in this area, characterized by the turn of the life form of grass and herb into a shrub life form in simba reserve forest. Species that

dominate in simba reserve forest area is *Eupatorium inulifolium*. We generally studied the distribution and abundance of ground vegetation and the morphometric condition of the *Eupatorium inulifolium* species in particular. Because this species can quickly colonizes open areas. It has large leaves and high stem. Therefore, observations of sub lethal effects caused by mercury are easier than grass or herb life form.

A very high content of mercury can cause damage to the body of organisms within a specified period. Ground vegetation can accumulate mercury rapidly. Ground vegetation that is mercury hyper accumulator will be able to survive and distributed widely in the area of waste contaminated with mercury. So that the problems that arise in this study were as follows: How does the distribution and abundance of ground vegetation in the traditional gold mining area of Sekotong? What species is mercury hyper accumulator? How is the response of ground vegetation communities in Sekotong to their physicochemical environmental factors? Morphometric analysis conducted for the species found in the three study stations. How does mercury content relation to the morphometric of species of ground vegetation?

The Purposes of this research were to examine the distribution and abundance of ground vegetation in traditional gold mining of Sekotong, and to study responses of ground vegetation communities to their environment.

Materials and methods

We used some materials in this research: a unit 1 m x 1 m of square plot, herbarium kit, med line for measuring species morphometric, thermometer, soil tester, zip lock plastic, and rubber band. This research was conducted at 3 location of Sekotong; Simba reserve forest, Selodong, and Pelangan in October 2009. Ground vegetation communities were taken by using the random quadrat methods with unit 1 m x 1 m. Sixty nine of square plots were placed in several regional studies with randomly stratified structure. In addition, we also performed morphometric measurements of *Eupatorium inulifolium*, mercury content in the quarry of sluice box and also in *Eupatorium inulifolium* leaves. Measurement of several physicochemical factors such as soil temperature, air temperature, soil pH, and soil humidity were also performed. Species identification of ground vegetation communities were carried out by comparing the herbarium and species photo with picture from the identification book, Backer (1973) and Steenis (1975). After raw data tabulation, we analyzed the data for counting some vegetation parameters such as density, relative density, frequency, relative frequency, and importance value (Barbour, 1987, Krebs, 2009). To know the significance of 3 studies areas, we analyzed the data of total species density by using one way ANOVA in 95 % level of confidence.

Results and discussion

The results of this research showed that grass life form, *Cynodon dactylon* dominated the quarry of gold's sluice box in Selodong (874 individual/ 24 m^2), and *Ischaemum* sp.2 dominated quarry in Pelangan (402 individual/ 16 m^2). In otherwise, shrub life form, *Eupatorium inulifolium* dominated the Simba reserve forest (706 individual/ 22 m^2).

Grass life form can dominate the Sekotong region because the grass has a stolon, rhizome or tuber roots. Therefore, the grass has an effective vegetative reproduction. In addition, the grass generative reproduction equipment modified into a small seed form and lightweight making it easier blown away into the surrounding environment and even to other areas. Thus, the grasses can grow quickly and properly distributed. For example, *Cynodon dactylon* dominated Selodong station. This species can reproduce generative and vegetative as well. Generative with very large number of seeds, and fine hairs are easily flown, while vegetative by forming stolon (Johnny, 2006). *Ischaemum* sp.2 dominated the other ground vegetation In Pelangan because it has the highest importance value.

In contrast to the life form of grass and herb, shrub rarely has the rhizome so that reproduce lasts longer and is less effective. However, shrub life form dominated Simba reserve forest region compared to herb and grass. Shrub that dominated is *Eupatorium inulifolium*. Because this plant is included of the plants that have seeds which are easily carried by the wind so it can be distributed to a more distant area. In addition, this plant is invasive species that very quickly colonizes open areas, such as former forest fires, clearing of agricultural land, and former mining area. Simba reserve forest is one which was originally used as farm land by local community, but eventually abandoned because the inhabitant has turned work into gold miners.

Abundance of grass and herb life form in the Simba reserve forest area is lower than Selodong and Pelangan. Because the Simba reserve forest dominated by shrub that won the competition among the three life forms. The types of shrubs are found such as *Eupatorium inulifolium*, *Erigeron annuus*, and *Synedrella nodiflora.* They can quickly colonize the Simba reserve forests which were opened by shifting cultivation.

The test results of analysis of variance (ANOVA) showed that there are significant differences between the total density of species at three study stations. Because the Simba reserve forest has been invaded by *Eupatorium inulifolium* that dominate the area. The presence of this species is able to suppress the presence of other species through competition for space and resources around the forest. *Eupatorium inulifolium* are able to absorb nutrients from the soil quickly and reproduce very quickly as well so that pressing the presence of other species. Therefore, there are not many other species that can grow in Simba reserve forests.

There are three patterns of plant distribution: clumped, random, and regular. This distribution pattern can be caused by differences in physicochemical factors and biotic factors in an ecosystem. But in general, clumped distribution pattern is a pattern of distribution that often occurs in nature (Barbour, 1987, Indriyanto, 2006). Spread of ground vegetation species that commonly occur in areas of Sekotong are randomly. A species can be dispersed randomly if the species have seeds that are anemochory, so that new individuals can spread far from its parent. Besides the relatively similar environmental conditions, resulting in all species has the opportunity to grow in the region. Clumped distribution occurs less frequent in this area. However, species that dominate each station of studies such as *Cynodon dactylon* in Selodong, *Ischaemum* sp.2 in Pelangan, and *Eupatorium inulifolium* in reserve forests have a clumped distribution pattern. Actually, clumped distribution can increase intra species competition, but the disadvantages are often compensated by an advantage that the plants that grow in groups can organize microclimate around as needed (Indriyanto, 2006).

Factors that led to the dominance of some species is the ability to compete for resources in the vicinity, such as nutrient sources and physicochemical conditions, in this case are an appropriate with the air temperature, soil temperature, pH, and soil moisture. The research areas are located in the traditional gold mining Sekotong which largely determined by the presence of mercury as a pollutant. This pollutant interacts with other environmental parameters in determining the distribution and abundance of ground vegetation. *Eupatorium Inulifolium* is found most abundant in the Simba reserve forest area which has lowest average value of soil temperature and soil moisture, but with the highest average value of soil pH. While the most dominate species in Pelangan was classified as *Ischaemum* sp.2 (grass life form). The grass morphology is more adaptive to high temperature environments like in Pelangan. Selodong which has average value physicochemical factors between Pelangan and Simba reserve forests are dominated by *Cynodon dactylon*. *Eupatorium inulifolium* that grows in Simba reserve forest is capable to grow higher and develop faster than other species. This situation led to the formation of shade for other species as well as covering the soil surface, therefore the average soil temperature measured in Simba reserve forest is lower than Pelangan and Selodong that have more open conditions. The highest soil temperature measured at Pelangan because there is no shade formed by the shrubs. Then, the soil pH and soil humidity value in Pelangan and Selodong are influenced by exist of sluice box, which are not exist in Simba reserve forest.

Eupatorium inulifolium is mercury hyper accumulator because it can absorb mercury from soil and air more than the life form of grass or herb. The content of mercury in

Eupatorium inulifolium leaves found highest in Pelangan, reaching 2798.13 ppb. Mercury can inhibit photosynthetic reactions causing metabolic disorders. As a result, plants may become stunted, yellowing leaves, and stems are not sturdy. The existence of mercury pollutant gives a sub lethal effect for which has mercury hyper accumulator character. It can therefore be estimated that the area heavily contaminated with mercury will cause the mercury content of the leaves also become increasingly high, as happened in the Pelangan.

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P-EC08

The Relationship of The Reef Fishes to The Coral Reef Ecosystem in Tengah Island, Karimujawa National Park, Indonesia

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ABSTRACT

The Tengah Island is one of favorite islands in the tourism zone of the Karimunjawa National Park. Tourism activities in the island can become threats to the sustainability of the coral reef ecosystem there. The aim of this study is to determine the abundance and diversity of reef fish and their trophic, which is to know the recent condition of coral reef ecosystems in the Tengah Island. This research utilizes the Line Intercept Transect (LIT) and Visual Swim Survey methods. The transects are 50 meters in the first station, and 20 meters in the second station. In the Visual Swim Survey method, the distance of each surveyor is 5 meters. Sixteen families of reef fish are found in this research. The most abundant families were Pomacentridae, Labridae and followed by Chaetodontidae, while the lowest were Balistidae, Centriscidae, and Ephiphidae. Based on the trophic levels, omnivores consist of 35.22%, herbivores 28.08%, inverts benthic 21.18%, carnivores 7.64%, corallivore 4.93%, and planktivore 2.96%. The percentage cover of coral reefs in the first station is 58.46% and 100% in the second station. Both stations are dominated by the branched (ACB) and digitate (ACD) growthforms from the genus Acropora. From this research, it is assumed that the diversity of reef fish in the Tengah Island is dominated by the Pomacentridae family and the most trophic levels are herbivores and omnivores. These evidences indicate that the current condition of the ecosystem is still good.

Keywords: Coral Reef Ecosystem, Tengah Island, Reef Fish, Karimunjawa

1. INTRODUCTION

Coral reefs are unique ecosystems typical to tropical ocean. As an ecosystem, coral reefs are composed by several components, namely the biotic and abiotic components. Changes in one of the component will have an impact to the ecosystem. From those two components, the amount of biodiversity can be used as an indicator for the stability of the ecosystem. An estimated from 12,000 marine fish species, 7,000 species inhabit the coral reefs. These reef fishes has low mobility, thus they need coral reefs as refuge for their survival and sustainability. Association between reef fishes and coral reefs are very close, making reef fishes possibble as bioindicator for the condition of coral reef ecosystems.

Karimunjawa Islands are an archipelago which has beautiful coral reefs which is potential as tourism spots. One of them is the Tengah Island. Because of it's diverse coral reefs, the Tengah Island has been managed in the tourism zone of the National Park. However, tourism activity can be threat to the preservation of the biodiversity in the area.

The aim of this study is to determine the abundance and diversity of reef fish and their trophic, which is to know the recent condition of coral reef ecosystems in the Tengah

Island. This research is expected to provide information as a data source for parties involved in conservation efforts of marine organism as Indonesia's natural wealth.

2. MATERIAL AND METHODS

The study was conducted in July 2011 in the waters of the Tengah Island, Karimunjawa National Park. There are two observation sites which are selected through a survey in advance by seeing the diversity and density of coral reefs.

Table 1. The location and conditions during the observation station at the Tengah Island

St	Latitude	Longitude	Temperature	Salinity	pH
1	5˚48'24.783"S	110˚30'24.350"E	30,25˚C	1.02	8.6
2	5˚48'12.651"S	11˚30'4.138"E	30˚C	1.02	8.6

The tools and materials used in this research are Global Positioning System (GPS) life vests, snorkel and fins, 50 meters roll, handcounter, water-resistant synthetic paper, pencils, digital cameras with underwater casing, and environmental parameters equipment.

This study used Line Intercept Transect (LIT) method and visual swim survey method at a depth of 1-5 meters along the length of the transects, which are 50 meters at Station 1 and 20 meters at Station 2. In the visual awim survey, observations were made by 2 surveyors that swim along the transect line at each point. The distance between the surveyors on the visual swim survey is 5 meters. The abundance of reef fish families are counted with handcounter and the results are reported. Data processing was done with the software MS Excel 2007 to compare the diversity and abundance among reef fish families and percent cover of the coral reefs.

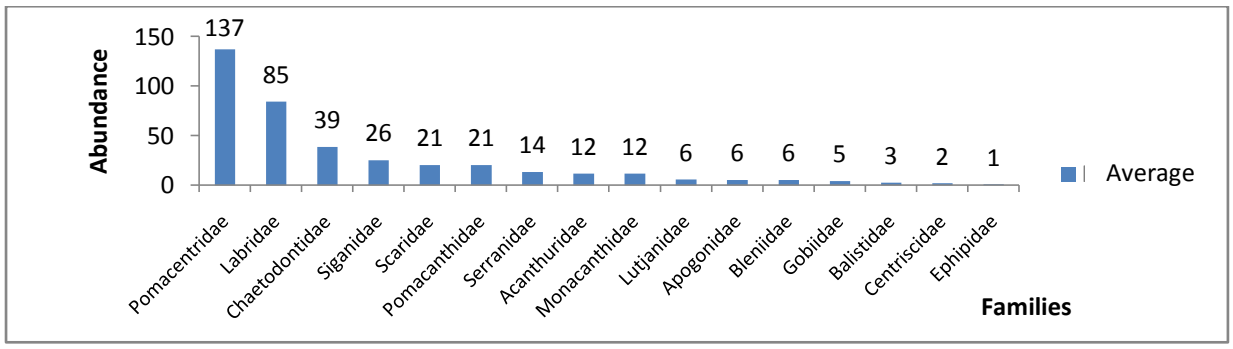
3. RESULTS AND DISCUSSION

The observations were made at a depth of 1-5 meters, with the assumption that reef fishes was the most abundant species as to the distribution of various types of coral growthforms. The diverse distribution of growhtform types, supported by the intake amount of sunlight, indicated that the environmental parameters in the waters was still considered good. Therefore, it becomes an ideal shelter for reef fishes [1]. From this research, sixteen families of reef fish were found in the Tengah Island. These results are based on the observed number of families referred from the National Parks Ecological Monitoring Karimunjawa phase 2, 2007 [2]. The most abundant families were Pomacentridae, Labridae and followed by Chaetodontidae, while the lowest were Balistidae, Centriscidae, and Ephiphidae (Figure 1).

Pomacentridae and Labridae family are reef fish members which is common to the coral reef ecosystem. From this observation, damselfishes (Pomacentridae) had average

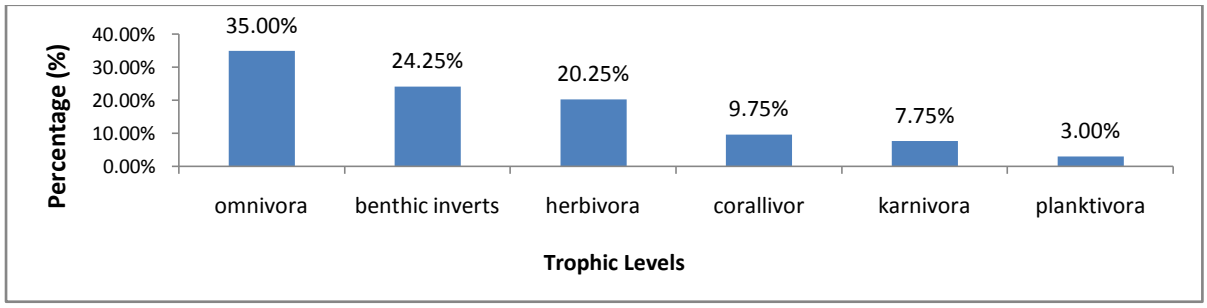
size less than 20 cm and the Labridae had an average size less than 40 cm. The fast reproduction cycle of the family Pomacentridae, approximately 20 days until larval stage, is very adaptive for it's population dynamics [3]. Different with the Pomacentridae, the Labridae are small "cleaner" fish (benthic inverts). The size makes the Labridae easier to hide from predators. This has allowed the two families to be the most abundant. As for the family Chaetodontidae (*kepe-kepe*), they were quite abundant in the Tengah Island for fish of this family are generally active during daytime and often found in shallow water with the depth of less than 18 m, which was in accordance with the observations that made at a depth of 1-5 m in daytime. The abundant amount of Chaetodontidae also due to their habits to form a large group while eating zooplankton.

Figure 1. Comparison of the average number of fish families in the Tengah Island.



In this study, the average size of the fishes observed were less than 1 meters. Families with large body size like the Acanthuridae, Caesionidae, Lutjanidae, and Serranidae were rarely encountered. This was because the observations were made in the region with high density of coral reefs and at low depth. At Station 1, the fish from the Family Lutjanidae were not found during data retrieval, but at Station 2, which are located deeper and farther from the coast, were found 12 fishes.

Figure 2. Comparison between the number of fish trophic groups



Based on the trophic levels, reef fishes in the Tengah Island can be divided into six trophic groups, which are carnivores, herbivores, planktivore, corallivore, omnivores, and benthic inverts. Omnivores consist of 35.22% which are the most abundant, herbivores

28.08%, inverts benthic 21.18%, carnivores 7.64%, corallivore 4.93%, and the least were planktivore 2.96% (Figure 2). Omnivores observed were mainly from the Family Pomacentridae. Members of this family diet on algae and scavenge on dead fishes remains [4].

Herbivorous fishes occupied the second highest trophic levels. Herbivorous fishes generally feed on algae (Grazer), which includes the *Ikan Semadar* (Acanthuridae), *Ikan Kambing* (Pomacanthidae), Parrot Fish (Scaridae), and *Ikan Beronang* (Siganidae). The grazing activity of Parrot Fish (Scaridae), as the largest component of herbivorous fishes, act to prevent excess growth of algae which can endanger the corals. The abundance of Parrot Fish are the main factors influencing the fish community structure due to it's grazing activity which can determine the condition of coral reef ecosystem [5]. The number of carnivorous fish found in this study was still in a reasonable amount, which has a quite far ratio with the herbivorous fishes. Carnivorous fishes have an important role to control the population of herbivorous fishes in the coral reef ecosystem. The population of coral-eating fishes (corallivore) was also still within normal range, which is less than herbivores and carnivores [5]. The number of plankton-eating fish (planktivore) were the lowest since the number of Caesionidae Family members (planktivore) were low as well. Benthic invertebrate eaters (benthic-invertivore) were the second most abundant. They were frequently found in the coral reef ecosystem. The members of this group were the Labridae Family which act as the "cleaner" fishes. These fishes ate the parasites in other fishes body surface [4].

The large portion of omnivorous and herbivorous fishes in the ecosystem, with fewer number of carnivores, showed that the ecosystem are still balanced and there are little mortality caused by parasites because of the benthic-invertivore abundance. The percentage cover of coral reefs in the first station is 58.46% and 100% in the second station, which are good. Both stations are dominated by the branched (ACB) and digitate (ACD) growthforms from the genus Acropora. The food pyramid composition and the density of the coral reef showed that the ecosystem is still in good condition. Although there were still coral reef found damaged by ship anchors, the problem has been done by active patrolling by rangers. There are also sanctions imposed on the ship that dropped anchor on the coral reef zones.

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P-EC09

Inventarization of Yeasts From Intestinal Tract of Gastropods Mangrove Forests of Rupat Island Riau

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not presented

P-EC10

GLASS EELS (*Anguilla* spp.) COMPOSITION AT THE ESTUARY ON THE ESTUARIES AT THE SOUTH COAST OF JAVA ISLAND

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ABSTRACT

The estuaries at the south coast of Java Island are the entrance of glass eels to the inland waters of Java Island. Until now, the information of the migration pattern of glass eel to the estuary of the estuaries is not yet available. This research aimed to determined the composition of glass eel were migrated at the estuary at south coast of Java Island. Glass eels were collected at the Cibuni, Bogowonto, Serayu, Cincinguling, Luk Ulo, Wawar, and Jali estuarine from March until July 2011 at night during the new moon. Glass eel identification was based on characters previously defined by Ege (1939), Watanabe et al., 2008; Tabeta et al.,1976; and Tabeta dan Ozawa, 1979. Among 6.382 specimens were collected, there are 3 species were identified. About 16,42% of the specimens were *Anguilla marmorata*, 82,96 % were *Anguilla bicolor bicolor*, and 0,62% were *Anguilla nebulosa nebulosa*. Peak of glass eel migration at the estuaries occured in May. The three species of glass eel migrated into the estuaries that have been sampled. *A. nebulosa nebulosa* migrated at estuaries only at March, whereas the other species migrated from March until July. There is a tendency of glass eel entering the estuaries in the west more than that goes into the river to the east.

Keywords: glass eel, *Anguilla*, Java, migration, estuary

P-EC11

Sea Turtle Hatchery in Trisik Beach, Yogyakarta: Current Report and Problematics

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ABSTRACT

Yogyakarta is a province of Indonesia that has many sea turtles breeding sites on its beaches but has a few sea turtle conservation activities. Trisik beach is frequently visited by sea turtles for landing and laying their eggs. Trisik beach also has a sea turtle conservation forum that manage sea turtle hatchery. The lack of informations and observations about sea turtle conservations activities in Trisik beach provide some dificulties to understand potentialities and problematics and also to make some improvement strategies. This research aimed to observe sea turtle hatchery problematics and potentiality in Trisik Beach and also to formulate problem solving strategies. Our research was carried out from April to August 2011 and continued until the end December 2011 for ensuring the sustainibility of research implementations. We used surveying, monitoring and interviewing methods to observed sea turtle hatchery activities in Trisik Beach. We also measured beach air temperature (29^0-35^0C) and humidity (48-76.5%), seminatural nest temperature (27.5^0-32^0C), humidity (15-25%) and acidity (pH 6.6-7.3), and the conditions of nursery water. The research results showed many problems during sea turtle hatchery activities in Trisik Beach in 2011. The main problems were generally classified as eggs robbery, low hatching success (50%), and high hatchlings mortality (16.51%). Then, we formulated the problem solving strategies and devided it into technicals and materials. Technical strategies included repairing the sea turtles hatchery techniques and make a guidance book of sea turtles hatchery procedure, while material strategies included improving facilities in eggs incubation aspect and nursery water filtration systems.

Keywords: Sea turtles hatchery, problematics, Trisik Beach, Yogyakarta

INTRODUCTION

All species of sea turtles reproduce on beaches around the world and migrate from foraging areas to mating areas, then the males return to the foraging areas while the females move to the nesting areas.[1,2,3] As a consequence of their food and habitat requirements, adult sea turtle are unevenly distributed troughout the circumglobal tropical and subtropical seas, included in Indonesia.[3,4] The sea turtle conservation global strategy, that addresses research, management, and conservation isues, required global, regional, and local conservation effort.[3,5] Yogyakarta is a province of Indonesia that has many sea turtles breeding sites on its beaches but has a few sea turtle conservation activities and scientific publications. Trisik beach is frequently visited by sea turtles for landing and laying their eggs. Trisik beach also has a forum, named Abadi sea turtles conservation forum, that manage sea turtle hatchery which is started in 2004. Informations and observations about sea turtle

conservations activities in specific beach, in our case is Trisik Beach, are very important to define potentialities and problematics and also to make some improvement strategies in local sea turtle conservation.[3] This research aimed to observe sea turtle hatchery problematics and potentiality in Trisik Beach and also to formulate problem solving strategies.

MATERIALS AND METHODS

Our research was carried out from April to August 2011 in Trisik Beach, Banaran Village, Galur, Kulon Progo, Daerah Istimewa Yogyakarta province. We involved local peoples, especially local hatchery volunteers, in this research. Surveying and monitoring of sea turtle hatchery activities were observed once a week. Data and informations about hatchery activities from previous year were collected from sea turtle hatchery data sheets that had been recorded by volunteers and also by interviewing the volunteers and the chairman of Abadi sea turtle conservation forum. Estimation of clutch size, hatching success, hatchlings mortality were carried out by using international standard formulas[6] with some modifications. Species identification of sea turtle hatchling used sea turtles identification key.[4,6,7] Persent Female was predicted by using indirect method (non invasive).[8,9] Informations about sea turtle conservation status were also collected from Balai Konservasi Sumber Daya Alam (BKSDA) Yogyakarta officers. We also measured weather conditions (temperature and humidity) of Trisik Beach, Semi natural nest conditions (temperature, humidity and acidity), and nursery water conditions. They were measured every two days start from the end of April until August 11, 2011.

RESULTS AND DISCUSSION

There are some indications of sea turtle conservation potentiality in Trisik Beach. One of them is enhancement of sea turtle nesting activities in Trisik Beach from 2004 to 2009 (Table 1.). Table 1 also discribe high hatchings success in that nesting periods. Clutches of sea turtles eggs typically have high hatching success (80% or more).[3] There are no certain nesting and hatching data in 2010, but the hatchery volunteers informed us that the number of eggs reach 1400 eggs and about 750 of them are successfully hatched. There are poor nesting activities in this year (until August 2011) which is only 2 to 3 nests in Trisik Beach. The more and detailed data are shown in table 2. The only 2 to 3 nests found in Trisik was caused by many problems. Based on information from local hatchery volunteers, the decrease of sea turtle nesting activies are caused by the season and the condition of sea this year. Their statement depends on Javanese calendar which indicate the beach has slow winds and waves and it is proved. Local knowledge is very useful for predicting nesting activities of sea turtles in certain beach, without ignoring the other aspects.[10] The increases

of artificial light and humans activities also have a contribution in lowering nesting activities in Trisik Beach.

Eggs robbery directly impact in number of eggs that are found and relocated in seminatural nest. Eggs robbery is caused by economical value of sea turtle eggs for consumptions, and it is common in worldwide.[11] Sea turtle hatchery volunteers in Trisik Beach always relocated sea turtle eggs in seminatural nest directly to decrease the impact of eggs robbery. The relocation of eggs in seminatural nest has some disadvantages and may brings another problems [3,6,9], but it is the best way to be implemented in Trisik Beach. The volunteers also persuade local peoples who have found sea turtles eggs to sell the eggs to them, but it is high in cost and sometimes does not work. Local government supports the sea turtle hatchery financial annually although it does not cover all off the operational cost.

Table 1. Nesting and hatching data of sea turtle hatchery in Trisik Beach

Years	N	E	HES	HS (%)	DH	HM (%)
2004	2	110	98	89.1	9	9.18
2005	5	517	495	95.74	25	5.05
2006	7	712	702	98.59	98	13.96
2007	8	720	706	98.05	29	4.1
2008	13	1352	1300	96.15	103	7.92
2009	17	1680	1587	94.46	261	16.44
2010	Unknown					
2011	2-3	264	132	50	35	26.51

Note: N is number of nest; E is number of eggs; HES is number of hatched egg shells; HS is hatching success; DH is number of dead hatchlings; HM is hatchlings mortality; data in 2010 were not documented well and some were missed.

Low hatching success and high hatchlings mortality are also found in this year (Table 2). Low hatchling success can be caused by improper treatment on eggs (include eggs collecting, transporting and handling), overheat, and the other factors.[3,6] The first clutch of eggs in seminatural nest was collected from two nests and brought by a local fisherman in bad conditions. They provide very low hatching success because improper treatments on eggs. The second clutch was collected from one nest and also brought by a local fisherman but treated more properly. Improper eggs treatments are still exist but little especially in eggs collecting, transporting and handling process. The relocation proccess in seminatural nest is by our guidance. They provide higher hatching success although can not reach 80% or more.

Table 2. Seminatural nest data of sea turtle hatchery in Trisik Beach, 2011

	CS	UE	HES	HS (%)	DH	HM (%)	Seminatural Nest			Beach	
							T (^{0}C)	Hum (%)	pH	T (^{0}C)	Hum (%)
1st Clutch	153	105	48	31.37	34	70.83	31.6	16.6	7.2	33.6	54.4
2nd Clutch	111	27	84	75.67	1	1.19	28.34	18.16	6.9	31.48	62.19

Note: CS is clutch size; UE is number of unhatched eggs; HES is number of hatched egg shells; HS is hatching success; DH is number of dead hatchlings; HM is hatchlings mortality; T is average temperature; Hum is average humidity; data of first nest were recorded from end of April to June 10, 2011 and seminatural nest parameters were measured at a 50 cm depth; data of second nest were recorded from June 23 to August 11, 2011 and seminatural nest parameters were measured at a 40 cm depth; The spesies of sea turtle in both first and second nest was *Lepidochelys olivacea*.

Hatchlings mortality is closely related to the condition of hatchlings and nursery. The hatchlings mortality is very high on the hatchling from first clutch. It is caused by bacteria infections. The hatchlings are only treat in bucket filled with seawater and the volunteers only change the seawater every two days. Uneaten food and hatchlings waste make the water conditions getting worse. Many opportunistic bacteria (*Vibrio, Flavobacterium etc.*) are naturally present in seawater and become pathogenic only when the animals are stressed, injured, or the environmental conditions are compromised.[12] The hatchlings from second clutch treat in aquarium with filtration and circulation system. The better nursery condition provides no hatchling mortality. The only one dead hatchling is found before nursery process (die after emerge from natural nest).

Air temperatures in Trisik Beach are varied in the range of 29 to 35^{0}C and can be lower at night (figure 1.a). Air humidity are also varied in the range 48-76.5% (figure 1.b). Temperature has such as a pervasive influence on the embryonic development of sea turtles. Clutch incubation temperature (natural or seminatural), beach temperature, and interaction between both of them are important to understand embryonic development of sea turtles, especially in hatching process and hatchlings sex ratio.[3] Seminatural nest temperatures are varied in the range of 27.5^0-32^{0}C with narrow fluctuations (figure 1.a). Seminatural nest humidities are varied in the range of (15-25%) with narrow fluctuations too (figure 1.b). The soil depth of seminatural nest keep the temperatures and humidities relatively constant. The soil acidity (pH) are also have narrow fluctuations in the range of 6.6 to 7.3. Average temperature and humidity in seminatural nest are different between first clutch and second clutch incubation (table 2). Both of them provide different hatchlings sex ratio (% female). The seminatural nest incubation of first clutch provide 60 % female (average incubation temperature are nearly above pivotal temperature) and the second

clutch provide 1.2 % female (average incubation temperature are widely below pivotal temperature of L. *olivacea*).

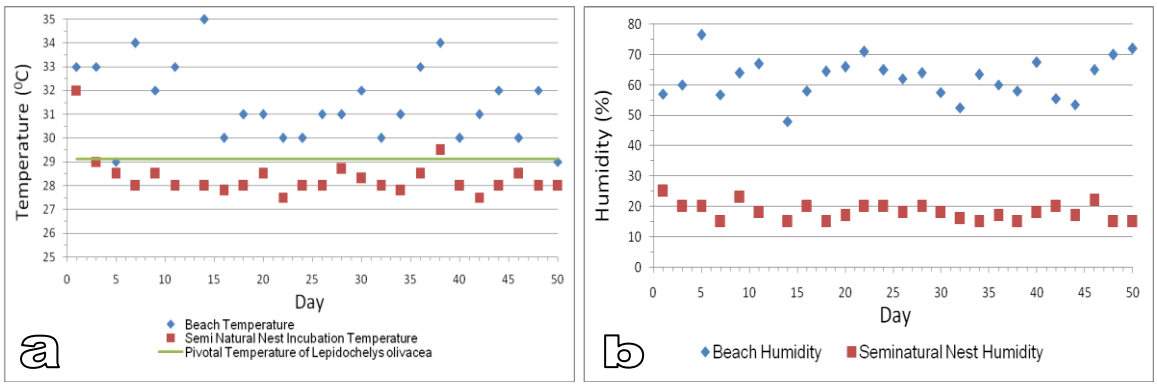

Figure 1. Average daily temperature (a) and humidity (b) of beach and seminatural nest from June 23 to August 11, 2011 in Trisik Beach. Seminatural nest humidity was measured every two days at a 40 cm depth.

The main problems of sea turtle hatchery in Trisik Beach recently were generally classified as eggs robbery, low hatching success and high hatchlings mortality. The short-term problem solving strategies need to be formulated. The problem solving strategies can be devided into technicals and materials. Technical strategies included repairing the sea turtles hatchery techniques and make a guidance book of sea turtles hatchery procedure, while material strategies included improving facilities in eggs incubation aspect and nursery water filtration systems.

Acknowledgment

We would like to thank to I-MHERE Project Faculty of Biology Universitas Gadjah Mada for funding this research. Many thanks to Abadi Sea Turtle Conservation Forum in Trisik Beach, Mr and Mrs Jarnudji, Dwi, and Joko Samudro for their immense contributions to this research. Many thanks to the Government of Daerah Istimewa Yogyakarta and Kulonprogo, and BKSDA for permitting this research. Also, thanks to Herpetology Study Club Faculty of Biology Universitas Gadjah Mada, the staff and students at Laboratorium of Animal Anatomy Faculty of Biology Universitas Gadjah Mada, and Prof. (ret) Dr. Nyoman Puniawati Soesilo, SU for their ongoing support.

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P-EC12

CONCENTRATIONS OF MERCURY IN GASTROPODS AND MUDSKIPPERS AT PELANGAN RIVER, SEKOTONG, WEST LOMBOK, INDONESIA

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ABSTRACT

A traditional gold mining there is in the district of Sekotong, West Lombok, NTB (West Nusa Tenggara); especially at southern area. One of a few rivers there is Pelangan River, which ending in the Lombok Strait. There were more than 7,000 peoples as miners and operators. The objectives of this research were found concentration of mercury in gastropods benthic in the length of river and in mudskippers at Pelangan estuary. The mercury contents to analysis for waters and substrates. The mercury sampling was conducted from five locations (up-stream until down-stream). The mercury concentration in gastropod at PS-3 (0.70055 ppm, highest) and PS-5 (0.00015 ppm, lowest); mudskippers (PS-5: 0.0567 ppm); sediment: 3.48 ppm; and waters: 0.00129 ppm. This is an environmental in dangerous condition because according to the government standard the limit of the mercury content was 0.001 ppm.

Keywords: gold mining, mercury, gastropod, mudskipper, Pelangan river

Introduction

A tropical stream characteristic can be used to define the way in which its can respons to human activities (Ramirez et al., 2008). A traditional gold mining there is in the district of Sekotong, West Lombok, NTB; especially at southern area. One of a few rivers there is Pelangan River, which ending in the Lombok Strait. There were more than 7,000 peoples as miners and operators. The objectives of this research were found concentration of mercury in gastropods benthic in the length of river and in mudskippers at the Pelangan estuary. Contamination of lead and alloy can be rise few problems of environmental pollution; other problem is mercury waste. Nico and Thamporn said, from gold-mining operations has impacted stream fishes (Winemiller et al., 2008).

An-organic and alkyl mercury was distributed in an animal tissues eq. brain and kidney. Mercury was chelated in sulf-hydril compounds and influenced in the cellular enzymatic systems. There is strong composition between alkyl-mercury and carbon-mercury and accumulation in the centre of nervous system. In the blood circulation, highest absorbed in red blood cells. Both an-organic and organic mercury can be transport by erythrocytes barrier in encephalon and placenta, and can secretion by breast milk. Biological and environmental correlates of water quality and against set standards (Norris & Georges, 1992).

Materials and Methods

Sampling site

This study was conducted at Sekotong, West Lombok, NTB, Indonesia. South Sekotong landscape is hilly and undulating geomorphology. Elevation of south Sekotong more than 70 meters above sea level. There are three rivers: Selodong, Blongas, and Pelangan. The end of the Blongas and Selodong rivers was to Sepian Gulf at south-eastern of Sekotong district; while Pelangan riverin into western (Lombok Strait). There are fisherman villages, secondary forest, mixture plantations, and deforestation areas.

Research object and sediment collection

The mercury sampling was conducted from five locations (up-stream until down-stream). Five locations were choose based on the amalgam processing activities presence a length the river and the characteristic gradient of water quality (Somlyody et al., 1983 cit. Devai, 1990), in October 2009. Gastropod was sampled at location-1 to 5 (up-stream to down-stream); but mudskipper was sampled at estuary only. Each location was sampled by using a quadrat plot 0.5 x 0.5 meter and purposive random sampling. Mudskipper was sampled by gill-net crossing Pelangan river. Sediment was taken with a simple gravity stainless-steel core 75 cm length and 2 inches in diameter. Concentration of mercury was measured for water and sediment.

Result and Discussion

The results of this research shown in Table 1., there are differences on concentration of mercury in gastropods among station each others.

Table 1. Concentration of mercury at Pelangan river, Sekotong, West Lombok

Location	Human activities	Concentration of mercury (ppm)		
		Gastropod	Sediment	Water
PS-1	No amalgam processing	0,02855	1,60	0.00047
PS-2	Little amalgam processing	0,12989	1,59	0.00325
PS-3	More amalgam processing	0,70055	2,83	0.00154
PS-4	Most amalgam processing	0,00122	1,86	0.00077
PS-5	Estuary, no processing	0,00015	3,48	0.00129

The mercury concentration in the waters at PS-1 (0.00047 ppm); PS-2 (0.00325 ppm); PS-3 (0.00154 ppm); PS-4 (0.00077 ppm) and PS-5 (0.00129 ppm). There is unusually PS-2 the highest than others, especially PS-3 and PS-4 maybe it's depend on

concentration of mercury in sediment makes influence to water surfaces. Nature of mercury will be going down to sediment because of gravitation. Beside that, there was water current influences to concentration of mercury both in the water and sediment. So accumulation in gastropod follows concentration in sediment because of as habitats.

Mercury contents in mudskippers was 0.0567 ppm); sediment: 3.48 ppm; and water: 0.00129 ppm. This is a environmental in dangerous condition because according to the government standard the limit of the mercury content was 0.001 ppm. Because its mostly toxic so U.S. Food and Administration (FDA) was limited concentration of mercury in the tissue of aquatic organisms not more than 0,005 ppm (Walter et al., 1973). The concentration of mercury 0.002 ppm in water bodies save in fishery activities but not for consumption (Anonimous, 2002).

Pollutans especially heavy metals can be makes accute and cronical diseases to fish and aquatic macro-invertebrates (snails, worms, insects, etc). Toxical condition will be rise a genetic and teratogenic effects to aquatic organisms. This is a environmental in dengerous condition because according to the government standard the limit of the mercury content was 0.001 ppm.

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P-EC13

QUALITY OF PELANGAN-SELINDUNGAN RIVER AS MERCURY WASTE DISCHARGING AREA OF GOLD PROCESSING IN SEKOTONG, LOMBOK BARAT

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not presented

P-EC14

EVALUATION OF SELODONG RIVER QUALITY AS WASTE DISPOSAL AREA OF TRADITIONAL GOLD PROCESSING IN SEKOTONG, LOMBOK BARAT

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not presented

POSTER - TOPIC 3

Systematic and Evolution (O-SE)

ICBS 2011

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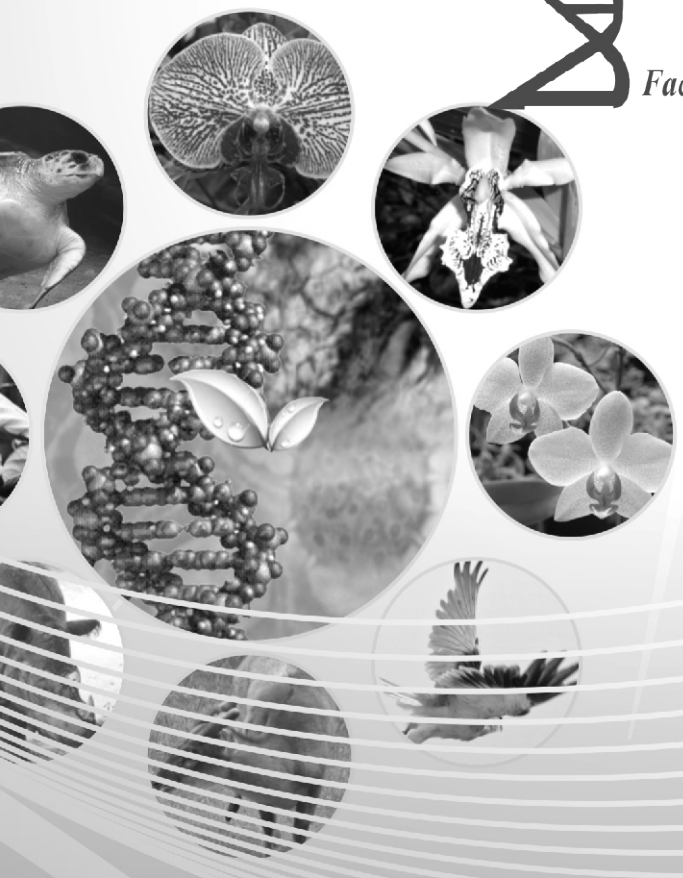

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

P-SE01

A COMPARATIVE STUDY OF STEK GROWTH OF *Tetrastigma glabratum* CULTIVAR THROUGH EX- SITU AND IN-SITU

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ABSTRACT

The aim of this study is to determine the differences of *Tetrastigma glabratum* in growth by cuttings, using Completely Randomized Design (CRD) with 3 treatments. In the cuttings, there are three terms used: SU for top cuttings, ST for central cuttings, SP for section base cuttings. *T.glabratum* are planted on 5 media containing a mixture of soil, sands and organic fertilizer with a ratio of 3:1:6. The five planting media are divided into A = manure, B = compost, C = Vermi Compost Fertilizer, D = Urea, and E = No fertilizer / Control. The result shows that the growth of *T.glabratum* meets the significant effect on all aspects which have been observed. Those are the first shoots appear, the number of shoots, root length, and live presentations. *T.glabratum* in situ growth is faster than the cuttings by eksitu. From the observation it is known that ST is growing better than SU and SP.

Keywords: Growth, Cuttings, Tetrastigma

INTRODUCTION

Walikadep (Tetrastigma glabratum Blume Planch) is a type of vines that live in protected forest areas in the district of Mount Prau Kendal. It is believed that the plant have the benefit as herb to treat some diseases. The part of the plant that is often used as the herb or medicine is the water produced by the trunk (Supangat 2009). According to K. Hyne (1987) in his book *Tumbuhan Berguna Indonesia* said that the descriptions of the plants are: shrubs that climb the length 10-20 m found mountain area with an altitude of 1600 m + dpal (from sea level). The fluid produced by the plant's refractive used as a cough medicine. The leaves are highly preferred as a replacement for "zurig" (Oxalissp.) Meanwhile, the Bogor Botanic Gardens (2010) categorizes the plant into Vitaceae Familia / Tetrastigma, Tetrastigma Genus, Species Tetrastigma glabratum(Blume) Planch.

The existence of walikadep in mount Prau is now rarely found. This is caused by the exploitation. Therefore this research is conducted to know the population of Walikadep in Gunung Prau. This research was conducted with the aim to: (1) obtain information about aspects of plant ecology of Walikadep, (2) analyze the factors threatening the population, (3) evaluate the social, economic and health, and (4) analyze the implications for the environment ecosystem (ex situ and conservation in situ).

METHODOLOGY

The method used in this research is pure experiment. Walikadep is planted by cuttings, using Completely Randomized Design (CRD). Each planting are given three treatments. In the cuttings, there are three terms, namely: Cuttings Edge section = SU, Central Cuttings= ST, and Cuttings section Jetty = SP. Walikadep planted on 5 media containing a mixture of soil, sand and organic fertilizer with a ratio of 3:1:6. Five planting medium is then called the medium A = Manure, medium B = compost, medium C = Vermi Compost Fertilizer, medium D =Urea, and medium E = control (no fertilizer). Planting walikadep with the in-situ performed in Prau mountain forests, and ex-situ growth in green house in the village Blumah which is the nearest village from Mount Prau forest. This research was conducted in 5 months, from January to May 2011.

DISCUSSION

After the five-month experiments, the five cuttings of walikadep showed different growth by ex-situ and In-situ.

Table 1. The Differences of Tetrastigma glabratum Growth by Ex-situ and in-situ for 5 months (January-May 2011)

No	Growth	In-situ	Per Month	Ex-Situ	Per Month
1.	Height	2,3 m	0,4 6 m	1,44 m	0,28 m
2.	Diameter	0,2	0,04	0,1	0,02meter
3.	Leaf	16,3	3,2	34,6	6,81 Tangkai
4.	Living(%)	100%	100%	75%	75%
5.	Shoots first	3 minggu	-	1bulan	-
6.	Biomassa	0,25 kg	0,50 kg	0,8kg	0,36 kg
7	Fertilizer	Casting		Casting	

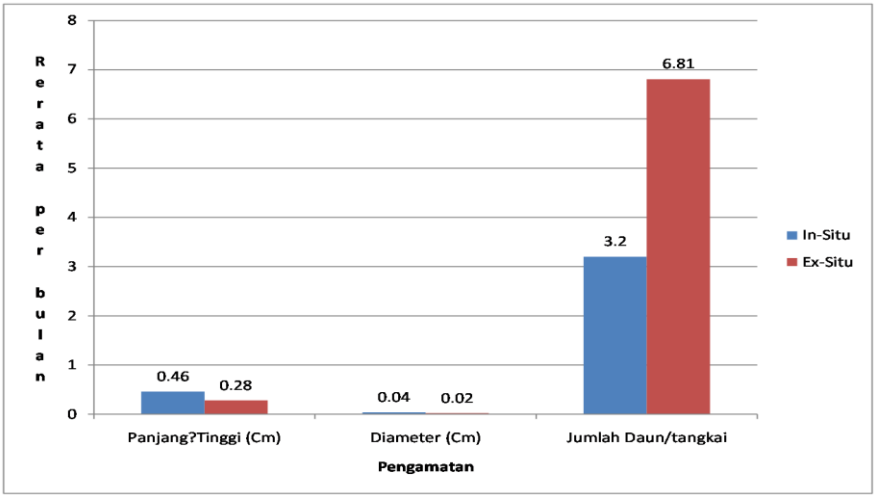

Figure 1. The Differences of Tetrastigma glabratum Growth in Ex-situ and in-situ for 5 months (January-May 2011)

The differences of Tetrastigma Glabratum growth between insitu and exsitu were clearly visible on the graph above. In in-situ, the stem length or height grows faster when compared with Ex-situ. For the five months, the plant grew with P = 2.3, 0.2 m diameter, leaf number 16, totaling 3 stalks of plants. From these data known that stem height growth each month with average of 0.46 meters. It is estimated that the length of plants in one year could reach 5.52 meters. In the next 10 years could reach 55.2 m and could be longer or higher than trees and could covered them then become a canopy.

Normally, these plants can grow rapidly, but its existence is rarely found as a result of excessive exploitation. Moreover the plant growth depends on such environmental parameters like temperature, altitude, humidity, light intensity, and hormones. Natural factors also influence the amount and growth of walikadep, such as landslides, erosion, forest fires, etc.

CONCLUSION

The results revealed that the growth of Tetrastigma glabratum has a real impact on all observed aspects such as the first emerging buds, the number of buds, stem height, stem diameter, biomass, and live presentations. Tetrastigma Glabratum which was planted in insitu cuttings grew faster than exsitu cuttings. The observation showed that ST (Middle Cuttings) could more survive than the SU (CuttingsEdge) and SP (Cuttings Jetty). Likewise the ST grew faster than in SU and SP. The best planting medium is the planting medium C (vermi compost).

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P-SE02

Diversity of Hermit Crabs (Crustacea: Decapoda: Anomura: Paguroidea) in Coastal Area of Gunung Kidul Regency, Yogyakarta, Indonesia

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ABSTRACT

Coastal Areas of Gunung Kidul Regency, Yogyakarta, Indonesia has sandy and rocky shores with strong waves, but some place calmer. The calm zone has seagrass bed (*Thalasia hemprichii*) beside seaweed bed. Seagrass and seaweed systems are important habitat for early life stages of many important species that seek protection from predators. These types of habitats provide a variety of ecological niches for a large number of decapods crustaceans including the hermit crabs, but there is no publication about species of hermit crabs in that location. Study was conducted to identify species of hermit crab in coastal area of Gunung Kidul Regency, carried out in June 2011. Samples were freely collected from Ngobaran beach, Nguyahan beach, Drini beach, Kukup beach, and Sepanjang beach. Cumulatively, Eight (8) species representing 3 families were discovered: *Aniculus ursus* (Olivier, 1812), *Calcinus elegans* (H. Milne Edwards, 1836), *C. latens* (Randall, 1840), *C.laevimanus* (Randall, 1840), *C. morgani* (Rahayu & Forest, 1999), *Clibanarius virescens* (Krauss, 1843), and *Dardanus megistos* (Herbst, 1804) which belong to *Diogenidae*; *Pagurus minutus* (Hess, 1865) which was a member of *Paguridae*.

Keywords : Crustacea, Hermit Crab, Gunung Kidul.

INTRODUCTION

Hermit crabs belong to Crustacean group. These carry and living in gastropod shell to survival and protect their soft-abdomen (Vance, 1972). Hermit crabs have asymmetry abdomen and uropodal rami not forming a tail-fan. 1st Pereopods are chelipeds, pereopods 2 and 3 were used to moving. Fourth pereopods is reduced and protected in the gastropod shell (Poore, 2004). None of the species, other than *Birgus latro*, have any fishery value, although many species of hermit crabs are regularly collected for the pet trade (Carpenter and Niem, 1998).

Hermit crabs play an important role in the marine trophic chain. Hermit crab acts as a detritus-scavenger with a consuming organic material remains submerged beneath the waters or a pictorial on the coast. Ability to adapt is very well made it can able to live in different types of habitats and ecosystems (Zaldi, 2009). Because of their diversity and their unique adaptations, these animals were interesting for observation.

Coastal area of Gunung Kidul Regency is naturally ecosystem which has high biodiversity. It has sandy and rocky shores with strong waves, but some place calmer. The calm zone has seagrass bed (*Thalasia hemprichii*) beside seaweed bed (Tanjung *et al.*, 2010). Seagrass and seaweed systems are important habitat for early life stages of many commercially important species that seek protection from predators (Humm, 1964). These

types of habitats provide a variety of ecological niches for a large number of decapods crustaceans including the hermit crabs (Moradmand and Alireza, 2007). Although Indonesian littoral hermit crabs are fairly well studied (Rahayu & Forest, 1992; Rahayu, 2003, 2005), but there have no record. So the purpose of this study was to determine the type of hermit crabs that live in coastal areas of Gunung Kidul and made a species list.

MATERIAL AND METHOD

Study was conducted in monthly June 2011, along coastal area of Gunung Kidul Regency at 5 beach (Fig.1). These localities are: Nguyahan beach (8°7'7.12"S, 110°30'9.90"E), Ngobaran beach (8°7'10.13"S, 110°30'18.25"E), Sepanjang beach (8° 8'12.88"S, 110°33'57.55"E), Drini beach (8°8'17.98"S, 110°34'41.48"E).

Figur 1. Sampling localities along coastal areas of Gunung Kidul Regency

Specimens were collected by direct hand picking at low tide from sandy and rocky beach. Collection conducted at night days. The first step to identify hermit crab was pulling it out from the shell by fire method and put it into salt water. Secondly, each species was photographed and released back after returning into their shells. Identification process conducted at Faculty of Biology, Gadjah Mada University based on morphology (Poore, 2004; Carpenter and Niem, 1998, Haig and Ball, 1988). Identification results were verified using web World Register of Marine Species (Türkay, 2011).

RESULT

In this section, a brief description on live coloration of each species, claws size, and habitat types are presented.

Diogenidae Ortmann, 1892

Aniculus ursus (Olivier, 1812)

Live coloration – Charapace green stripes, red lines and white lines. Occular peduncles white with red ring medially; cornea black. Antennules peduncles green; flagellum orange. Antennae all green. Chelipeds terminated by a black claw, have green stripes in red lines and yellow silks; full with red setae. Merus and carpus of pereopods 2 and 3 terminated by a black claw, have green stripes in red lines and yellow silks; carpus survace have red color; full with white and red setae.

Claws size – Left bigger.

Habitat – This species was collected in rocky beach with algae.

Records in the region – Ngobaran Beach and Nguyahan Beach

Calcinus elegans (H. Milne Edwards)

Pagurus fasciatus Bell, 1853

Live coloration – Carapace white with brown spots. Ocular peduncles bright blue with narrow black area at base; cornea black. Chelipeds dark brown with white tubercles on fingers and distal part of palm. Antennules and antennae all orange. Merus and carpus of pereopods 2 and 3 bright blue in proximal and black in distal. Propodus bright blue at both ends with black band medially. Dactyl bright blue with black spots.

Claws size – Left bigger.

Habitat - This species was collected in the shallow subtidal with algae.

Records in the region – Sepanjang Beach and Drini Beach.

Calcinus latens (Randall, 1840),

Pagurus cristimanus H. Milne Edwards, 1848
Calcinus intermedius De Man, 1881
Calcinus terrae-reginae Haswell, 1882
Calcinus abrolhensis Morgan, 1988 {6}

Live colouration – Carapace dark greenish, shading off to mottled white. Ocular peduncles pale pink with black cornea. Basal segment of antennular peduncles blue but distal segment orange; flagellum orange. Antennal peduncles green with yellow flagellum. Basal chelipeds dark greendish, gradually graying to white distally; fingers white. Merus and carpus of pereopods 2 and 3 dark green; propudus much lighter brownish purple proximally and white distally; dactyl brown proximally and white distally with setae.

Claws size – Left bigger

Habitat – This species was collected in rocky beach with algae.

Records in the region – Sepanjang Beach and Drini Beach.

Calcinus laevimanus (Randall, 1840)

Pagurus tibicen H. Milne Edwards, 1836 (preoccupied name)
Pagurus lividus H. Milne Edwards, 1848
Calcinus herbstii De Man, 1887

Live coloration – Carapace greenish white or gray green. Basal ocular peduncles brown; ocular peduncles orange with bright blue proximally; cornea black. Antennules blue except for narrow orange band at distal end of basal article; flagellum orange. Antennal peduncles with basal segment blackish green, rest of peduncle and flagellum orange. Chelipeds dark brown; fingers and distal part of palm of left cheliped with varying amounts of white. Pereopods 2 and 3 with ground colour of merus and carpus golden brown with dark brown longitudinal stripe, propodus brown; dactyl white, with dark brown subdistal ring and subproximal spot.

Claws size – Left bigger

Habitat – This species was collected in rocky beach with algae.

Records in the region – Nguyahan Beach, Ngobaran Beach, Sepanjang Beach, and Drini Beach.

Calcinus morgani (Rahayu & Forest, 1999)

Calcinus areolatus Rahayu & Forest, 1999

Live coloration – Charapace white to gray. Ocular peduncle dark brown proximally, distal half blue, narrow blue dark ring close to cornea; cornea black. Distal antennular peduncle and basal segments black; flagella yellow. Distal antennal peduncle all yellow. Chela all dark brown with narrow white area distally. Pereopods 2 and 3 dark brown with narrow white area distally.

Claws size – Left bigger

Habitat – This species was collected in rocky beach with algae.

Records in the region – Sepanjang Beach and Drini Beach

Clibanarius virescens (Krauss, 1843)

Clibanarius philippinensis Yap-Chiongco, 1937

Live coloration – Charapace shades of dark and light brown. Ocular peduncles solid olive drab, except for narrow white ring just proximal to cornea; cornea black with white spots. Antennular peduncles with basal segment olive drab or dark brown, terminal segment olive drab or dark brown fading to orange distally; flagellum orange. Antennal flagella uniform blue. Chelipeds olive drab or brown with white tubercles and white fingers. Pereopods 2 and 3 olive drab with darker band distally of propodus; dactyl white with setae.

Claws size – Equal

Habitat – This species was collected in rocky beach with algae and sandy beach.

Records in the region – Drini Beach, Sepanjang Beach.

Dardanus megistos (Herbst, 1804)

Cancer megistes Yap-Chiongco in Estampador, 1937
Cancer megistos Herbst, 1804
Dardanus megsitos Ward, 1942
Dardanus spinimanus H. Milne Edwards, 1848
Pagurus megistos Herbst, 1804
Pagurus punctulatus Olivier, 1812
Pagurus spinimanus H. Milne Edwards, 1848

Live coloration – Charapace orange-red with many white spots ringed with black. Ocular peduncles solid reddish except for narrow gray ring just proximal to cornea; cornea dark brown. Antennular peduncles and flagella uniform red. Antennal peduncles red; flagella uniform white. Cheliped, pereopods 2 and 3 all red with white spots ringed with black; red setae in the middle of the spots.

Claws size – Left bigger

Habitat – This species was collected in rocky beach with algae.

Records in the region – Nguyahan Beach and Ngobaran Beach.

Paguridae Latreille, 1802

Pagurus minutus (Hess, 1865)

Eupagurus dubius Ortmann, 1892
Pagurus dubius (Ortmann, 1892)

Live coloration – Charapace yellow with red-brown 2 longitudinal stripes. Each lie have spots medially. Occular peduncles white with red ring medially; cornea black. Antennules and antennae all yellow. Chelipeds greenish yellow, palm with red spot surface; full with setae. Merus and carpus of pereopods 2 and 3 greenish yellow, merus survace with red spot; propudus red proximally and distally.

Claws size – Right bigger

Habitat – This species was collected in rocky beach with algae.

Records in the region – Drini Beach

DISCUSSION

The hermit crab of Gunung Kidul Regency comprises mainly specis with widespreas distribution in the Indo-West Pacific region (WIWP). This is caused the location belong to Indonesia country.

Table 1. Species recorded from coastal areas of Gunung Kidul Regency

	Nguyahan	Ngobaran	Sepanjang	Drini
Aniculus ursus	+	+		
Calcinus elegans			+	+
C. latens			+	+
C.laevimanus	+	+	+	+
C. morgani			+	+
Clibanarius virescens			+	+
Dardanus megistos	+	+		
Pagurus minutus				+
Total species	3	3	5	6

All samples were collected from 4 beaches that have easy access. Cumulatively, 8 species collected during the study. Quantity is not recorded, because the weather was impossible so just did the recording of species found in the coast. Six species found in Drini, five in Sepanjang, and each tree species in Nguyahan and Ngobaran. Nguyahan-Ngobaran and Sepanjang-Drini share the same species of Diogenidae, it is demonstrating that adjacent beaches have the same kind species. All species, associated with seaweed in submerged areas. It is caused the fragments of algae are the mainly diet for hermit crab.

Drini and Sepanjang beach has higher number of species because the beach has large intertidal zone with large masses of seaweed and seagrass. Both of beaches also provide many gastropod shells. In second hand, Kukup has less number of species because has few intertidal zone.

Calcinus laevimanus was collected from all beach. This species lived in rocky beach with algae. Interestingly, Pagurus minutus which collected in Drini beach, was not found in other beach. It may be caused by habitat suitability of Drini Beach for Pagurus minutus.

In summary, ten hermit crabs found in the coastal area of Gunung Kidul Regency, Indonesia. Their geographical distribution is under effect of their habitat preferences. Zoogeographically, most species of the present study show the WIWP pattern of distribution. Further studies should be carried out fortaxonomic and zoogeographical analyses of hermit crabs of the region. Further investigation in same and other location also no doubt reveal the existence of even more species than those reported here.

ACKNOWLEDGEMENTS

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P-SE03

MORFOLOGICAL CHARACTERISATION AND IDENTIFICATION OF FUNGI THAT CAUSED SAPROLEGNIASIS ON THE GORAMY (*Osphronemus goramy* Lac.)

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ABSTRACT

Saprolegniasis is a commonly problematical fungal diseases on the freshwater-cultured fish. Goramy (*Osphronemus goramy* Lac.) is the prominent freshwater-cultured fish in Indonesia. Goramy frequently infected with saprolegniasis. Saprolegniasis is decreasing the productivity of the Goramy cultured, so control of saprolegniasis must be done. Characterization and identification of the fungi caused saprolegniasis can be help to controlled this disease. The identification of the fungi causes saprolegniasis on the goramy has not done yet. Based on the problem, a study to characterize and identify the fungi cuases saprolegniasis by the morphological characteristic of the fungi on the goramy had been studied. The aimed of the research was to study the morphological characters of the fungi that caused saprolegniasis on the goramy. Characteristic of the somatic structures, asexual characters, and sexual characters were identified. The isolates were obtained from saprolegenisis lesions on the goramy. The fungi were isolated by inoculating sample onto glucose-yeast extract agar (GY agar), GY broth, and hemp seeds-sterilizing tap water media. Characterization and identification resulted two fungal isolates from goramy, and identified as *Achlya heterosexualis* dan *Saprolegnia diclina* type 3.

Keywords: characterization, saprolegniasis, goramy,

INTRODUCTION

Saprolegniasis is a commonly problematical fungal diseases on the freshwater-cultured fish. Goramy (*Osphronemus goramy* Lac.) is the prominent freshwater-cultured fish in Indonesia. Goramy frequently infected with saprolegniasis. Saprolegniasis is decreasing the productivity of the Goramy cultured, so control of saprolegniasis must be done. Characterization and identification of the fungi caused saprolegniasis can be help to controlled this disease. The identification of the fungi causes saprolegniasis on the goramy has not done yet. *Aphanomyces invadans* had identified from saprolegniasis lession on goramy in Thailand[1]. Based on that problem, a study to charactersize and identify the fungi cuases saprolegniasis by the morphological characteristic of the fungi on the goramy had been studied.

MATERIALS AND METHODS

The isolates were obtained from saprolegenisis lesions on the goramy. The fungi were isolated by inoculating sample onto glucose-yeast extract agar (GY agar)[2,3,4], GY broth[2,3,4] and hemp seeds-sterilizing tap water media[3,4]. The fungal isolates then characterisize and identified. Characteristic of the somatic structures, asexual characters, and sexual characters were identified.

RESULTS AND DISCUSSION

Characterization and identification resulted two fungal isolates from goramy, and identified as *Achlya heterosexualis* dan *Saprolegnia diclina* type 3. Based on somatic structures, asexual characters and sexual characters, the isolates were description.

1. Description of *Achlya heterosexualis* isolate.

   Monoecious or dioecious; cylindrical, clavate, or fusiform zoosporangia; *renewal* of secondary zoosporangia are *sympodial* and / or *cymose*; zoosporangial discharge are *achlyoid*; *monoplanetic* zoospore; 7,5-9,5 µm in diameter of zoospore cyst; *fusiform, cylindrical, clavate*, or *irregular* gemma ; single or catenulate gemma; >1 week on timing of gemma shaped; spherical, subglobose, pyriform, dan clavate oogonium; oogonial origin are lateral, terminal, or intercalary; 52,5 µm - 100 µm on diameter of oogonia; 20 µm - 500 µm on the length of oogonia stalk; a few of oogonia were sessile; inner wall of oogonia are *smooth*; *oogonial pitting* are under the antheridium appresion on oogonium wall; 3-18 oospores in an oogonium; 17,5 µm-50 µm in diameter of oospore; *Oospore centricity* are subcentric (type I and II) and eccentric; *Antheridial origin* are diclinous and hypogynous; there are fertilization tube from antheridium to oogonium; methodes of antheridium appresion on oogonium are *apical* and / or lateral (fig. 1)

2. Description of *Saprolegnia diclina* type 3.

   Monoecious; cylindrical, clavate, or fusiform zoosporangium; *renewal* methods of secondary zoospore are *Internal proliferation*; *zoosporangial discharge* are saprolegnoid; diplanetic zoospore; 6,25-7,5 µm on diameter of zoospore cyst; zoospore ornamentation like a short hair; perkecambahan kista zoospora secara *direct*; pyriform, cylindrical, clavate, or irregular gemma; single or catenulate gemma; >1 week on the times gemma shaped; subspherical (subglobose) oogonium; *oogonial origin* are terminal; 45 µm on diameter of oogonia; oogonia shaped after 4 weeks in incubation; *Antheridial origin* are diclinous; antheridium appresion on oogonium are lateral (fig 2).

A. heterosexualis characters that describe from goramy almost like the characters that describe by Braksdale (1965)[5]. There are any variation characters in sexual and asexual characters *A. heterosexualis* from goramy. The variation characters *A. heterosexualis* from goramy are clavate sporangium, cymose on renewal of sporangium, pyriform and clavate gemma, sessile or catenulate oogonium, subcentric type I and II on oospore centricity, and hypogynous antheridium. *A. heterosexualis* that describe by Braksdale didn't have that characters. The other way, *A. hetrosexualis* from goramy didn't have monoclinous or androgynous antheridium like the characters that describe on *A. heterosexualis* by Braksdale. Thereby, isolates of *A. heterosexualis* from goramy lesion more variative on sexual and asexual characters then *A.heterosexualis* that describe by Braksdale.

Isolates *A. heterosexualis* from goramy have more dominan in diclinous antheridium then hypogynous antheridium. Hypogynous antheridium on the isolates didn't penetrate into oogonium, and not fertilized the oospore (disfungction antheridia). Hypogynous antheridia in *Achlya* commonly as a reducted antheridia, and on the other species like *Saprolegnia hypogyna*, the antheridia didn't formed a fertilize duct to oospore. Thereby the hypogynous antheridia on those species are not realy antheridia, but they are just like a latent cells with reducted on the sexual function[5].

S. diclina characters that describe from goramy almost like the characters that describe by Jhonsons Jr. *et.al* (2002)[5]. *S.diclina* that describe by Jhonson Jr. *et.al* more variative in sexual and asexual characters than *S.diclina* from goramy lesion. *S.diclina* that describe by Jhonson Jr. *et al* have sphaerical, subsphaerical, obpyriform, napiform, dolioform or few irregular oogonium, lateral, intercalary, or terminal, single or catenulate; antheridium diclinous, rarely monoclinous or androgynous.

Thereby the fungal isolates from saprolegniasis lessions in goramy can be identified as *Achlya hetrosexualis* and *Saprolegnia diclina* type 3. The differences character with the other *A. heterosexualis* or *S.diclina* type 3 that describe by Braksdale or Jhonsons Jr. et.al just a variation characters. Molecular characterization can be doing to ensure any difference characters of one species with the others.

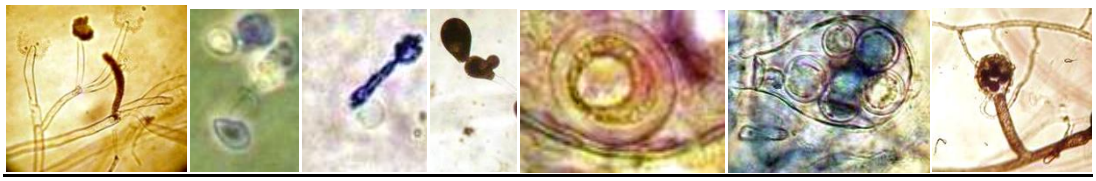

Figure. 1. Morfology of *Achly heterosexualis*. A. Zoosporangium cylindrical- achlyoid type, sympodial, with spore-balls on the mouth of sporangium. B. Zoospore diplanetic. C. Zoospore germination. D. Catenulate gemma. E. Ecentric oospore. F. Oosporangium with oospore and hypogynous antheridia inside. G. Oosporangium with diclinous antheridia

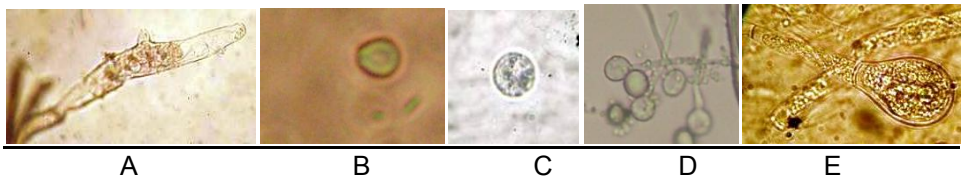

Figure.2. Morfology of *Saprolegnia diclina*. A. Zoosporangium cylindrical- Saprolegnioid type. B. Zoospore diplanetic. C.Zoospore cyst. D. Zoospore cyst germination. E. Oogonium pyriform with ospore inside

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P-SE04

Bacterial Diversity In Milkfish (*Chanos chanos*) Gastrointestinal : Potential Candidate For Probiotic And Cellulose Degrading Agent

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ABSTRACT

Milkfish is a herbivore which eat phytoplankton. In milkfish digestive tract, there are many variety of normal bacteria which help milkfish digestion process. These Bacteria has potential as a probiotic and cellulose degrading agent. The purposse of this study were to explore bacterial in milkfish digestive tract, to study bacteria which potential as a probiotic, and to find bacteria that can degrade cellulose. Milkfish acclimatized then dissected aseptically. Samples of stomach and intestine that have been mashed, suspended into physiological saline solution at pH 2, then inoculated on Tryptone Soya Broth (TSB). Isolates that able grew on pH of 2 suspected as probiotic bacteria. Furthermore culture was plated on Tryptone Soya Agar (TSA). Cellulolytic bacteria selected by inoculated bacteria on Carboxil Methil Cellulose (CMC), then dropwise a congo red. The ability of bacteria to degrade cellulose indicated by clear zone around the colony. Characterization and identification of bacteria used *Bergeys Manual of Determinative Bacteriology*. The result showed that three bacterial isolates were potential as probiotics and eight bacterial isolates were cellulolytic. Based on colony morphology, cell morphology and biochemistry test indicated that isolates B1-BSA, BSA-E2, NS-LBA, and NS-BSA-D1 have similarity with *Pseudomonas* sp. NS-LBC isolates have similarities with *Micrococcus* sp. Isolates LBA-4-5 have similarities to *Alkaligenes* sp. NS-LBD isolates have similarities to *Enterobacter* sp. The result of bacteria isolates from stomach milkfish (*Chanos chanos*) obtained isolates that have similarity with *Pseudomonas* sp., *Micrococcus* sp., *Alkaligenes* sp. and *Enterobacter* sp. *Pseudomonas* sp.and *Micrococcus* sp. potential candidate for probiotic. *Alkaligenes* sp *and Pseudomonas* sp were potential candidate for cellulose degrading agent.

Keywords: *Milkfish gastrointestinal, Probiotic, Cellulolytic Bacteria*

INTRODUCTION

Milkfish (*Chanos chanos*) is kind of herbivore fish that consume phytoplankton and has a long digestive track (Lathifah, 2009). There are so many floral normal bacteria that help milkfish in digestion process. Flora normal bacteria have potential as a probiotic and cellulolytic bacteria. Probiotic break down complex molecule into simple molecule that easy to digest. Cellulolytic bacteria break down phytoplankton (their food) cell wall that contains cellulose. Probiotic and cellulolytic bacteria from the milkfish digestive track have potential to be used by human. Probiotic can be balancing bacteria in the digestive tract (Fuller,1987). Cellulose enzyme can be used for composting agriculture waste, bioethanol and bifuel production (Alexander, 1965 ; Maryandini *et al*, 2009 ; Bansal *et al.,* 2009). Based on the background, the purpose of this research are to explore bacterial diversity in milkfish

digestive tract, to study bacteria which potential as a probiotic, and to find bacteria that can degrade cellulose.

MATERIALS AND METHODS

Acclimatization Milkfish

Milkfish was gotten from Center of Aquaculture Development Briny / BBPBAP (*Balai Besar Pengembangan Budidaya Air*), Jepara. Then, sample was acclimatized for two days in Animal Taxonomy Laboratory, Biology Faculty, Universitas Gadjah Mada, by giving phytoplankton as the feed.

Isolation Bacteria

Milkfish dissected aseptically to taken the digestive organ (stomach). One gram samples of stomach and intestine that have been mashed, suspended into 9 ml of physiological saline solution pH 2 then inoculated on enrichment media Trypotone Soya Broth (*TSB*) for 24 hours, Isolates that able grew on TSB with a pH of 2 suspected as probiotic bacteria. Furthermore culture was plated on Tryptone Soya Agar (TSA). After that isolates tested the ability of the growth in the medium pellet extract.

Cellulolytic bacteria selected by inoculated bacteria on Carboxil Methil Cellulose (CMC). The colonies that have been grown, washed by congo red. Colonies that showed the clear zone after spilled by congo red can be categorized as the isolates that have potential as cellulolytic bacteria. After that isolates tested the ability of the growth in the medium *Chlorella* extract.

Figure 1. Milkfish dissected aseptically

Identification of the bacterial strains

The cultures were identified according to their cell morphology, coloby morphology (shape colony,shape of entire colony**,** elevation, and surface texture.) , gram reaction, and biochemical characteristics (acid production from glucose, fructose, lactose ; amylum

hydrolysis ; catalase test ; indol test ; sierra twin test ; and simmon sitrat test. Identification of bacteria used *Bergeys Manual of Determinative Bacteriology*.

RESULTS AND DISCUSSION

The result showed that three bacterial isolates (NS-BSA-D1, NS-LBC, NS-LBA) grew on TSB with a pH of 2 and pellet extract, indicates the bacteria were potential as probiotic. The probiotic bacterium can live in acidic condition, habitat of these bacteria resistant to acid to reach the intestines alive to maintain harmonious fish digestive tract. Not all bacteria are able to grow in the acid pH.

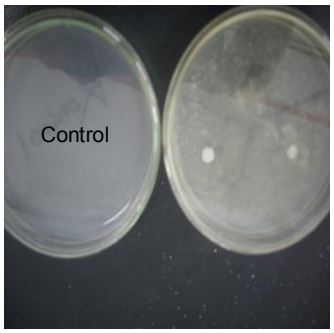

Figure 2. Colonies that grew in pellet extract

Eight bacterial isolates (BSA-B1, BSA-B3, BSA-E2, BSA-D1, LBA-4, LBA-5, LBC and LBD) were cellulolytic.

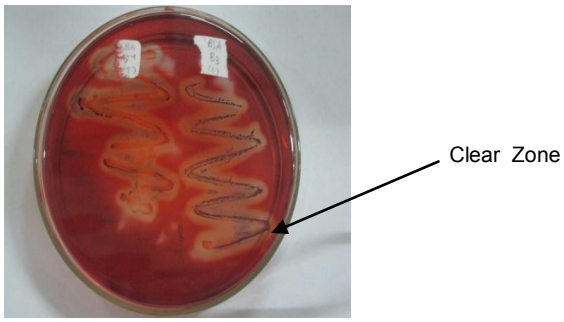

Figure 3. Colonies that grew in CMC agar
(→ : Clear Zone around bacteria colonies)

When congored reagent dropped into cellulose medium, cellulose medium became red, but in the bacteria colonies that grew in CMC medium after spilled by Congored reagent showed the clear zone around the colonies. Clear zone indicate cellulose was degrade because of cellulose enzyme produced by cellulolytic bacteria.

Table 1. Colony morphology isolates

No	Isolates Code	Colony Morphology				
		Colour	Colony Shape	Shape of Entire Colony	Internal Structure	Elevation
1.	NS- BSA-D1	Cream	Circulair	Entire	Smoth	Low convex
2.	NS-LBA	White	Rhizoid	Undulate	Coarsley Granular	Raised with concave
3.	NS-LBD	Cream	Circulair	Entire	Smoth	Convex
4.	NS-LBC	Cream Yellowish	Circulair	Entire	Smoth	Low convex
5.	BSA B1	White	Circulair	Lacerate	Coarsley Granular	Raise with concave
6.	BSA E2	Cream	Rhizoid	Lobate	Coarsley Granular	Effuse
7.	LBA 4	White	Circulair	Entire	Finaly Granular	Raise with concave
8	LBA 5	White	Circulair	Entire	Finaly Granular	Raise with concave

Table 2. Characteristic Isolates

No	Isolates Code	Gram Stain	Growth	Cell Morphology	Motility
1	NS-LBD	Gram -	Fakultatif anaerob	Rodd	+
2	NS-BSA-D_1	Gram -	Aerobik	Ovoid	+
3	NS-LBC	Gram +	Aerobik	Coccus	+
4	NS-LBA	Gram -	Aerobik	Rodd	+
5	BSA B1	Gram -	Aerobik	Rodd	+
6	BSA E2	Gram -	Aerobik	Rodd	+
7	LBA 4	Gram -	Aerobik	Coccus	+
8	LBA 5	Gram -	Aerobik	Coccus	+

Table 3. Biochemical test

No	Isolates Code	Glukose	Fructose	Lactose	Amylum Hidrolysis	Simmon Citrat Test	Indol Test	Catalase Test	Nitrat Test	Sierra Test
1	NS-LBD	+	-	-	+	+	-	+		+
2	NS-BSA-D_1	-	+	+	-	+	-	+		+
3	NS-LBC	+	-	-	+	+	-	+		+
4	NS-LBA	-	+	+	+	+	-	+		+
5	BSA B1	-	-	-	+		-	+	+	+
6	BSA E2	-	+	+	+		-	+	+	+
7	LBA 4	+	-	-	+		-	+	+	+
8	LBA 5	+	-	-	+		-	+	+	+

Identification of bacteria used *Bergeys Manual of Determinative Bacteriology*.Based on colony morphology, cell morphology and biochemistry test indicated that isolates B1-BSA, BSA-E2, NS-LBA, and NS-BSA-D1 have similarity with *Pseudomonas* sp. NS-LBC isolates have similarities with *Micrococcus* sp. Isolates LBA and LBA-4-5 have similarities to *Alkaligenes* sp. NS-LBD isolates have similarities to *Enterobacter* sp.

The result of bacteria isolates from stomach milkfish (*Chanos chanos*) obtained eight isolates that have similarity with *Pseudomonas* sp., *Micrococcus* sp., *Alkaligenes* sp. and *Enterobacter* sp. *Pseudomonas* sp.and *Micrococcus* sp. potential candidate for probiotic. *Alkaligenes* sp *and Pseudomonas* sp were cellulolytic bacteria and potential candidate for cellulose degrading agent.

Acknowledgment

Our sincere thanks for the research grant from Student Innovations Grants / GKI (*Grant Karya Inovasi Mahasiswa*) PPKB UGM.

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P-SE05

MORPHOLOGICAL CHARACTERS OF *Ichthyophis* sp. (AMPHIBIA: GYMNOPHIONA: ICHTHYOPHIDAE) IN PETUNGKRIONO, DIENG PLATEAU, CENTRAL JAVA

Chomsun Hadi Kurniawan, Trijoko, Rury Eprilurahman

Biology Faculty - Gadjah Mada University

not presented

P-SE06

THE TREES SPECIES DIVERSITY AROUND SPRING WATER AT TWO AREAS IN PURWODADI, PASURUAN

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ABSTRACT

The aim of this research is to know the trees species diversity around water springs at two areas in Purwodadi, Pasuruan, East Java. The data was gathered by vegetation analysis, in order to observe the diversity, density and diameter of trees. Eleven plots for each area with one ha in extent were observed. The data was analyzed using Mueller-Dombois's method to calculate importance value index, while the similarity of trees community was analyzed by Oosting's method. The coordinate and altitude of every spring waters or its group's site were determinated using Geographical Position System (GPS) to know where the position on the map is. Result indicated that at least 28 families, 37 genera which consisted of 69 species of trees grown around water springs at the first area with 5.06 of diversity index, while the second area, consisted of 23 families, 42 genera and 54 species of trees with 4.50 diversity index. The diversity of trees species from Moraceae was the highest among other families, however, the importance value index were dominated by *Bambusa blumeana* from Poaceae, both at the first and the second area. Those areas had 46.07 % of trees similarity index of communities. We hope that this information, can be used for alternative guidance to restore or to conserve such areas in order to save and sustain of trees diversity and their habitat.

Keywords: water spring, plant diversity, purwodadi, pasuruan.

INTRODUCTION

Indonesia is extremely rich in vegetation diversity[7]. Of the estimated 250,000 plant species occuring in the southern hemisphere, around 25000 species exist in this country[10]. However still very little of those plants have been managed properly for the welfare of Indonesian people. Even, some of them have been seriously in threatening prosses caused by many factor. It was reported that habitat destruction has been occured everywhere and often included in protected areas. Therefore, conservation of plants and ecosystems both in-situ and ex situ is very important to avoid existing plants diversity loss. In international forums, Indonesia with 189 countries in the UN membership in year 2000, has been determined to implement sustainable development through the Millennium Development Goal's (MDGs). One of eight targets to be achieved by 2015 is ensuring the sustainability of natural resources and environment. To achieve the MDGs targets more quickly, a summit on sustainable development (2002) organized by the United Nations has agreed to more be focused and integrate efforts in the field of water, energy, health, agriculture and biodiversity (WEHAB)[2]. The aim of this research is to know the trees species diversity around spring water at two areas in Purwodadi, Pasuruan, East Java.

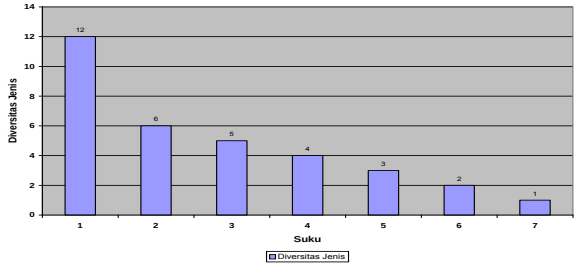
MATERIALS AND METHODS

Water springs location were informed by local comunities. The data was gathered by vegetation analysis, in order to observe the diversity, density and diameter of trees. Eleven plots for each area with one ha in extent were observed. The data was analyzed using Mueller-Dombois's method to calculate importance value index, while the similarity of trees community was analyzed by Oosting's method. The coordinate and altitude of every spring waters or its group's site were determinated using Geographical Position System (GPS) to know where the position on the map is.

RESULTS AND DISCUSSION

a. Tree Diversity

Result indicated that at least 28 families, 37 genera which consisted of 69 species of trees grown around spring water at the first area with 5.06 of diversity index, while the second area, consisted of 23 families, 42 genera and 54 species of trees with 4.50 diversity index. The diversity of trees species from Moraceae was the highest among other families, however, the importance value index were dominated by *Bambusa blumeana* from Poaceae, both at the first and the second area (Figure 1 and 2).



. Figure 1. Diversity of tree species in the First Area (Gajahrejo)
1 Moraceae; 2. Lauraceae; 3. Meliaceae, Mimocaceae; 4. Myrtaceae, Poaceae; 5. Euphorbiaceae, Sterculiaceae, Tiliaceae; 6.Annonaceae, Arecaceae, Bombacaceae, Malvaceae, Verbenaceae; 7. Fourteen other species, each single species.

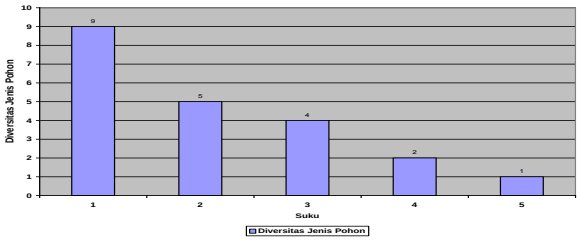


Figure 2. Diversity of Tree Species in the second area (Parerejo)
1. Moraceae; 2. Anacardiaceae, Mimosaceae; 3. Meliaceae, Myrtaceae; 4. Arecaceae, Bombamcaceae, Clusiaceae, Euphorbiaceae, Myristicaceae, Poaceae, Rubiaceae, Sterculiaceae, Tiliaceae; 5. Nine other species, each single species.

From Figure 1 and 2 seem that the diversity of tree species of Moraceae is higher than other families which reach 12 and 9 species. Moraceae is one of the family of flowering

plants, the tribe of Rosales. In this tribe, including the genus of *Ficus*. Characteristic of this genus can be seen from the fruit that is not true because the fruit is formed from the bottom of the enlarged flower then closes to form a circle like a fruit. The flowers are hidden inside the "fruits" and is pollinated by certain insects, usually from members of the Hymenoptera[3]. Most of the Moraceae family grown in the lowland tropics and even the genus of *Ficus* distribution center thought to be located in Indo-Malesia region that includes Indonesia, Malaysia, the Philippines, Brunei and Papua New Guinea. Some *Ficus* species can be classified as a key species (keystone species) because of its fruit. It is preferred to eat by animals, so that potential if planted as a material for improving of the environment quality [5,6,8,11]. In accordance to the restoration and maintenance of water resources, some species of the genus of *Ficus* have specific characteristics, such as, deep and broad rooting, many branching in low posisition, broad canopy, that are potential to reduce the speed of rainfall grains. Thus the destructive force on the surface layer of soil is low, and the infiltration of water into the ground is better. As a result water is retained relatively longer in the soil and released slowly, allowing the continuity of spring and reduce erosion or landslides. However, the diversity of tree species with the highest importance value index, both in the first and the second area, is *Bambusa blumeana* from Poaceae. *Bambusa blumeana* is commonly known growing in tropical Asia[1]. The recorded plants that grow around the springs is not known certainty whether they were planted by local communities in the past and has grown naturally or the existing native plants or both,. Unless *Bambusa blumeana*, there are also some species that are co-dominant such as, *Dendrocalamus asper, Ceiba pentandra, Gigantochloa atter, Ficus benjamina, Cananga odorata, Syzygium javanicum and Ficus virens.* The twenty most important tree species from two observed areas are listed in figure 3 and 4.

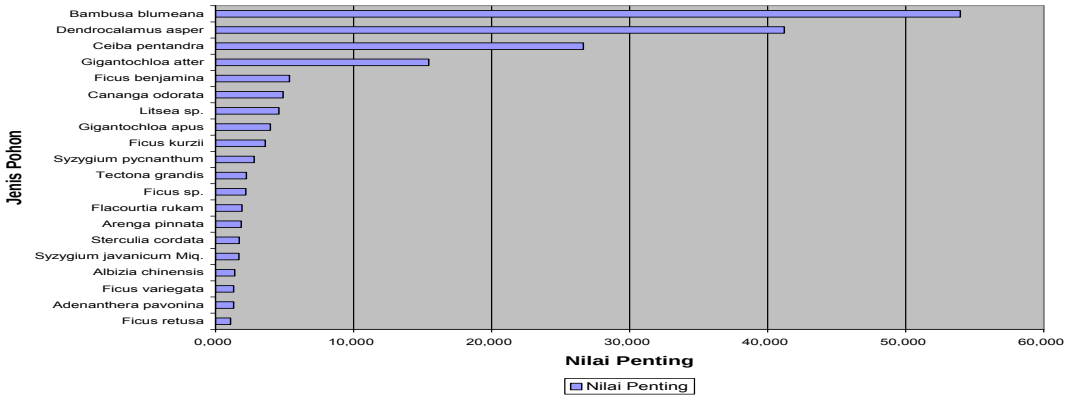


Figure 3. Tree Species Diversity at the First area (Gajahrejo), Twenty most important tree species of 69 species

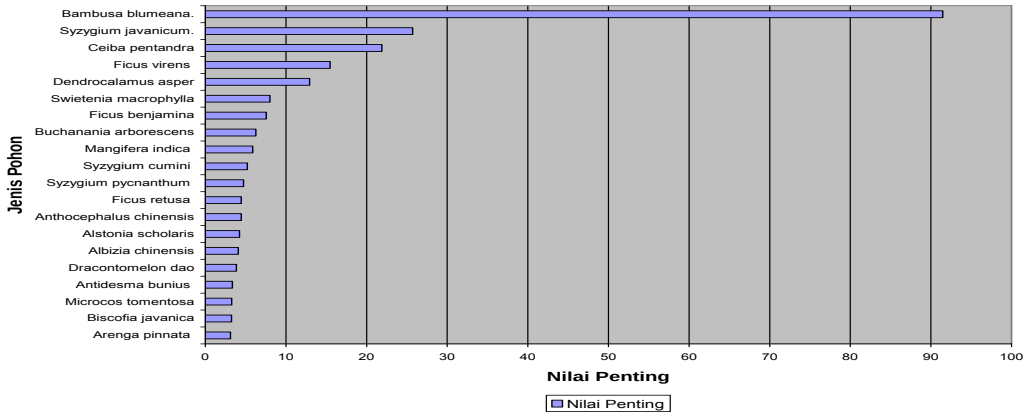


Figure 4. Tree Species Diversity at second area (Parerejo), Twenty most important tree species of 54 species.

b. Community Similarity

Coeficien similarity can be calculated according to the following formula: C = 2W / a + b, where, C = coefficient of community similarity; W = Number of equal value or the lowest (≤) of the types contained in the areas compared; a = Number of quantitative value of all species contained in the first standing; b = Number of quantitative values of all species contained in the second standing. The coefficient similarity value may indicate the level of species composition similarity of the two communities compared. Community coefficient values ranged from 0 -100 with the understanding that if the coefficient of similarity has come closer to number 100 then the level of species composition similarity is high[9]. Based on the amount of the same and the lowest of importance value index of tree species in two communities being compared that equal 120.229 and the amount of the cumulative value of a + b = 600, so that the coefficient similarity can be calculate (C) = (120.229 x 2) / 600 x 100% = 40.07% . This means that the degree of similarity between the two communities is 40.07% with an indication that the two locations compared are quite different. However both these areas have similarity in terms of species diversity richness, i.e. from the family of Moraceae.

c. Tree Density

Tree density in the first area (Gajahrejo) is 110.7 trees / ha, while in the second area (Parerejo) 80.4 trees / ha. To obtain better environmental services will require the addition of plants around the springs by prioritizing diversity of native and / local species which has been adapted to the habitat. It is expected that this tree species diversity information can also be used as an alternative reference to the provision of material restoration or conservation of biodiversity around the springs, especially to area with similar altitude and

similar climatic conditions to restore or to conserve such areas in order to save and sustain of trees diversity and their habitat.

d. The Spring Position on the Map

The results of measurements with the GPS coordinates indicate that the position of springs in the two areas within sub districts of Purwodadi lies in the range 7 º 48'101 " south latitude; 112 º 42'000" longitude up to 7 º 48'120 " south latitude; 112 º 43'628" longitude at an altitude between 388 and 491 m above sea level. The position of spring and spring groups are listed in Figure 5.

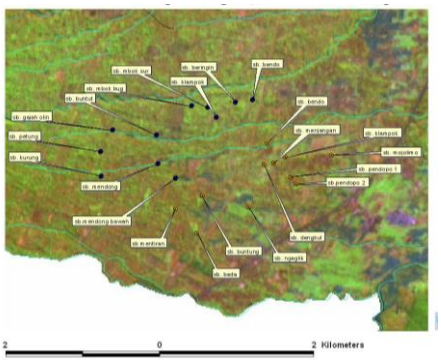


Figure 5. The position of Springs or Spring groups in Two Areas in the Sub District of Purwodadi
Blue dot : The first area and Orange dot : the second area
Map quoted from: Pasuruan Simtaru[4]

CONCLUSION

- At least 28 families, 37 genera which consisted of 69 species of trees grown around spring water at the first area with 5.06 of diversity index, while the second area, consisted of 23 families, 42 genera and 54 species of trees with 4.50 diversity index.
- The diversity of trees species from Moraceae was the highest among other families, however, the importance value index were dominated by *Bambusa blumeana* from Poaceae, both at the first and the second area.
- Those areas had 40.07 % of trees similarity index of communities.
- Tree density in the first area (Gajahrejo) is 110.7 trees / ha, while in the second area (Parerejo) is 80.4 trees / ha.
- We hope that this information, can be used for alternative guidance to restore or to conserve such areas in order to save and sustain of trees diversity and their habitat.

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P-SE07

Diversity and Feeding Habit of Anura in Plawangan Hill, Yogyakarta After Mount Merapi Eruption 2010

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ABSTRACT

Plawangan Hill, southern slope of Mount Merapi suffered great damage after Mount Merapi eruption in 2010. Plawangan Hill ecosystem was dramaticaly changed after the eruption. This change of ecosystem affected all organism in this place, including the Anuran. This research aimed to study Anuran diversity and feeding habit after the eruption in Plawangan Hill, Yogyakarta. The research was carried out on April to July 2011, VES (Visual Encounter Survey) method was used to collect samples of Anurans while stomach dissection method was applied to determine the feeding habit of the Anurans. Nine Species of Anurans were identified, they were *Hylarana chalconota, Odorrana hosii, Polypedates leucomystax, Rhacophorus margaritifer, Limnonectes kuhlii, Limnonectes macrodon, Limnonectes microdiscus, Duttaphrynus melanostictus* and *Megophrys montana.* The *Megophrys montana* only found in it larval stage. *Hylarana chalconota* was the most abundant species found in this research. Twenty two stomachs were taken during this research. Insects were the most common food item found during this research. *Limnonectes kuhlii* was the only species not use insect as their main prey.

Keywords: Plawangan Hill, Anuran, Merapi eruption, VES, Stomach Dissection

INTRODUCTION

Southern slope of Mount Merapi was natural habitat for thirteen Anuran species. They were *Megophrys montana, Leptobrachium haseltii, Odorrana hosii, Hylarana chalconota, Limnonectes microdiscus, Limnonectes kuhlii, Rhacophorus reinwardtii, Polypedates leucomystax, Philautus aurifasciatus, Philautus sp., Rhacophorus margaritifer, Phrynoidis aspera* and *Duttaphrynus melanostictus* (Eprilurahman and Kusuma, 2011). This place was damaged by Merapi eruption in 2010. However, Plawangan Hill, part of Gunung Merapi National Park, located in southern slope of Merapi Mountain was not entirely damaged. This research aimed to study Anuran diversity and feeding habit after the eruption in Plawangan Hill, Yogyakarta.

MATERIALS AND METHODS

Tools used in this research were torch, Plastic bag, Flakon bottle, Syringe, Dissecting kit, microscop, caliper, and digital camera. Chemical used in this research was 70% alcohol.

Anuran survey was conducted by Visual Encounter Surveys (VES) according to Kusrini (2009). Survey was carried out on April to July 2011 in Plawangan Hill Yogyakarta. Individuals captured during the survey were identified, measured and counted. Uncaptured Individuals were noted if visualy identified. Some specimens were taken for feeding habit analisys. Feeding habit analisys was conducted by Stomach Dissection according to Kusrini (2009). This method was carried out by firstly kill the specimen by injected 70% alcohol to the hind brain. After the specimen was died the stomach was removed and preserved with 70% alcohol in flakon bottle. In the lab, stomach contents were identified and counted.

RESULTS AND DISCUSSION

Nine Anuran species were identified during this research. Eight species were found on it adult form. They were *H. chalconota*, *O. hosii*, *P. leucomystax*, *R. margaritifer*, *L. kuhlii*, *L. macrodon*, *L. microdiscus*, *D. melanostictus.* (Fig. 1). *Limnonectes macrodon* was never reported found in the southern slope of Merapi before this research.

Fig. 1. Up from left to right *R. margaritifer, P. Leucomystax, O. Hosii, H. Chalconota*. Down from left to right *L. Macrodon, L. Kuhlii, L. Microdiscus, D. melanostictus*. (image of *L. Kuhlii* and *D. melanostictus* by Asti, *i*mange of *H. Chalconota* by Atmaja and the other image by Kuswatoro).

Four species were found as a tadpole. They were *M. montana, R. margaritifer, H. chalconota and L. microdiscus.* During this research *M. montana* was only found as a tadpole and never found on it adult form. (Fig. 2)

Fig. 2. Mouth of *M. montana* tadpole (image by Asti)

Hylarana chalconota was the most abundant species found in this research. While *M. Montana*, *P. leucomystax* and *O. Hosii* were considered as the rarest species found during this research (fig. 3). *Hylarana chalconota* was the most abundant species in Plawangan hill

because it could life in almost all habitats in Plawangan Hill, such as man distrubance area, on small tree or grass, land and little pond.

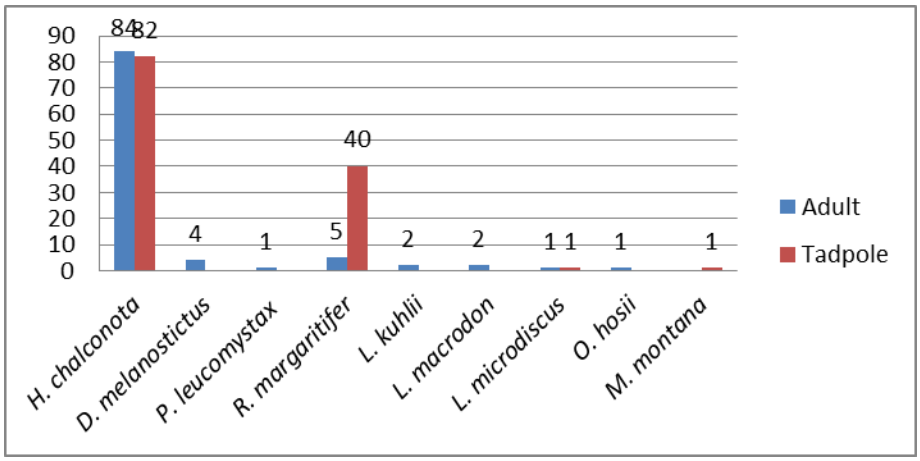

Fig. 3. Number of Anura Individuals found during the research

Twenty two stomachs from eigth Anuran species were taken in this research. Insects were the most common food items found in the stomach. (Table 1.). Ants (Formicidae : Hymenoptera) were the most common insect found. Usually ants were found in a great number in one stomach, this was because ant life in colony so one frog could ate ant in great number at once.

According to Young (1995) Amphibian ate prey that easily found in it habitat. It was proved by this result. *Limnonectes kuhlii* mainly ate Crustacean, because it usually life in the stream, *Duttaphrynus melanostictus* was a terrestrial toad, mainly ate ants and dung beetle (Scarabidae : Coleoptera) that usually life on land. *Hylarana chalconota* could life in little trees, land and pond, so they could ate many kind of Insects and also spiders.

Tabel 1. Food Items Found in Anuran Stomach.

Prey Taxon	Anuran Species																	
	Limnonectes kuhlii		*Dutaphrynus melanostictus*		*Hylarana chalconota*		*Polypedates leucomystax*		*Rhacophorus margaritifer*		*L. macrodon*		*L. microdiscus*		*Odorrana hosii*	
	n=3		n=2		n=11		n=1		n=2		n=1		n=1		n=1	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Crustacea	2	50														
Chilopoda					2	4										
Arachnida	1	25			4	8					1	50	1	25		
Coleoptera			5	23,81	4	8							1	25	1	100
Scarabidae			6	28,57	2	4										
Hymenoptera					2	4			1	50						
Formicidae			9	42,86	23	46							2	50		
Diptera					3	6										
Muscidae					1	2										
Hemiptera																
Cicadellidae					1	2										
Orthoptera					1	2					1	50				
Dermaptera					1	2										
larva	1	25														
Mantodea					3	6										
unidentified			1	4,76	3	6	1	100	1	50						
Number	4	100	21	100	50	100	1	100	2	100	2	100	4	100	1	100

CONCLUSION

Nine Anuran species were identified during this research. They were *H. chalconota*, *O. hosii*, *P. leucomystax*, *R. margaritifer*, *L. kuhlii*, *L. macrodon*, *L. microdiscus*, *D. melanostictus and M. Montana. Limnonectes macrodon* was never reported found in the southern slope of Merapi before this research. *Hylarana chalconota* was the most abundant species found in this research. Twenty two stomachs from eigth Anuran species were taken in this research. All Anura found in this research use Arthropoda as their main prey. Insects were the most common food items found in the stomach.

Acknowledgment

The authors wish to express their gratitude to Faculty of Biology Gadjah Mada University for financing the research. The authors also would like to thanks the head of Gunung Merapi National Park for the research permission. Special thanks to Hastin ambar Asti, Ikhsan Lukmana Indra Putra and the member of Herpetology Study Club Faculty of Biology Gadjah Mada University for all the help and discussion.

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P-SE08

HABITAT ANALYSIS OF EPIPHYTIC CLIMBER *Hoya purpureofusca* Hook.f. AT CIBODAS MOUNTAIN, WEST JAVA, INDONESIA

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not presented

P-SE09

Comparison of Decapods (Crustacean) Biodiversity Between Rocky Beach and Sandy Beach in Tengah Island, Karimunjawa National Park

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ABSTRACT

Intertidal areas have high biodiversity. At the same time, biodiversity can be used as main criteria to establish protection policy priorities, or to propose management actions especially in National Park. Study on Crustacean conducted to compare biodiversity of crustaceans on rocky beach and sandy beach in coastal areas of Tengah Island, Karimunjawa National Park. The study was carried out in 10-12 July 2011. Sampling was carried out by the cruising method and trap within 0-3m depth. The biodiversity index will be calculated by Shannon-Wiener index. From the study, can be collected 12 species of decapods included into 8 family. Tengah Island biodiversity index is 1.267 in sandy beach and 1.258 in rocky beach. It mean sandy beach have higher biodiversity than rocky beach. More than it, Tengah Island have low biodiversity of crustacean at all habitat.

Keywords : biodiversity, crustacean, Tengah Island

Introduction

Karimunjawa National Park is an island that has a type of lowland rain forest ecosystem, seagrass, algae, coastal forests, mangroves, and coral reefs. The island was located on the southern Java Island. Tengah Island is one of the islands part of the Karimunjawa National Park who have high biodiversity. Basically the biodiversity of a species can be used for basic biological resource protection priorities. Tengah Island Ecosystems include white sandy beaches, coastal forests, and coral reefs. So that the diversity of crustaceans can be seen from the rocky shore habitats and sandy beaches. Crustacean has a high diversity of substrates that contain more food resources.

Figure 2. The Coast of Tengah Island

Material and method

The tools used in this research is trap, bucket, millimeter blocks that have been laminated, cork, raffia, a digital camera. While the materials used in this research traping crustacean is the fish. The research was conducted on 10-13 July 2011. The study was conducted for 2 times in 3 days using traping method and methods of cruising. Trap that has been tied up with cork mounted as many as 20 points along the coast of Tengah Island. Traps installed at depths of 0 - 3 m below sea level. Trap used at 09.00 am and the next day made the arrest crustaceans. Method of cruising is done every day at 11.00 am. Once it catches on photos using a digital camera above the millimeter blocks that have been laminated. Crustaceans are not used as a specimen because of the central island is a conservation area, so the catches are released back into nature. Identification is done using the book The Living Marine Resources Of The Western Central Pacific Volume 2: cephalopods, crustaceans. holothurians and sharks and A Guide To The Decapod crustaceans Of The South Pacific.

Result and discussion

There are the result of this research is :

Table 1. Data of some species were identified from Rocky beach

1	P7100176-P7100186	Grapsidae	Metopograpsus messor	Rocky	9
2	DSC03858-DSC03874	Diogenidae	Dardanus logopodes	Rocky	2
3	DSC03875-DSC03882	Diogenidae	Diogenes pallescens	Rocky	1
4	-	Alphidae	Alpheus strenuus	Rocky	3
5	P1000725-P1000734	Parthenopidae	Daldorfia horrida	Rocky	1

Table 2. Data of some species were identified from Sandy beach

1	P1000659-P1000665	Pilumidae	Pilumnus caerulescens	Sandy	1
2	P1000666-P1000671	Xantidae	Etitus laevimanus	Sandy	1
3	P7100672-P1000673 & P7100125-P7100130	Pilumidae	Pilumnus longicornis	Sandy	1
4	P1000736-P1000742	Potunidae	Thalamita danae	Sandy	10
5	P1000756-P1000760	Portunidae	Thalamita spinimana	Sandy	3
6	P1000743-P1000755	Xanthidae	Atergatis floridus	Sandy	1
7		Ocypodidae	Ocypode pallidula	Sandy	1

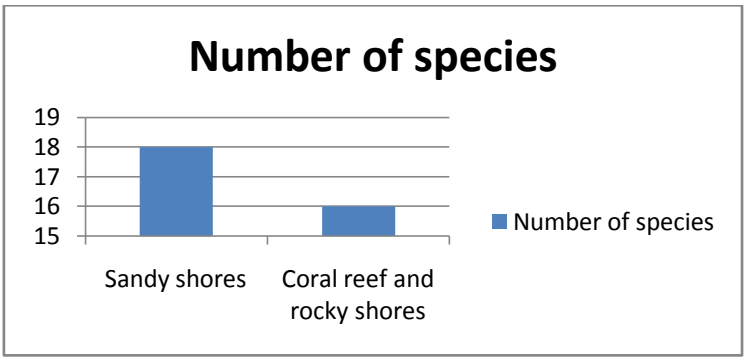

Figure 3. Table number of spesies biodiversity crustaceans in Rocky beach and Sandy beach, Tengah Island, Karimunjawa

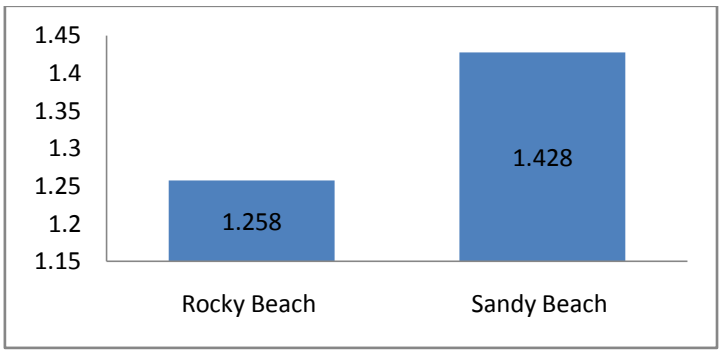

Figure 4. Table of Shannon-Wiener index biodiversity crustaceans in Rocky beach and Sandy beach, Tengah Island, Karimunjawa.

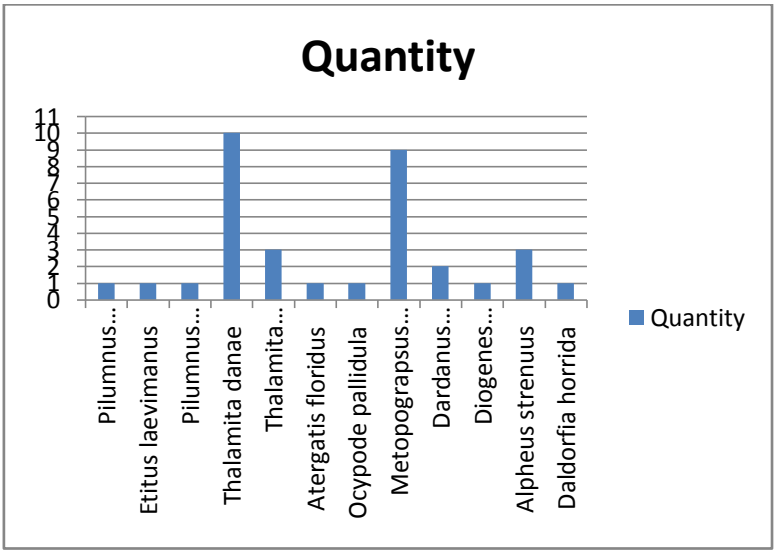

Figure 4. Biodiversity of Crustacean in Tengah island, Karimunjawa National Park.

Diversity on the sandy beach crustaceans is higher due to a food source on the sandy beach is higher compared to the rocky beach. Based on the results of Shannon-Wiener index,

crustacean diversity in the sandy beach and rocky beach included the medium category. Tengah Island has medium biodiversity crustacean.

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P-SE10

IDENTIFICATION THE LOCATION OF METHATHORACIC GLAND OPENING IN RICE BUG *Leptocorisa oratorius* F. AND *Leptocorisa acuta* T. (HEMIPTERA : ALYDIDAE)

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[2]. MTs YAPI Pakem, Sleman
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INTRODUCTION

The Rice bug, *Leptocorisa oratorius* F. and *Leptocorisa acuta* T. are insect pest of rice. Adult male and female produce chemical compounds that contain octyl acetate (OAc), 2-(E)-octenyl acetate (2EOAc), octanol (OL), 2-(E)-octenol (2EOL) and 3-(Z)-octenyl acetate (3ZOAc), as a defensive odour secreted from metapleural scent gland and spray out from the opening gap at methathoracic gland. The aim of this study is to distuingish location of the opening gap at methathoracic gland between *L.oratorius* and *L. acuta*.

MATERIAL AND METHODS

L. oratorius were sampled from the paddy field ecosystem of Kepitu Village, Sleman, DIY Yogyakarta. The identification process was at The Entomology Laboratorium, Puslit-Biology, LIPI, Bogor. The location of opening gap at methathoracic gland was identified by taking a photograph with camera-microscop, then compared with the location of opening gap from *L. acuta*.

RESULT AND DISCUSSION

Result of this study indicate that location of the opening gap both *L. oratorius* and *L. acuta* are in the same places.

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P-SE11

ANATOMY AND MORPHOLOGICAL ANALYSIS OF *Pandanus tectorius* Parkinson AGAINST TENSILE STRENGTH

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ABSTRACT

The Local names of pandanus are pandan laut(Sundanesse); pandan nipah (Maluku); pandan ponelo (Gorontalo); pandan abu(Sumatera). The local names indicate that distributed of pandan in Indonesia is wide enough. There are several central production of pandan that have vital role for community empowerment in Java.

Pandan that usage for handicraft usually growth at coastal. It is soft, long, easy to colored, easy to weaved that commodity is spread in Indonesia. The Product not only for carpet, but it developed to hat fashion, women hand bag, chair, laundry box, pillow cover, krey, etc.

The spread of usage specially to furniture is need tensil strength enough. Morphology and Anatomy analysis to tensile strength need research from several growth central location. The growth central locations are Gunung Kidul; Bantul; Kebumen; dan Tasikmalaya.

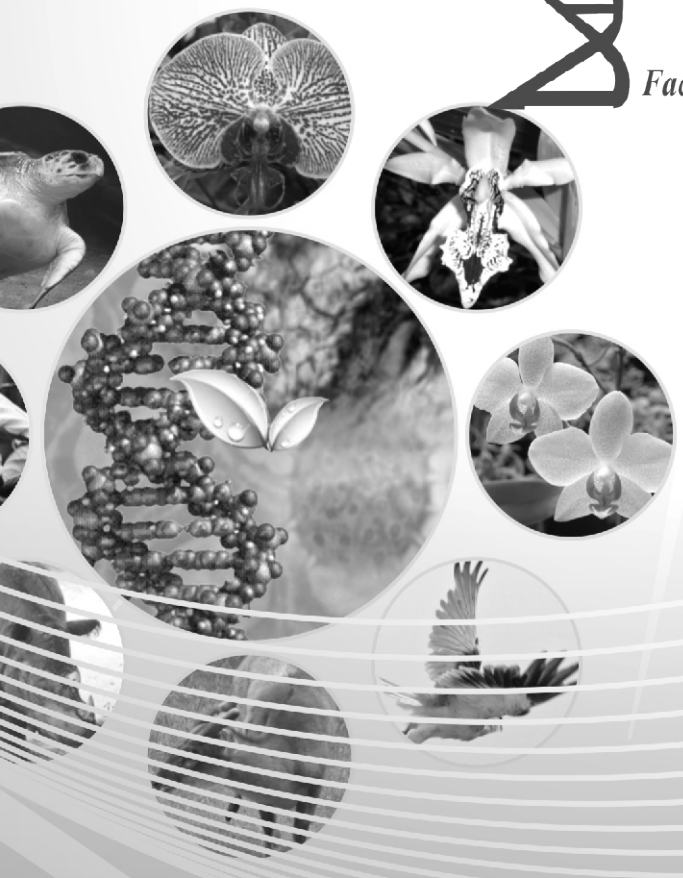
The result of research can use to recomendate for optimal usage of pandan from own location.

POSTER - TOPIC 4

Physiology and Developmental Biology (O-PD)

ICBS 2011

Faculty of Biology Universitas Gadjah Mada



FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

P-PD01

The Growth of *Chryseobacterium indologenes* ID 6016 on Filtrate's Culture of *Enterococcus faecalis* ID 6017 Which Continuously Growing on Orange II Containing Medium

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ABSTRACT

C. indologenes have been reported to grow in the medium which contained degrading orange II by *E. faecalis*. This phenomena generates a hypothesis that *C. indologenes* could grow and use intermediate products of orange II degradation as their growing medium. To verify this hypothesis, this research was done. Objective of this research was to determine the growth of *C. indologenes* ID 6016 on filtrate's culture of *Enterococcus faecalis* ID 6017 which continuously growing on orange II containing medium. Research was done by inoculating *C. indologenes* on the rest medium from growth of *E. faecalis* on orange II containing medium. This medium was assumed to contain sulphanilic acid and 1-amino-2-naphtol. The results showed that *C. indologenes* was able to grow on the medium filtrate and to decrease or degrade the content of sulphanilic acid and 1-amino-2-naphtol. The biomass production was increased according to the decrease of sulphanilic acid, 1-amino-2-naphtol and COD. The specific growth rate (μ) varied from 0.0092 – 0.0129. Suphanilic acid, 1-amino-2-napthol, and COD consumption were 33 - 37, 24 – 28.9, and 68 – 100.27 $mg.l^{-1}$, respectively.

Keywords*: decolorize of orange II, Sulphanilic acid, 1-amino-2-naphtol, the growth of C. Indologenes*

INTRODUCTION

Development of textile industry in Indonesia gives negative impacts for environmental quality. One of these negative impacts is effluent of dyeing process, especially caused by synthetic dye which relatively resistant to microbial degradation. During dyeing process, approximately 10-15% of the dyes are released into the environment through effluent of wastewater treatment (WWT) (1). So, the existence of these dyes in water ecosystem could inhibit light and create problems to photosynthetic aquatic plants and algae. In addition to their visual effect and their adverse impact in terms of chemical oxygen demand, many synthetic dyes are toxic, mutagenic and carcinogenic (2).

Orange II is one of synthetic azo dyes which could be decolorized by *Enterococcus faecalis* ID 6016 and produce intermediate products identified as sulphanilic acid and amino-naphthol (2). Although they are colorless, they are toxic and mutagenic. *Chryseobacterium* as one of flavobacteria could grow in the medium contained aromatic compound as sole carbon source and consume it (3). Several researchers reported that *C. indologenes* could grow in the medium contained suphanilic acid, even until 500 mg/l sulphanilic acid in the medium (4,5). There is no information about the growth of *C. indologenes* in medium contained decolorized azodye. Therefore, objectives of this research are to study the growth

of *C. indologenes* in several cell concentrations in filtrate's culture of *E. faecalis* which continuously growing on orange II containing medium.

MATERIALS AND METHODS

Microorganism and Composition of Growth Medium

C. indologenes ID6016 was obtained from Laboratory of Microbiology, Faculty of Biology, Satya Wacana Christian University, Salatiga, Indonesia. *C. indologenes* was maintained in basal medium which contained 0.250 g/l sulphanilic acid and agar 20 g/l. Composition of 1 l basal medium was K_2HPO_4 7.095, KH_2PO_4 3.574, $(NH_4)_2SO_4$ 1.980, $MgSO_4.7H_2O$ 0.250 g, and 1 ml of trace element (composed of $CuSO4.5H_2O$ 0.234, $ZnSO_4.7H_2O$ 0.11, $MnCl_2.4H_2O$ 0.35, $FeSO_4.7H_2O$ 0.63 g/100ml). For growing medium, we used fíltrate culture of *E. faecalis* which was continuously growing in orange II containing medium. Outlet of continuously culture of *E. faecalis* was filtration using 0,2µm membrane filter and put into 250 ml sterile flaks. The filtrate culture of *E. faecalis* was collected when the growth of *E. faecalis* raised steady state condition and orange II was decolorized.

Culture condition

The 48 hours slant culture of *C. indologenes* was inoculated into 200 ml semisynthetic medium as preculture and incubated in shaker incubator with 120rpm during 24 hours. Concentration of inoculums tested were 10%, 20% and 40%. Mediums were inoculated aseptically by precultures according to the treatments. Then, *C. indologenes* was grown by batch system and agitated. Five ml of bacterial culture were taken by sterile syringe every 24 hours. It started at the injection of preculture into growth media, and stopped after the bacterial growth reached stationary phase.

Analytical methods

Samples were centrifuged at 3326 g for 30 min to separate supernatant and cell mass. The supernatant was used for determining sulfanilic acid, 1-amino-2-naphthol, and COD concentrations. The concentration of sulfanilic acid and 1-amino-2-naphthol were determined by HPLC (6,7). The concentration of COD was determined by colorimetric method using a standard curve at 600 nm (8). Cell mass concentration was determined by turbidimetric method using a standard curve of cell absorbance at 500 nm against cell mass. All measurement of absorbance was done in a Shimadzu UV–Vis 1201 Spectrophotometer.

RESULTS AND DISCUSSION

The results showed that *C. indologenes* could grow on filtrate's culture of *E. faecalis*. During its growth, biomass concentration increased and sulfanilic acid and 1-amino-2-naphthol concentration decreased (Fig. 1). The increasing of biomass concentration was

followed by decreasing of COD concentration too. The decreasing of COD concentration which indicated organic content of medium has a significant relationship with consumption of organic matters. From growth curve of *C. indologenes* (Fig. 1), we could see that in the all treatments of inoculum concentration, decreasing of sulfanilic acid and 1-amino-2-naphthol content will followed by decreasing of COD concentration. These results answer assessment of previously experiments (4,5) which presumed *C. indologenes* could use intermediate products of orange II degradation as their growing substrate.

Commonly, bacterial growth will refer lag, logaritmic, and stationary phase, but in the growth of *C. indologenes* relatively didn't show lag phase (Fig. 2). These phenomenon might caused of *C. indologenes* was cultured in sulphanilic acid containing medium previously, so they didn't need long time to grow. Lag phase of growth only occurred in 40% inoculum culture caused there was take palace competition in the high cell density. In the high cell density population will occurred competition for resources (9).

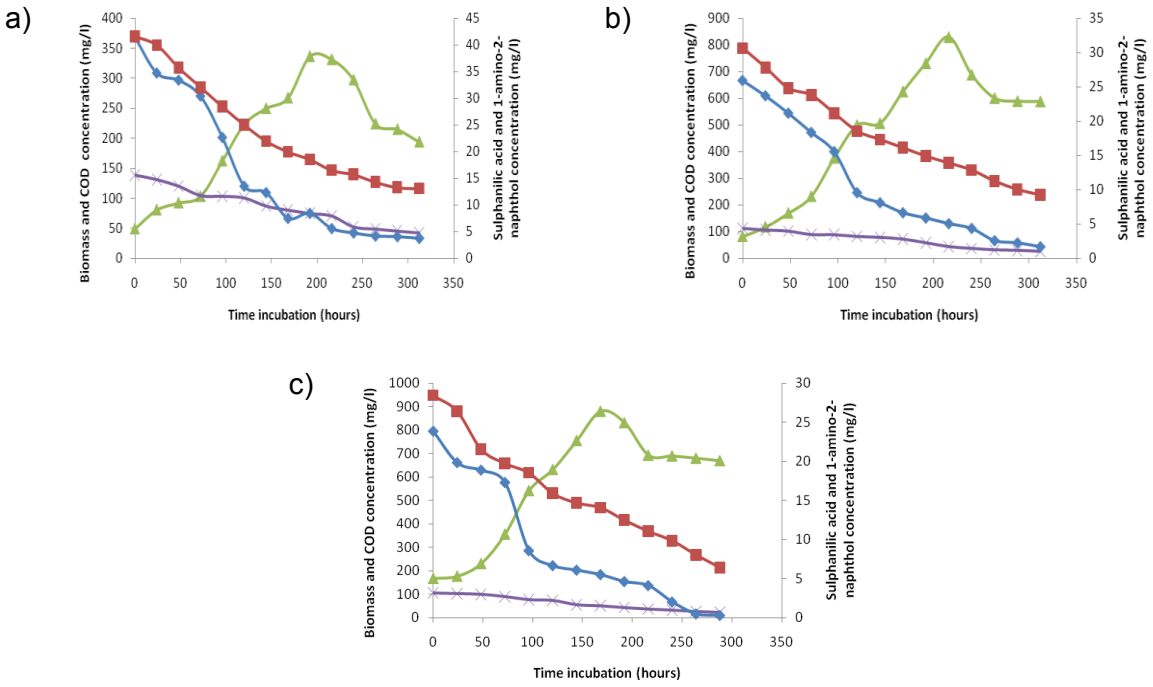

Fig.1. The change of cell biomass of *C. indologenes* (%), COD (x), sulphanilic acid (!), and 1-amino-2-naphthol (∀) concentration on the medium with 10 (a), 20 (b), and 40 (c) % inoculum

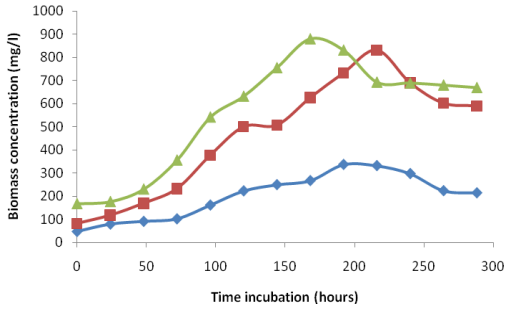

Fig.2. The growth of *C. indologenes* in the medium with 10 (∀), 20 (!), and 40 (%) % inoculum

The results showed that *C. indologenes* showed the optimum growth in medium with 20% inoculum. In this culture, specific growth rate of *C. indologenes* was the highest, i.e 0.015/hour (Table 1). In this culture, sulphanilic acid and 1-amino-2-naphtol consumption were the highest. So, this inoculum concentration could give best condition for growth of *C. indologenes* and consumption of intermediate products of orange II degradation.

Existence of intermediate products consumption give evidence ability of *C. indologenes* to use intermediate products of orange II decolorization for bacterial growth, despite of these intermediate products were resistant to microbial degradation. It was proven to be decreasing of sulphanilic acid and 1-amino-2-naphthol concentration during cell growth phase. Several researchers also reported that *Sphingomonas* sp. and *Ps. paucimobilis* could use sulphanilic acid as sole carbon source (10,11).

Table1. The parameter of *C. indologenes* growth in culture with several inoculum concentrations

The growth parameter	Unit	Inoculum concentration (%)		
		10	20	40
Specific growth rate	/hour	0,010	0,015	0,011
Biomass production	mg/l	11,1	29,97	31,28
Sulphanilic acid consumption	mg/l	33,69	37,06	36,61
1-amino-2-naphthol consumption	mg/l	24,23	28,88	28,68
COD consumption	mg/l	68,44	100,27	92,44

CONCLUSIONS

Based on experimental results, we concluded that *C. indologenes* could growth on filtrate's culture of *E. faecalis* which continuously growing on orange II containing medium. The inoculum concentration which gave the best growth of *C. indologenes* was 20%. It was caused by in this inoculum concentration bacterial growth faster and consumes intermediate products higher than 10 and 40 % inoculum concentration.

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P-PD02

PLANT GROWTH PROMOTING BACTERIA ISOLATED FROM MERAPI, TIMIKA, PALANGKARAYA AND SUKABUMI

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not presented

P-PD03

IDENTIFICATION, COMPOSITION AND CHARACTERIZATION CAROTENOID PIGMENTS IN SWEET POTATOES (*Ipomoae batatas* L.)

Helena T. Tuririday[1] Martanato Martosupono[2] Soenarto Notosoedarmo[2] Lisiard Dimara[3]

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Email; vona2003@yahoo.com

not presented

P-PD04

PLANT RESPONSE AND NITRATE REDUCTASE ACTIVITY IN VIVO ON RICE (*Oryza sativa* L.) CULTIVARS IR-64 TO BIOFERTILIZER APPLICATION AND DROUGHT

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ABSTRACT

Water stress affects growth, development and productivity. Biofertilizer expance an absorbtion zone of root which increase resistance of plant against drought. The objective of this research was to study plant response and Nitrate Reductase Activity in vivo on flag leaf to drought stress and biofertilizer application. Research was conducted in green house using complete randomized design (CRD) with 2 factors. First factor is field capacity 100%, 75%, 50% and 25%). Second factor is biofertilizer doses (0 liter/Ha, 5 liter/Ha, 10 liter/Ha and 15 liter/Ha). Each combination with 5 replications. The growth parameters measured were plant height, leaf number, tiller number fresh and dry weight and length of root. Anatomy parameters were stomata index, diameter of root, cortex, stele and xylem cells. Biochemistry parameters were chlorophyll content, prolin acumulation and Nitrate Reductase Activity. Productivity parameters were number of panicle and number of grain per plant. Environtment parameters were temperature, pH, humidity and light intencity. The data was analyzed with ANOVA and followed by DMRT at significant level of 5%.

The result revealed that the growth parameters, diameter of cortex, number of panicle and number of grain per plant decreased due to water capacity decreasing; length of root, diameter of xylem cell induces by the lower water capacity; chlorophyll content increased by the doses of biofertilizer; biofertilizer seems to elevate the effects of stress due to water stress and NRA was not decreased by water capacity and the doses of biofertilizer.

Keywords : drougth, biofertilizer, rice cultivars IR-64, NRA.

P-PD05

ISOLATION OF CHITINOLYTIC FUNGI FROM RHIZOSPHERE

Nur khikmah[1)], Sebastian Margino[2)], and Rina Sri Kasiamdari[3)]

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ABSTRACT

The aim of this research was to obtain fungi as biological control agents of pathogenic fungi. The isolation of fungi was done using *surface plating* on colloidal chitin agar (CCA) medium. Selection is based on the hidrolysis activity on CCA medium and specific activity of chitinase in liquid chitin medium. Specific activity of chitinase was measured on substrates reduction.

The result of isolation was found 33 isolates. Based on the hidrolysis activity, it was obtained 13 isolates with hidrolysis activity ≥ 1,50. Three among 13 isolates have higher chitinase spesific activity than *Trichoderma viride* as reference isolate (210,14 U/mg). RTW1 isolate had the highest chitinase specific activity 444,24 U/mg. Therefore, it was regarded a potential as biological control agents.

Keywords : chitinolytic fungi, chitinase enzyme, biological control

INTRODUCTION

Chitinolytic fungi have been described have high mycoparasitic ability in inhibiting phytopathogenic fungi. In those intercations, chitinolytic fungi produce chitinase enzyme that degrade cell wall of phytopathogenic fungi. Chitin is main stuructural component of fungi cell wall, which ranges from 22-40% [2]. The aim of this study was to obtain chitinolytic fungi as biological contol agents.

MATERIAL AND METHODS

Soil samples were taken from rhizhosphere of solanaceae. Isolation of fungi was done using surface plating method on colloidal chitin agar (CCA) medium. Qualitative selected based on the hidrolysis activity on CCA medium, by comparing clear zone diameter around colony with colony diameter [2]. Selected isolates were quantitative assayed specific activity of chitinase (chitinase activity per mg protein) in liquid chitin medium. The enzyme separated from medium by sentrifuge at 3000 *rpm*, 4^{o}C for 30 minutes. The supernatant was used as a source of crude enzymes. Chitinase activity was measured on substrates reduction of colloidal chitin using spectrophotometric method. Protein concentration was determined according to Bradford Method [4].

RESULT AND DISCUSSION

The total number of fungi obtained were 33 isolates. All of those isolates can produce clear zones around colonies on CCA medium. Clear zone is an indicator of chitin degradation by chitinase in solid medium [1]. Of those 33 isolates, 13 isolates have hidrolysis activity ≥ 1.50 (Table 1). Based on quantitative selection showed that 3 isolates were had higher specific activity than *Trichoderma viride* 6128 FNCC (210.14 U / mg) as reference isolates (Table 2). The results showed that there was no correlation between hidrolysis activity with specific activity of chitinase. It was assumed due to the use of different medium. The growth of fungii in liquid medium was rapidly than the growth on solid medium [5]. RTW1 isolate had the highest chitinase specific activity 444.24 U/mg. Therefore, this isolate had a potency as biological control agents.

Table 1. Hidrolysis activity of chitinolytic fungi on colloidal chitin agar medium

No	Isolate Code	Ø Clear zone (cm)	Ø Colony (cm)	Hidrolysis activity
1	RTM3	3,75	1,80	2,08
2	RTM4	7,10	4,00	1,78
3	RCM4	6,00	4,00	1,50
4	RTW1	3,60	1,80	2,00
5	RJM2	2,10	1,00	2,10
6	RJM5	6,50	2,93	2,21
7	RJM6	5,90	2,80	2,11
8	RKT6	2,50	1,50	1,67
9	RKD1	7,90	5,00	1,58
10	RKD2	5,60	3,70	1,51
11	RKD3	8,50	5,40	1,57
12	RLF1	3,10	1,40	2,21
13	RLF3	5,90	2,80	2,11

Table 2. Chitinase specific activity of chitinolytic fungi

No	Isolate Code	Protein Concentration (mg/ml)	Chitinase activity (U/ml)	Chitinase spesific activity (U/mg)
1	RTM3	0,0829	32,4468	391,40
2	RTW1	0,0637	28,2979	444,24
3	RJM5	0,1148	26,2766	228,89
4	*T.viride*	0,0893	18,7660	210,14

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P-PD06

Interaction between *in vitro* culture *Aquilaria* spp. shoots and Hyphomycetes (*Acremonium* spp. and *Fusarium* spp.) from Bangka

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ABSTRACT

Agarwood resin was formed as a response of agarwood tree (*Aquilaria* spp.) towards hyphomycetes infection. Eight isolates of hyphomycetes i.e 5 isolates *Acremonium* spp. and 3 isolates *Fusarium* spp. from Bangka's agarwood were interacted in dual culture with shoot of *Aquilaria crassna* klon AC8, *A. malaccensis* klon PD, *A. microcarpa* klon CD in 50% modified-Murashige-Skoog agar media. Hyphomycetes response towards the presence of shoot and shoot response towards hyphomycetes, fragrance index and the presence of terpenoid substance were observed for 4 weeks interaction. The presence of shoot affected the growth of hyphomycetes. All hyphomycetes caused shoot death of all clones at fourth week of interaction. *Acremonium* L, *Fusarium* A, B, and D induced fragrance formation in different shoot clone. The maximum fragrance was in moderate level (score 1 out of 3). Of those isolates, only *Acremonium* L might induce fragrance formation (1,22-1,33) in all clones of shoot. Sterol was detected both in interacted and control shoots of all clones. Sterol in *A. crassna* generally increased due to dual culture, but not in *A.malaccensis* and *A. microcarpa* in dual culture.

Keywords: *Acremonium* spp., *Fusarium* spp., *Aquilaria* spp., shoot , dual culture

P-PD07

Growth Promoting of Soybean Plant By Coinoculation *Pseudomonas* sp. and *Bacillus* sp. Strain with *Bradyrhizobium japonicum*

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ABSTRACT

Plant growth promoting rhizobacteria (PGPR) are root colonizing bacteria that exert beneficial effects on plant development. This study was designed to examine *Pseudomona*s sp. and *Bacillus* sp. ability to enhance soybean growth. Isolates used in this study were *Pseudomonas* sp. Crb 3, Crb 17, and *Bacillus* sp. Cr 24, Cr 66. These strains were coinoculated onto soybean plants with *B. japonicum* under greenhouse conditions. *Pseudomonas* sp. and *Bacillus* sp. were observed on soybean seedling as a plant growth promoting. Coinoculation of *Pseudomonas* sp. and *Bacillus* sp. strains with *B. japonicum* were tested for their in vitro-antagonistic activities. All isolates did not show any antagonistic activity each other. Coinoculation with *Pseudomonas* sp. Crb 17 and *Bacillus* sp. Cr 24 increased nodule number, dry weight of root, and dry weight of shoot. Therefore, we recommended that *Pseudomonas* sp. Crb 17 and *Bacillus* sp. Cr 24 would be suitable for use as a PGPR.

Keywords: PGPR, *Pseudomonas* sp., *Bacillus* sp.

INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are root colonizing bacteria that exert beneficial effects on plant development [1]. PGPR belonging to *Pseudomonas* sp. and *Bacillus* sp. were isolated from the rhizosphere of soybean. This study was designed to examine *Pseudomona*s sp. and *Bacillus* sp. ability to enhance soybean growth.

MATERIAL AND METHODS

Isolates used in this study were *Pseudomonas* sp. Crb 3, Crb 17, and *Bacillus* sp. Cr 24, Cr 66. Coinoculation of *Pseudomonas* sp. and *Bacillus* sp. strains with *B. japonicum* were tested according to Kirby-Bauer method [2] for their in vitro-antagonistic activities. These strains were coinoculated onto soybean plants with *B. japonicum* under greenhouse conditions [3]. *Pseudomonas* sp. and *Bacillus* sp. were observed on soybean seedling as a plant growth promoting. During the growth process under greenhouse condition, the plants were watered with N-free solution [4], The plants were harvested at 42 d after inoculation. After harvesting, data on nodule number, shoot weight and root weight were collected. All the samples were weighed after not less than 48 h of drying at 70 °C.

RESULT AND DISCUSSION

According to in vitro antagonistic activities, all isolates did not show any antagonistic activity each other (Data not shown). PGPR belonging to *Pseudomonas* sp. Crb 3, Crb 17, and *Bacillus* sp. Cr 24, Cr 66. Co-inoculated with *B. japonicum.* Result of nodule number, dry weight of root, and dry weight of shoot are given in Tabel 1. Nodule number and total dry weight under greenhouse condition were significantly increased by *Pseudomonas* sp. Crb 3, Crb 17, and *Bacillus* sp. Cr 24, Cr 66. Co-inoculated with *B. japonicum*. Nodule number in Crb 17 + Cr 24 + Bj 11 strains was increased by 83.8%, compared to Crb 3 + Cr 66 + Bj 11 strains. Total dry weight in treatment containing Crb 17 + Cr 24 + Bj 11 strains was increased by 31.7%, compared to Crb 3 + Cr 66 + Bj 11 strains. Coinoculation of *Pseudomonas* sp. and *Bacillus* sp. strains with *B. japonicum* had beneficial effects on soybean plant growth. Presumably these *Pseudomonas* sp. and *Bacillus* sp. strains increased nodule number and total dry weight of soybean plant because of their IAA (*indole acetic acid*) and siderophore production. In fact, these *Pseudomonas* sp. and *Bacillus* sp. strains produce IAA (*indole acetic acid*) and siderophore [5]. Crb 17 + Cr 24 + Bj 11 strains were given the best effects, presumably because of their optimum IAA concentration on soybean plant growth. Therefore, we recommended that *Pseudomonas* sp. Crb 17 and *Bacillus* sp. Cr 24 would be suitable for use as a PGPR.

Table 1. PGPR Strains effects on nodul number of soybean seedling grown for 42 days under green house

No	Strains	Nodule number (plant^{-1})	Dry weight (g plant^{-1})		
			shoot	Root	Total
1	Control	0.00a	0.221a	0.072a	0.293a
2	Bj 11	21.3b	0.357b	0.090b	0.447b
3	Crb 3 + Cr 66 + Bj 11	30.3c	0.603c	0.138c	0.741c
4	Crb 17 + Cr 24 + Bj 11	55.7d	0.781d	0.195d	0.976d

*DMRT at the 5% level

Acknowledgement

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P-PD08

Optimation of Staining method of Pancreatic β cell granules on Normal and Diabetic White Rats (*Rattus norvegicus* L.) with Victoria Blue

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ABSTRACT

Alloxan monohydrate is often used for diabetic induction. These substances can damage pancreatic β cells of animal test, resulted changes of β cell islets of Langerhans. These changes can be seen through observation of slides stained with Victoria Blue. Specifically, changes of difference absorption and extensive dye stained the islets of Langerhans. The purpose of this experiment was to determine the optimum staining time and to know the changes in β cell islets of Langerhans with Victoria Blue staining in normal and diabetic rats. The research was done using male white rats, Wistar strain, aged 2 months, weight of about 150-200 grams. Pancreatic organs were taken from normal and diabetic rats as animal models, three rats for each. Rats were induced with alloxan monohydrate single dose of 130 mg /kgbw. Pancreas organ was fixed using Bouin solution. Microanatomical slides were performed with paraffin method and stained with Victoria Blue and Phloxine. The research data were analyzed descriptively. The results showed that the optimum staining time in Victoria Blue was for about 45 minutes. The results showed the color of Victoria blue staining in normal rats was sharper and more widespread because of Victoria Blue dye granules of insulin. Therefore, up to 45 minutes the Victoria Blue staining can differentiate between normal and diabetic condition on histological slides of pancreatic β cells.

Keywords: pancreatic β cells, islets of Langerhans, paraffin method, Victoria Blue, alloxan monohydrate

Introduction

Diabetes is a major degenerative disease in the world today, the number of sufferers continues increasing, and thus it encourages an increase in experimental search for better new drugs to treat this disease[1]. A diabetes experiment using induced rats was one of the easiest and the most secure method to search for new drugs [2]. Alloxan routinely used to induce diabetes in experimental animals since its ability to induce diabetes was known. Alloxan be toxic to pancreatic β cells that are often used for the induction of diabetes in the study [3]. Alloxan rapidly and selectively accumulated in pancreatic β cells and was known to induce DNA chain of rat pancreatic islets [4]. Alloxan effectively damages the pancreatic β cells that caused diabetic condition in test animals, can caused insulin dependent diabetes mellitus in animals with characteristics similar to type 1 diabetes mellitus in humans [3,5].

Damage or changes in pancreatic β cells because of induced alloxan can be viewed or analyzed by using microanatomical slide of pancreatic islets of Langerhans. To see the damage of these cells special staining can be used. [6] It is suggested that something that can do staining of insulin granules of pancreatic β cells was Victoria Blue with Phloxine as

counter-stain, with a range of 15 minutes to 24 hours. The range of staining was very long time, and it still has not had a certain optimum time yet. In terms of staining, the duration of the time is still within the range so that the results could not be ascertained. Thus, in a study to see the damage of pancreatic β cells, it needs a lot to trials. Based on the experience of staff of histology and cell biology laboratory Faculty of Medicine, Universitas Gadjah Mada , the time of Victoria Blue to stain ranges from 15-30 minutes. To obtain optimal results and to know the optimum time in the pancreatic β cell staining with Victoria Blue, it is required a preliminary study. Therefore, specific research is needed to determine the right duration to obtain the best staining.

Materials and Methods

Induced Animal Test

Male white rats (*Rattus norvegicus* L.), Wistar strain, aged 2 months, weight of about 150-200 grams of as many as 10 heads obtained from preclinical unit LPPT UGM used as test animals. Five white male rats made hyperglycemic by induced alloxan monohydrate solution 130 mg dose / kgbw intraperitonially in physiological fresh saline solution 0.9% volume of 1ml. Five normal rats injected with physiological saline solution. Normal group were given distilled water, diabetic group given un-distilled water, for 30 days.

Slide Microanatomical

After 30 days of treatment, three animals from each group were randomly anesthetized using chloroform. Peritoneum dissected animal and organ pancreas were taken and then next they were fixed in Bouin solution. Pancreatic organs were processed for histological preparation through paraffin method and cut of about 6 µm. The slides which have not stained yet latter will get treatment in a different time when immersed in the Victoria Blue 15, 30 and 45 minutes and repeated 3 times.

The staining steps were as followed: results of sectioning soaked in Xylene I and II for 8 minutes, followed by absolute alcohol for 8 minutes, and leveled alcohol of 96%, 90%, 80% and 70% for 1 minute and washed using running tap water and distilled water. Then slide into the mordant solution for 24 hours at 37 º C. After that, slide washed under running tap water until they become colorless and washed with distilled water again. Slide sections were placed in the oxidation mixture for 3 minutes, washed well in water. Section was placed in sodium bisulphite for 1 minute or until clear. Then, it washed under running tap water for 3 minutes and then rinsed with distilled water. Section dipped in 70% alcohol for 1 minute, immersed in a solution of Victoria Blue dye for 15, 30 and 45 minutes. After that, washed with distilled water and then stained with aqueous solution of Phloxine for 90 seconds. Then,

wash in distilled water and dehydrated in 95% alcohol , absolute alcohol was continued for 1 minute. Clear with xylene I, II and III for 5 minutes. Mount in canada balsam and covered with glass cover. After that analyzed descriptively.

Results and Discussion

Having done observation and analysis descriptively in each pancreas preparations, the results obtained was pancreatic β cell staining with Victoria Blue and phloxine as counterstain group of normal rats as shown by Figure 1(a,b and c), and the group of diabetic rats in Figure 2 (a, b, and c).

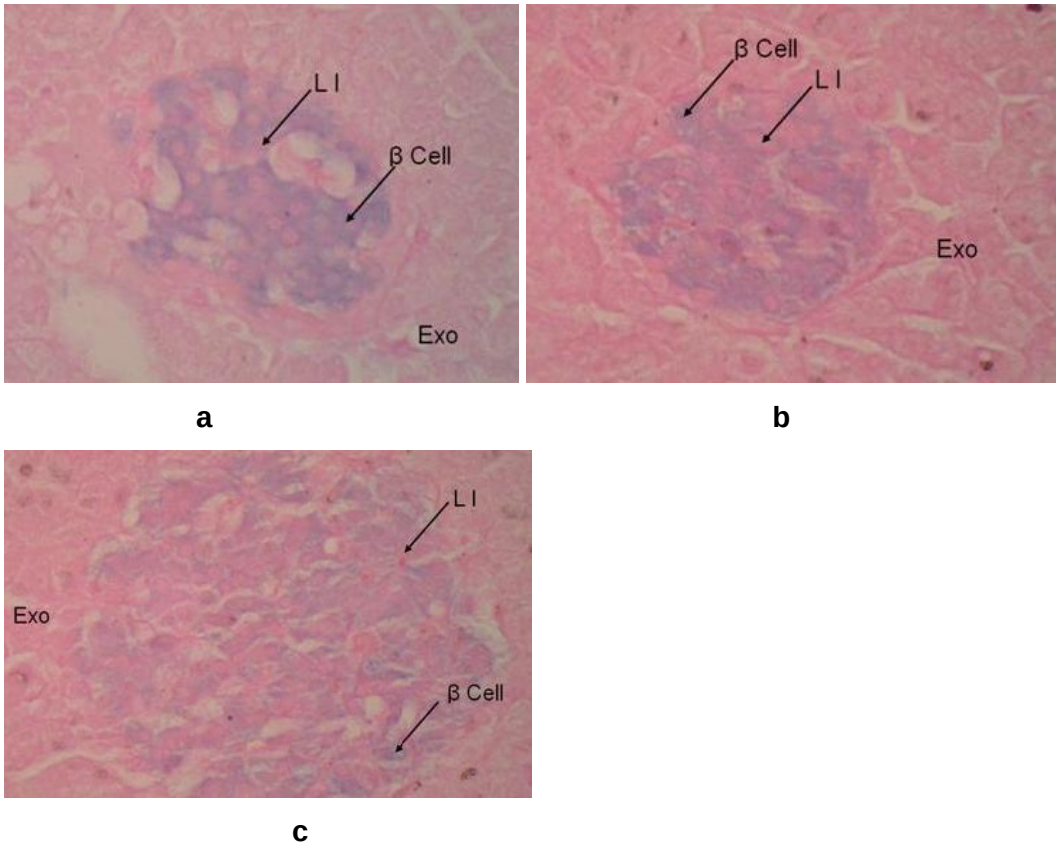

Figure 1. Micronatomy of Langerhans islets in normal rats pancreas were stained with Victoria Blue. Magnification is 400 ×. (a) stained for 15 minutes; (b): 30 minutes and (c): 45 minutes. (LI) : Langerhans islets; (Exo): Exocrine areas

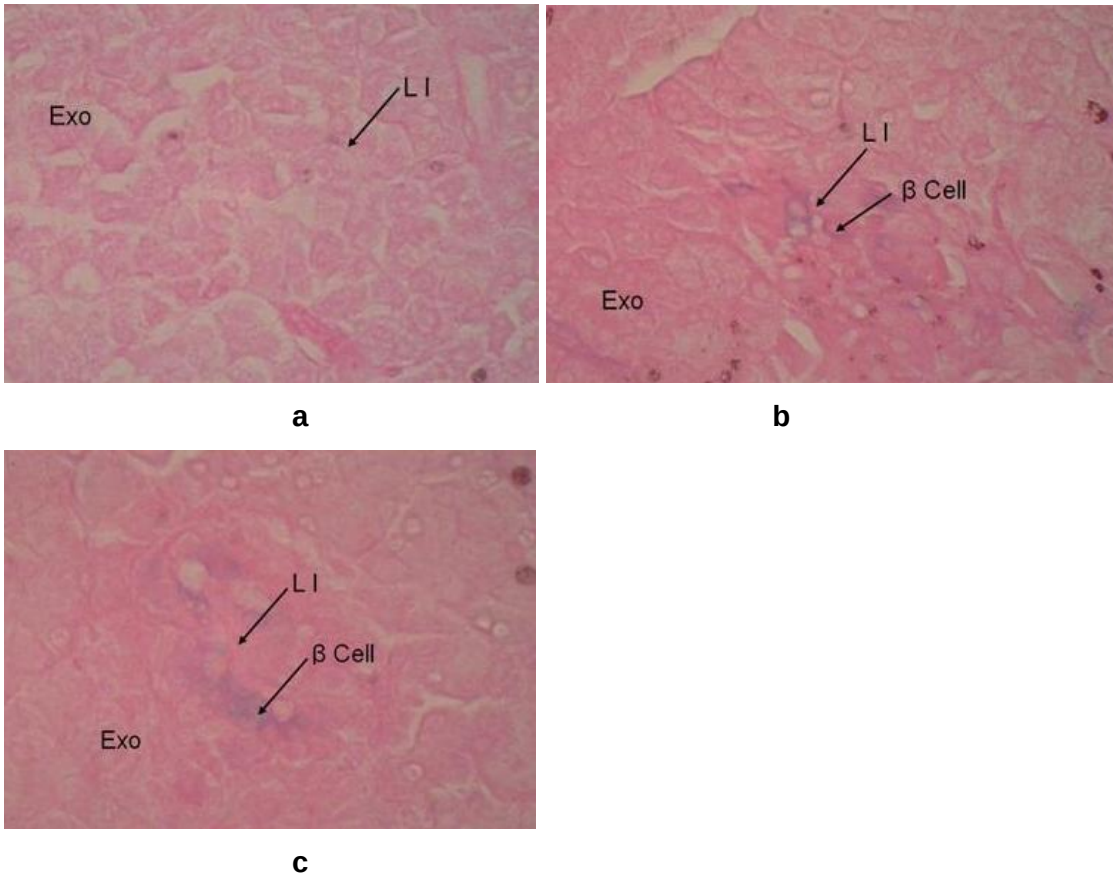

Figure 2. Micronatomy of Langerhans islets in diabetic rats pancreas were stained with Victoria Blue. Magnification is 400 ×. (a) stained for 15 minutes; (b): 30 minutes and (c): 45 minutes. (LI) : Langerhans islets; (Exo): Exocrine areas

Based on Figures 1 and 2, the results of staining in the group of normal mice showed that the region of Langerhans stained was wider than in the group of diabetic rats. In 15 minutes of staining, the color of Victoria Blue would fade back when rinsed with water so it seems that it was like not stained, whereas at 45 minutes the color of Victoria Blue was sharper andmore widespread than at 30 minutes.

Such results are caused by induction alloxan monohydrate in experimental animals to produce hyperglycemia conditions which selectively destroy β cells of pancreatic islets of Langerhans. Whereas the exocrine cells showed no difference in normal and diabetic groups. This can be caused by alloxan properties that do not damage the pancreatic cells as a whole, but they are selective toxic that damage pancreatic β cell only[7]. The Damage of β cells in the pancreas of diabetic group is higher because the conditions of diabetes or hyperglycemia do not inhibited by certain substances such as drugs. The damage of pancreatic β cell leads to reduce insulin granules carrier in pancreatic β cells. This selective toxic nature is caused by the alloxan which accumulate in particular through the glucose

transporter GLUT 2 [8]. Thus, fewer group of diabetic β cells can be stained by the Victoria Blue.

Alloxan induction will increase the release of insulin and proteins from pancreatic β cells but give no effect on glucagon secretion. Alloxan cytotoxic action is mediated by free radicals. Dismutase underwent by radical change into hydrogen peroxide. The action of free radicals with high stimulation increases cytosolic calcium concentration that causes destruction of β cells rapidly[8]. Research on the mechanism of action in vitro showed that alloxan expenditure induces calcium ions from the mitochondria resulting in impaired cell oxidation process. The exit of calcium ions from the mitochondria is disrupted cell homeostasis efforts, so that initiate cell death[5].

[4] states that the action of alloxan damaged the DNA nucleus of pancreatic β cells using the accumulation of oxygen radicals or alkalization of DNA. The efforts of β cell to repair DNA appears as a response to suicide, because the repairing induction of DNA involves the activity of poly ADP-ribose polymerase using the substrate NAD^+ cell, consequently levels of NAD^+ intracellular dropped dramatically and inhibits the activity of cellular synthesis and secretion of insulin, and ultimately causes pancreatic β cell death.

Diabetic group had a greater β cell damage than the normal group, so that β cells are able to absorb very little color of Victoria Blue than in the normal group. This is because Victoria Blue seems specifically dye insulin granules of pancreatic β cells[6].

Acknowledgments

We would like to thank to the staff of histology and cell biology laboratory, faculty of medicine, Universitas Gadjah Mada University who helped dye material procurement. The conclusion of this study is the optimum time for staining the pancreas with Victoria Blue is 45 minutes. Victoria Blue staining can be used to see the difference between β cells of the pancreas in normal and diabetic conditions.

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P-PD09

Effectivity of Oryzalin on Seed Germination of Garden Balsam (*Impatiens balsamina* L.)

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ABSTRACT

The research was conducted to induce seeds germination by soaking seeds in oryzalin solution. Oryzalin is a herbicide product that can be applied to induce polyploid in plant. Seeds of *Impatiens balsamina* were soaked in oryzalin concentrations (0%, 0.01%, 0.02%, 0.03%, 0.04%) for 0 hour, 12 hours, 24 hours, 36 hours and 48 hours. After 7 days of germination, germinated seeds was counted to know the LD 50. Germinated seeds after soaking in 24, 36 and 48 hours in oryzalin treatment are higher than 50%. Soaking seeds for 48 hours showed the highest percentage in germination for all treatment concentration of oryzalin. However, 0.01 % oryzalin concentration showed as optimum concentration for seeds germination. After 7 days of germination, control treatment and 0.01% oryzalin showed the growth of roots and hypocotyl. In contrast, concentration of oryzalin higher than 0.01 % only showed the growth of hypocotyl, while the roots was stunted. Oryzalin can delay the growth of roots due the radicle retardation.

Keywords: *Impatiens balsamina,* oryzalin, seed germination

Introduction

Impatiens balsamina is a flowering plant that has been used for offering in Balinese ceremonial. The flower can be used for colouring agent, for example nail polish. Parts of the plants are also give advantage for health due to antioxidant in anthocyanin pigmen of flower. The colour of flowers are red, pink, purple and white. Farmers usually grow the plants in the field. During harvesting, when it occurs in wet season whereas rainfal is high, the flowers is easy to decay because the flower petal is thin. To overcome the problem, oryzalin is applied for *Impatiens* seedlings.

Oryzalin is a herbicide that can affect microtubulin performance. Oryzalin is also known as antimitotic inhibitor, so it can be applied to induce polyploidy in plants. Ploidy levels (triploid, tetraploid, hexaploid or mixoploid) can affect crossability process, fertility of hybrids, plant vigor, and gene expression (Ranney, 2006). Oryzalin was more efficient in polyploid induction (18% of the surviving plants at 0.005% with the 24 hr exposure) on *Rhododendron* sp.(Jones, *et. al,* 2006). The use of mitotic inhibitors often produces polyploids. Polyploid plant has enhanced the size of leaves and flowers. Thick flower may give benefit in order to overcome problem due to the rain.

There are some techniques to apply oryzalin in plant. One of the method is treat the seeds in oryzalin solution to know the effect of oryzalin in germinated seeds.

Materials and Methods

Seeds of *Impatiens balsamina* was soaked in oryzalin solution (0%, 0,01%, 0,02%, 0,03%, 0,04%) for 12, 24, 36 and 48 hours. After soaked, the seeds were germinated in Petri Dishes based on the treatment combination. Each day, number of germinated seeds were counted. Percentage of seed germination was calculated after 7 days.

Results and Discussion

After 7 days of germination, each treatment showed different effect on seeds. The results of this study demonstrate that the method of applying soaking seeds for 24, 36, and 48 hours could increase germination until 50% for all treatment, including control (0%). Imbibition occured for the seeds during soaking thus physiological process started including activated of hormon to start germination. Based on Figure 1, soaking seeds for 48 hours showed the highest percentage for germinated seeds.

Oryzalin 0.01 % was optimum for germination test on *Impatiens* seeds, because by increasing time of soaking, there were an enhancing percentage in germination. In addition, germinated seeds showed a continue in growth of hypocotyl and roots for seeds soaked in 0.01% Oryzalin.

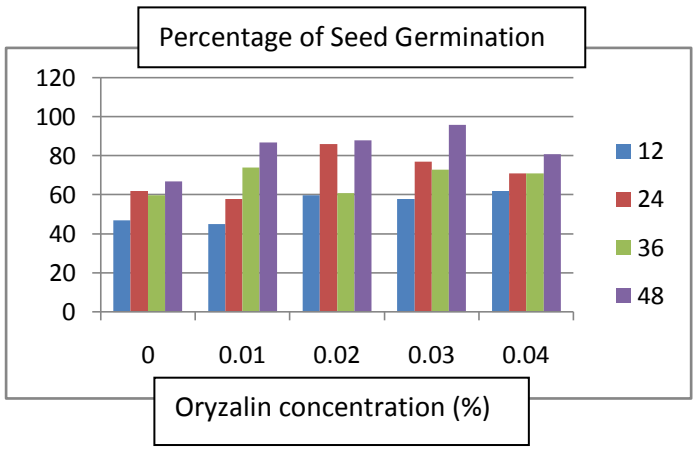

Figure 1. Germinated Seeds

The growth of germinated seeds was inhibited by increasing of oryzalin concentration. The radicle and cotyledon showed stunted growth for 6 days after germination (Fig. 2).

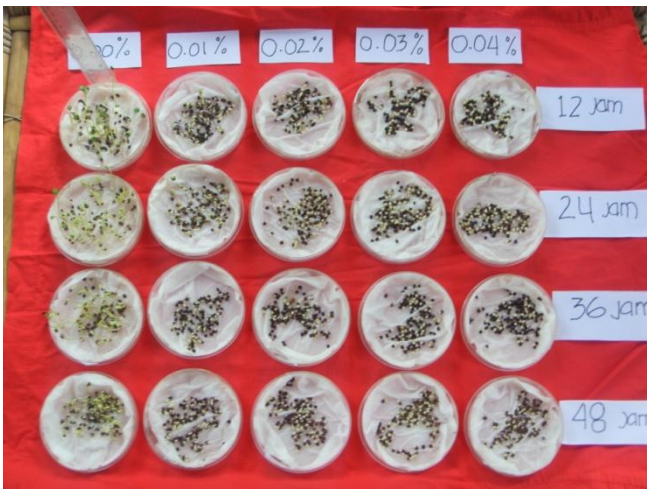

Figure 2. The Growth of Seedlings

Radial swelling on radicle is also stimulated. Cells in the zone of division were slightly more sensitive to oryzalin than were cells in the zone of pure elongation (Baskin *et al*., 1994).

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P-PD10

EFFECTIVENESS OF PAPAYA JUICE (*Carica papaya* Linn.) TO DELAY SENESCENCE OF CUT ROSE FLOWER (*Rosa hybrida* L.)

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ABSTRACT

Rose (*Rosa hybrida* L.) is a cut flower which possess value as top-rank-sale of cut flower in Indonesia. This is because roses have beautiful morphology and diverse varieties. Cut rose flower freshness can not last long. Therefore, there should be a way to maintain freshness or delay its senescence. This experiment was aimed to determine the effectiveness of papaya juice (*Carica papaya* Linn.) in delaying senescence of the cut rose flower (*R. hybrida* L.) and to know the optimum concentration of papaya juice (*C. papaya* Linn.) for maintaining freshness of cut flower roses (R. *hybrida* L.). This experiment used cut rose flowers (*R. hybrida* L.) whose condition are still fresh and half-bloom (two days old after cutting from the parent plant). Solution in the form of papaya juice (*C. papaya* Linn.) with concentration of 0%, 10%, 20% and 50% are used to soak the rose stems by pulsing method, within 3 hours. After that aquadest was used as holding solution for roses. Each treatment used 5 replicates. The parameters observed were fresh flowers age, the percentage of petals senescence, percentage of bent neck flower, percentage of leaves senescence and transpiration rate. The Results showed freshness of cut rose flower (*R. hybrida* L.) is the longest in the treatment of juice papaya (*C. papaya* Linn.) with 10% and 20% concentration which was 10 days whereas in the control (0%) only 5 days. Percentage of petals senescence, percentage of bent neck flower, percentage of leaf senescence and transpiration rate in the treatment of 10% and 20% significantly different from control (0%). Based on this research it can be concluded that the pulsing method with papaya juice (*C. papaya* Linn.) can maintain the freshness of cut flower roses (*R. hybrida* L.) 5 days longer than controls and the optimum concentration of papaya juice (*C. papaya* Linn.) is 10% or 20%.

Keywords : rose (*Rosa hybrida* L.), papaya juice (*Carica papaya* Linn.), pulsing, senescence.

Introduction

Rose (*Rosa hybrida* L.) is the most popular cut flower in Indonesia due to its beautiful morphology and various cultivars. The fresh cut roses can not survive for a long time. A good flower display with freshness of flowers become the expectations of the consumens.The flower senescence is characterized by the freshness less of the flower, not bright in colors and the change of color as well as the condition of the crown began to weak due to the low of water level caused by transpiration. The level of cut rose flower freshness are influenced by temperature, humidity, light intensity, accumulation of ethylene, transpiration, respiration, bruising or injury, lack of nutrition and metabolic processes. By using water, cut flowers rose only survive in fresh condition for 4-5 days. Therefore, there should be an alternative way to maintain freshness or delay its senescence. Papaya fruit contains many kinds of nutrients that can be used by cut flowers to survive without soil and enough media. Allocating some refreshing solution using pulsing or holding method to the cut flowers are intended to supply the energy needs in the advanced development during

transportation, storage, and display. The aims of this experiment was to determine the effectiveness of papaya juice (*Carica papaya* Linn.) in delaying senescence of the cut rose flower (*R. hybrida* L.) and know the optimum concentration of papaya juice (*C. papaya* Linn.) for maintaining freshness of cut roses (R. *hybrida* L.).

Materials and methods

This experiment used a fresh cut rose (*R. hybrida* L.) obtained from the florist in Kota Baru Yogyakarta with a half old blooms and two days old. Flowers are chosen in the same conditions and relatively same in size including the length of the stalk, leaf number, and other morphology. The concentration levels of ripe papaya juice (C. papaya) are made in many kinds variety such as 0% (control), 10%, 20% and 50%. The ripe papaya juice 10% is made by blending papaya fruit without water about 100 ml and adding of aquadest until the volume of solution becomes 1 liter. Papaya juice solution 20% is made by blending ripe papaya without water about 200 ml and adding of aquadest until the volume of this solution become 1 liter. Solution of 50% papaya juice is made by blending ripe papaya without water about 500 ml and adding of aquadest until the solution volume of 1 liter. Aquadest is used to control the treatment. Each treatment conducted in 5 replications. In this study used the pulsing method to soak the cut rose flower with a solution containing distilled water or papaya juice with different concentrations for 3 hours. Next cut flowers placed in a bottle experiment filled with aquadest was used as holding solution for cut rose flowers. Aquadest medium was replaced every two days. The deadline for the experiment is determined from the treatment started to show the senescence and no longer worthy of display. The percentage of crown senescence is scored by 1 to 4, ie score 1 = less than 10%, score 2 = 10-20%, score 3 = more than 20% and score 4 = not worthy of display. The percentage of bent neck flower, scored by the score of 1 to 4, ie score of 1 = erect; score 2 = ducking less than 45 °; score 3 = ducking over 45 ° and score 4 = not worthy of display. Percentage of leaf senescence, scored by 1 to 4, ie score of 1 = fresh, score 2 = slightly wilted, 3 = wilting, and score 4 = dry. Daily transpiration velocity is assumed by the amount of solution absorbed (ml stalk / day), ie by calculated an average reduction of aquadest every day. Temperature and humidity of the room. The data obtained were tabulated, then to determine whether there is a real difference in the samples tested, analyzed by analysis of variance (ANOVA). If there is a real difference then the difference determined by Duncan's Multiple Range Test (DMRT) a direction on the level of 5% (Hossain *et al*., 2007).

Result and discussion

The Results showed freshness of cut flower rose (*R. hybrida* L.) is the longest in the treatment of papaya juice (*C. papaya* Linn.) with 10% and 20% concentration which was 10 days whereas in the control (0%) only 5 days. Percentage of petals senescence, percentage of bent neck flower, percentage of leaf senescence and transpiration rate in the treatment of 10% and 20% significantly different from control (0%). Soaking in the juice of ripe papaya (*C. papaya* Linn.) for 3 hours is intended to provide nutrients for cut flowers, roses (*R. hybrida* L.). This is because cut flowers are no longer getting nutrients from the soil as it has not been cut. Cut rose flower that has been cut from the stem is still doing metabolic activity. Holding solution of cut flowers generally need carbohydrates whose function as an energy source as well as to replace water lost through transpiration (Halevy and Mayak, 1979).

Figure 1. Freshness of cut flower rose (*R. hybrida* L.)
Different alphabet determine there is a real difference in the samples tested on the level of 5%

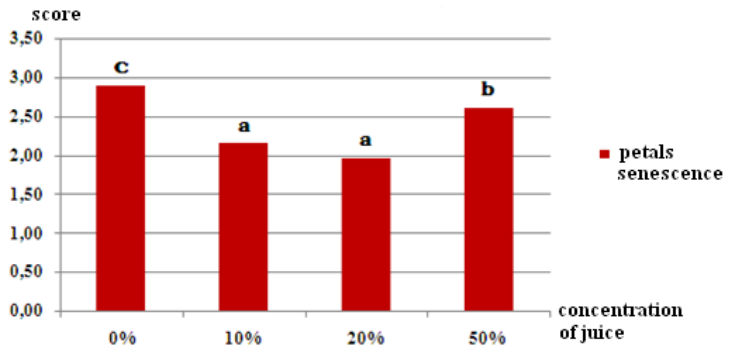

Figure 2. Percentage of petals senescence of cut rose flower (*R. hybrida* L.)
Different alphabet determine there is a real difference in the samples tested on the level of 5%

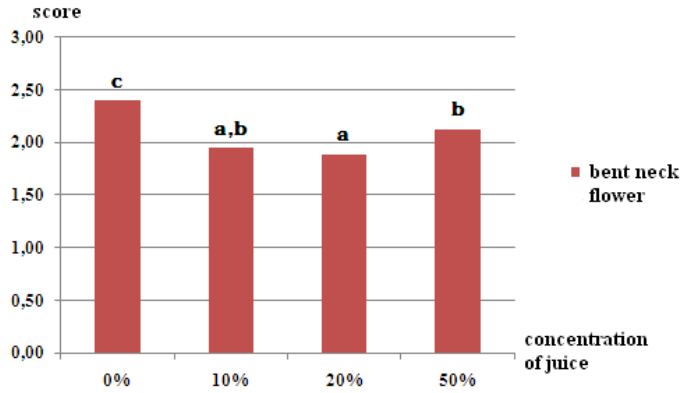

Figure 3. Percentage of bent neck of cut rose flower (*R. hybrida* L.)
Different alphabet determine there is a real difference in the samples tested on the level of 5%

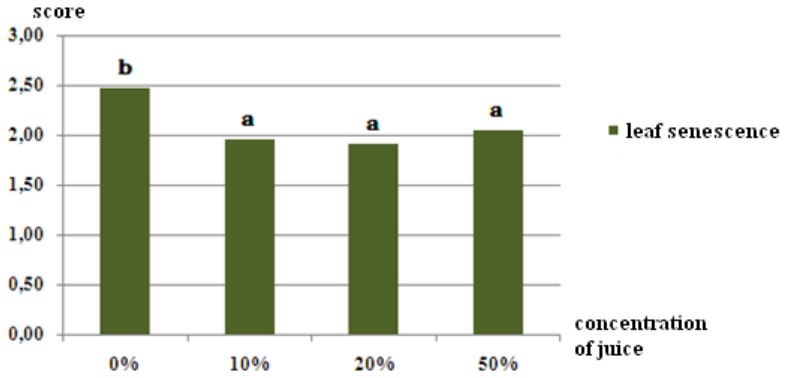

Figure 4. Percentage of leaf senescence of cut rose flower (*R. hybrida* L.)
Different alphabet determine there is a real difference in the samples tested on the level of 5%

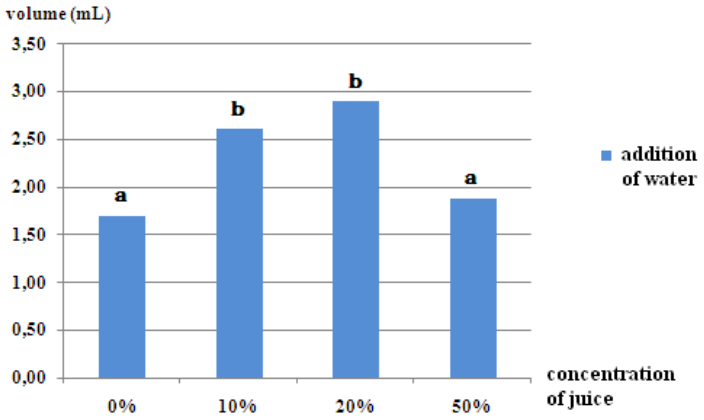

Figure 5. Transpiration rate of cut rose flower (*R. hybrida* L.)
Different alphabet determine there is a real difference in the samples tested on the level of 5%

Papaya juice (*C. papaya* Linn.) concentration of 10% and 20% is the optimum concentration as pulshing medium. Nutrient solution contained in the juice can be absorbed

properly due to the condition of the solution to the fluid cell is not hipertonis. So that the balance between water content and nutrient content causes the solution easily absorbed and easily transported throughout the plant. While the concentration of 50% is a fairly dense concentration. This caused the hipertonis solution and reduces water osmosis into the cell on the stalk cut flowers, which causes the nutrient is also difficult to be absorbed. Besides the concentration is too high is also a good medium for microorganisms, so it will cause the base of the stalk rot and block the absorption of water. Turbidity indicates the existence of microorganisms that have been breeding so spread to all parts of the solution and the base of the submerged plants become slippery due to microbes. According Suyanti (2002) decay that occurs in the stalk crops due to the microorganisms causing the water absorption is also inhibited. Based on this research it can be concluded That the pulsing method with papaya juice (*C. papaya* Linn.) Can maintain the freshness of cut flower roses (*R. hybrida* L.) 5 days longer than controls and the optimum concentration of papaya juice (*C. papaya* Linn.) is 10% or 20%.

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P-PD11

Enzyme Characterization Of Cellulolytic Bacteria Isolated from the Stomach of Milkfish *(Chanos chanos)* As Potential Agent to Degrade Organic Waste

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Introduction

Enzyme is produced by living things, include bacteria. There are various sources of cellulose enzymes produced by microbes. In the herbivorous fish digestive, such as milkfish (Chanos chanos) consuming Chlorella, there is cellulolytic bacteria. The bacteria is predicted producing the cellulose enzyme, so it can digest the algae. The presence of cellulolytic bacteria that produce cellulose enzyme can be used for various needs, like composting for organic waste, increase the digestibility of animal feed, bio-ethanol production. This research aim to characterize the cellulose enzyme from bacteria that isolated from stomach of milkfish (Chanos chanos) that has potential agent to degrade organic waste. The bacteria will characterize are bacteria that obtained from previous research *Bacterial Diversity In Milkfish (*Chanos chanos*) Gastrointestinal : Potential Candidate For Probiotic And Cellulose Degrading Agent* (Lathifah *et al*., 2008). From four cellulolitic bacteria are BSA B1, BSA E2, LBA and BSA B3 but just isolate that have biggest activation of cellulolitic enzyme will characterize .

Materials and Methods

Selection of cellulolitic index .

Selection for isolates BSA B1, BSA B3, LBA, BSA E2 that have biggest cellulolitic index. This measurement looked from activation to reducing sugar and clear zone on CMC media .

Making of Inoculate Starter

A total of two bacteria full loops were inoculated in 100 ml of 1 % CMC liquid medium and incubated on the shaking incubator for 24 hours. That starter would be used to measure growth curve and cellulase activity testing.

Growth Curve Measurement.

Bacteria growth measurement and enzyme activity testing were done by interval within 24 hours. Growth of bacteria is measured by using spectrophotometer by wave length

Cellulase Activity Testing

Taken 5 ml from culture. Further, the sample was centrifuged by using speed 10.000 rpm for 10 minutes, until gotten pellet and supernatant. That obtained supernatant is the crude enzyme that will be tested the activity. The cellulase enzyme activity is measured by Miller method (1959) by adding 1 ml of crude enzyme into 1% CMC substrate in phosphate buffer of pH 7 for 15 minutes. The reaction was stopped by adding of 2 ml of Dinitro Salicylate Acid (DNS) and boiled for 15 minutes, and then the sample awaited until the temperature down become room temperature and measured by wave length 540 nm. One unit of cellulase activity is defined as number of enzyme that produces 1 µmol of glucose per minutes.

Enzyme Characterization

Cellulase enzyme characterization includes determination of pH, optimum temperature, and the suitable substrate. Characterization of cellulose activity is gotten by mixing 1 ml of crude enzyme with 1 ml of substrate, then incubated for 10 minutes and the reaction is stopped by heating, level of reduction sugar production stopped by heating, and then the reduction sugar product that has been created, measured by spectrophotometer.

Determination of Optimum pH. Determination of optimum pH was done by dissolving of the crude enzyme into 1% of CMC substrate in the different pH condition, (acid condition in pH 2 and 4, neutral condition in pH 7, and alkali condition in pH 8 and 10) by using citrate acid buffer (pH 2 and 4), phosphate buffer (pH 7), and NaOH Glycine buffer (pH 8 and 10).

Determination of Optimum Temperature. Done by testing cellulase activity on different temperature (20°C until 50°C within interval 10°C) in 1% of CMC substrate in the optimum pH buffer and incubated for 30 minutes.

*Determination of Substrate.*Done by testing cellulase activity in different substrate (CMC, AVICEL, Whattman Paper) in the suitable buffer and optimum pH, incubated in optimum temperature.

Enzyme Activity Testing for Each Organic Waste Substrate

The procedure that done in this stage is takes 5 ml of CMC substrate and AVICEL, is added 5 ml of crude enzyme. For filter paper substrate, a number of 2.5 pieces of filter paper 1x6 cm^2, added 2.5 ml of buffer and 5 ml of crude enzyme. The reaction between substrate and crude enzyme were done in 100 ml of Erlenmeyer tube for 60 minutes in the optimum temperature. After that, the reaction was stopped by adding 50 µl of NaOH 0.2 M or by incubating at 100°C. For CMC substrate, composition of substrate-enzyme was transferred as much as 2 ml into reaction tube, and then added 2 ml of DNS and incubated at 100°C immediately. A number of 1 filter paper 1x6 cm^2 and 3 ml of crude enzyme and buffer mixture, transferred into reaction tube and added 2 ml of DNS, the incubated at 100°C immediately. After the incubation between enzyme and substrate, AVICEL, straw, and banana peel mixture finished, added 50 µl of NaOH 0.2 M immediately. Further, that suspension centrifuged at 2500 rpm for 25 minutes. As much as 2 ml of supernatant was taken and added 2 ml of DNS, then incubated at 100°C immediately. All the samples were measured by spectrophotometer by wave length 540 nm

Result and Discussion

Table 1. Cellulolytic Index and Sugar Reduction BSA B1, BSA E2, LBA, BSA B3

No	Isolat Code	Sugar Reduction (%)	Cellulolytic Index
1.	BSA B3	0.213	0,667
2.	LBA	0.21	0,463
3.	**BSA E2**	0.231	0,705
4.	**BSA B1**	0.281	1,036

BSA B1 showed the ability in producing reduction sugar for 0,281% and cellulolytic index for 1.036. As for BSA E2 isolates showed the second biggest for reduction sugar and cellulolytic index, were obtained level of reduction sugar 0.231% and cellulolytic index 0.756. Determination of reduction sugar level was done to decide how the ability of isolates in breaking cellulose that has been contained in CMC substrate into monomer-monomer of simple sugar. Cellulose is the polysaccharide that consist of glucose monomer that has been bound by -1,4 glycoside bond. By cellulase enzyme, that bond will be degraded until cellulose become monomers. Cellulolytic index is decided by calculation of clear zone diameter that has been created by colonies of bacteria isolates after the isolates were spilled by Congored reagent.

Bacteria Growth Curve and Cellulase Enzyme Activity

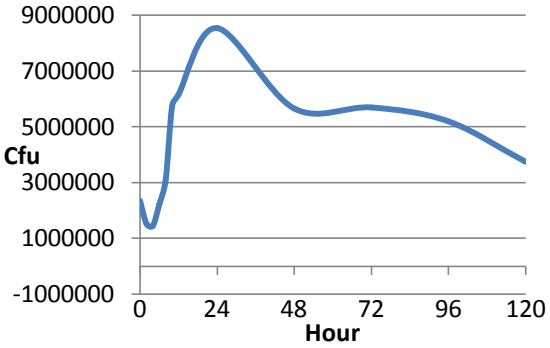

Figure 2. Bacteria BSA B1 Growth Curve

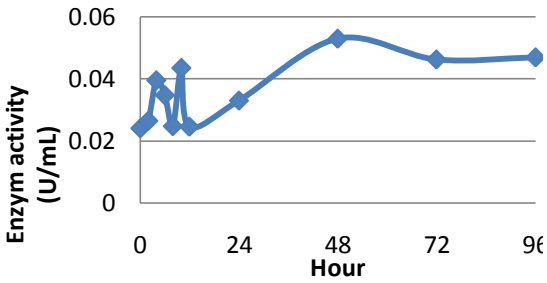

Figure 3. BSA B1 enzyme activity

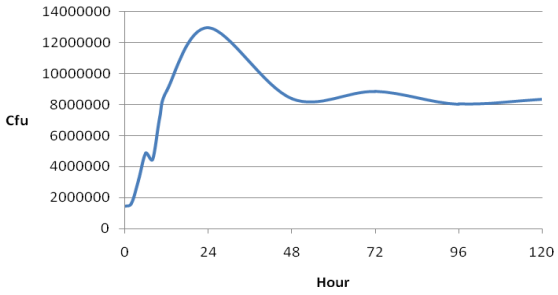

Figure 4. Bacteria BSA E2 Growth Curve

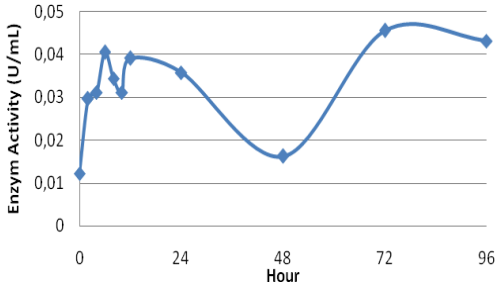

Figure 5. BSA E2 enzyme activity

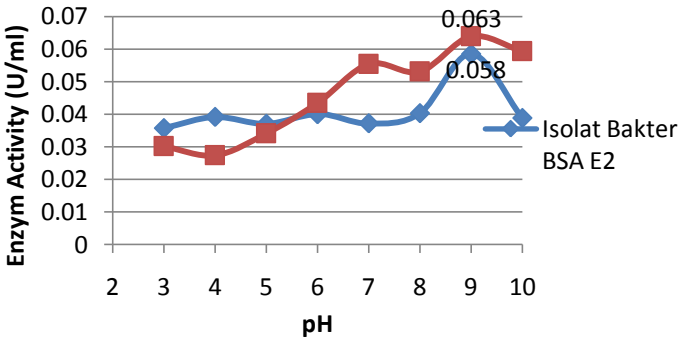

Figure 6. Enzym activity by BSA B1 dan BSA E2 in different pH

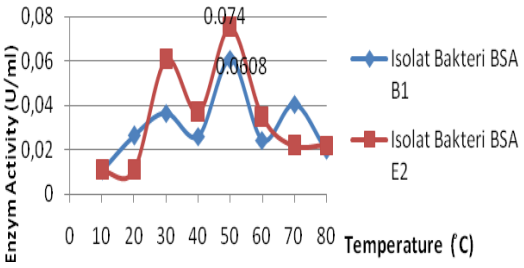

Figure 7. Enzym activity by BSA B1 dan BSA E2 in different temperature

These two isolates have the enzyme optimum activity at pH 9 and temperature is 50°C.

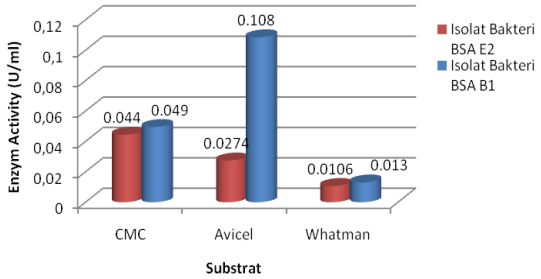

Figure 8. Enzym activity by BSA B1 dan BSA E2 in different substrat

Isolates of BSA B1 matched at AVICEL substrate, while BSA E2 matched at CMC substrate.

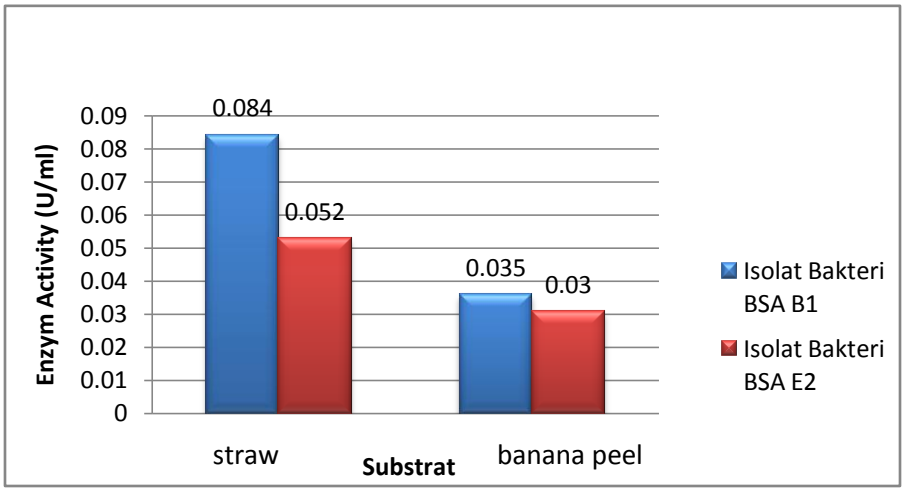

Figure 9. Ability cellulase enzyme in degrade waste organic

The potency of two isolates in degrading of organic waste likes straw and banana peel can be considered good enough. Enzyme Activity of BSA B1 at straw substrate as big as 0.084 unit/ml, while BSA E2 enzyme isolates 0.052 unit/ml. Enzyme activity of BSA B1 at banana peel substrate as big as 0.35 unit/ml, while BSA E2 enzyme isolates as big as 0.03 unit/ml.

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P-PD12

THE DIGESTIVE TRACTS STRUCTURE OF *Cuora amboinensis* (DAUDIN, 1802)

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ABSTRACT

Cuora amboinensis is endangered turtle species in Indonesia. C. *amboinensis* is commonly known as a facultative herbivorous turtle. This assumption still needs further data from the structure of digestive tracts study. Studying the structure of digestive tracts ensures the compatibility of digestive system to process any kinds of foods. This research aimed to study the structure of digestive tracts of C.*amboinensis* and the relation with its food preference. Food preference observations were done by feeding 10 specimens of C *amboinensis* with many kinds of food at the same time, and observed the most favorable food chosen. Three specimens were sacrificed and dissected to observe macroscopic structure of their digestive tracts. Digestive tract regions observed were lingua, esophagus, ventriculus, intestinum tenue, intestinum crassum and cloacae. For histological preparation of digestive tracts was used standard paraffin method and stained with Hematoxylin-Eosin. The microscopic structure of digestive tracts of C *amboinensis* was observed with microscope and photograph, and it was also compared with other species. The result of food preference observations showed that *C amboinensis* had an equal tendency of choosing both vegetables and meats as their food. The observations of digestive tracts structure were showed that C *amboinensis* digestive tracts have omnivorous characteristics with an herbivorous tendency. We can conclude that digestive tracts of C. *amboinensis* are compatible to process both vegetables and meats equally thus C. *amboinensis* were omnivorous.

Keywords: digestive tracts, food preference, *Cuora amboinensis*

P-PD13

The Effect of Organic Matters Toward The Growth of *Casuarina equisetifolia* var. incana Seedlings in Various Sites of Coastal Area

Winastuti Dwi Atmanto, Sri Danarto, Widaryanti Wahyu Winarni, Hery Priyanto

ABSTRACT

Numoreous vegetation destructions affect the ecosystem along coastal area. An effort to overcome further destructions is cultivation. The obstacles are the extreme environmental factors of the coastal area. The research aimed to figure out the influence of organic matters on the seedling of *Casuarina equisetifolia* in several sites.

The study was carried by using RCBD with compacted organic matters and without organic matters in three different sites: non cover crops, few cover crops, and site with cover crops. Each site consists of three blocks, plot is in square. Every unit experiment covers 16 trees. The observation focused on 4 trees in the centre of the plot. Growth parameters observed are survival percentage, the increase of height and diameter, total dry weight, and the amount of nodules. The environmental factors observed are temperature, humidity and light intensity.

Non cover crop site with compacted organic matters showed better result for the growth of seedlings with 100% life percentage, increasing height average up to 26.6 cm, diameter 1.06 cm and total dry weight 394.95 g. The use of compacted organic matters in coastal area increases the amount of nodule and multiplied 6 times compared with seedling without organic matters.

Keywords: organic material, *Casuarina equisetifolia* var. Incana, sites

P-PD14

Microscopic Examination on the Mobility of *Amoeba Sp* Based on Timelapsed Imaging Technique

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ABSTRACT

Microscopic examination on the *Amoeba sp* motion from pond water has been carried out. The examination was using a digital optical microscope that adopting a time-lapsed imaging technique developed at the Department of Physics Gadjah Mada University. A set of sequential images of an *Amoeba sp* has been obtained. Then, the central positions based on the cell nucleus from time to time were obtained. The graphic analysis showed that the average speed of the *Amoeba sp* for 18 second examination was about (0,57 ± 0.03) μm/s.

Keywords: cell movement, *Amoeba sp*, *time-lapsed* recording, digital microscope

INTRODUCTION

Amoeba sp has a unique movement in which its body shape changes while its moves. When the amoeba changes its body shape, some parts extend to form pseudopods or false legs [1]. These pseudopods are responsible for the mobility of the amoeba. The development of pseudopods is due to the viscosity differences between anterior and posterior cytoplasm [2].

The amoeboid movement is also found in human body, such as in Kupffer cells of liver, various kinds of white blood cells like- monocytes and neutrophils, cancerous cells showing metastasis and macrophages [3]. Therefore, this amoeboid movement is interesting to learn.

To start this study, *Amoeba sp* has been chosen. Amoeba sp has simple shape cell and easy to found in freshwater area. *Amoeba sp* is a unicellular microorganism in Protist Kingdom. The amoeboid protists form an important part of eukaryotic diversity, covering about 15,000 described species [4]. *Amoeba sp* can be found as a free-living organism in freshwater, salt water and terrestrial. They can also be found in animals and human body.

The time-lapsed imaging technique has been developed at the Department of Physics Gadjah Mada University. Using this technique, object or cell movement including the amoeboid movement may be recorded and observed periodically as sequential images.

Numerous studies about the mobility of Amoeba reported that the average speed of the microorganism were different [5]. It is influenced by many factors such as cell size, chemical material, temperature [6], light-shade difference and substrate type [7]. In this paper, analysis on the *Amoeba sp* movement is presented. This study may provide the basic way to explore amoeboid and other microorganism in the future.

Material and Methods

The basic material was the freshwater amoeba, *Amoeba sp*. This was obtained from the pond water. The *Amoeba sp* was introduced on the concave glass with a pipette prior examination under the optical digital microscope. The magnification was set to 20X. The digital microscope was a modification of an analog microscope that was coupled with a charge-couple-device (CCD) video camera. The camera output was sent to the frame grabber card that was installed to the computer. Using an image capturing software developed at the Department of Physics Gadjah Mada University (Fig.2), the computer may capture sequential images capturer the digital images and then analyzed frame by frame using a free image processing software, e.g. *ImageJ*.

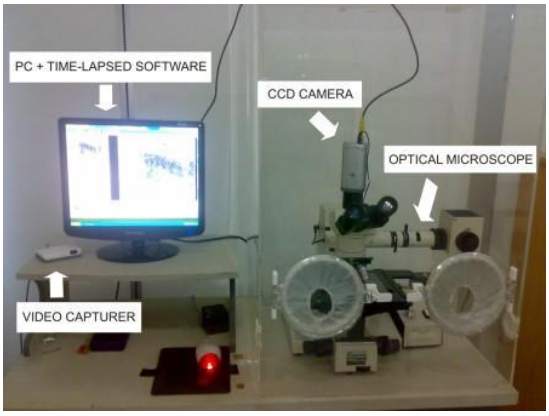

Fig.1 The modified digital microscope.

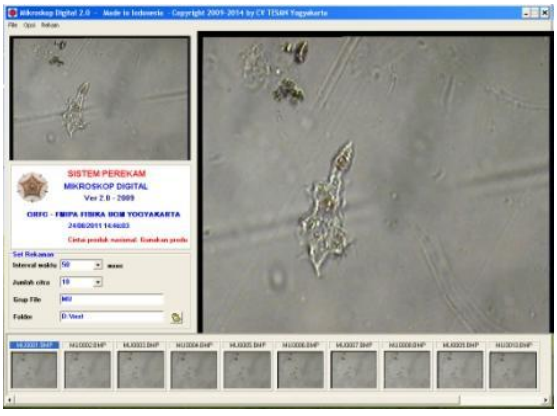

Fig.2 The time-lapsed imaging software

Results and Discussion

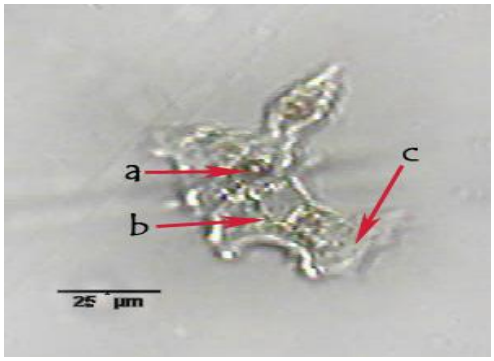

Fig 3. The anatomy of *Amoeba sp.* a. nucleus, b. cytoplasm, and c. pseudopodia

Microscopic anatomy of cell of *Amoeba sp* is shown in Fig.3. The cell nucleus is regarded as the centre of the cell, while pseudopodia as an extension of the cytoplasm define the body shape. The image sequence showing *Amoeba sp* movement as indicated by the changing of cell shape is shown in Fig.4. A set of 50 images of *Amoeba sp* in a time interval of 500 ms was recorded. The speed of movement was determined based on the cell nucleus position from time to time.

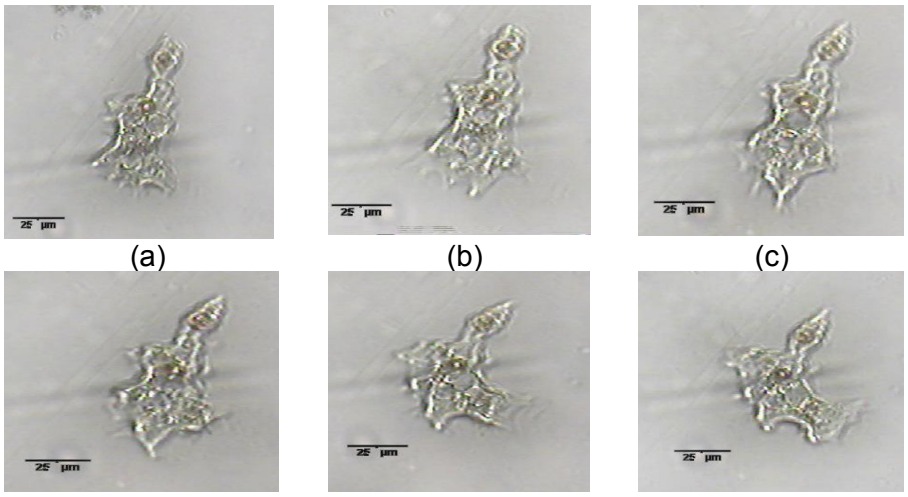

Fig.4 The sequence of *Amoeba sp* cell movement (a) 1st second, (b) 5th second, (c) 10th second, (d) 15th second, (e) 20th second, (f) 25th second

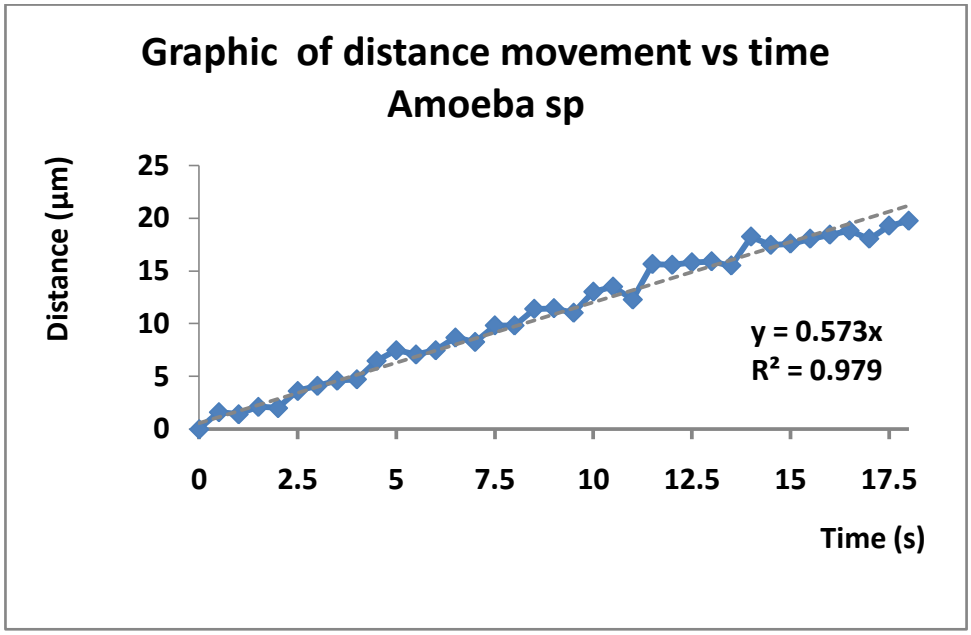

Fig. 5. The graph indicating the relation of *Amoeba sp* movement to time.

The graphic in Fig. 5 showed that the average speed of the *Amoeba sp* for 18 second examination was about (0,57 ± 0.03) µm/s. In general, the sequential images resulted from time-lapsed imaging technique leads to study about amoeboid mobility clearer and more quantitative in compare to the simple visualization data, such as in video or single picture.

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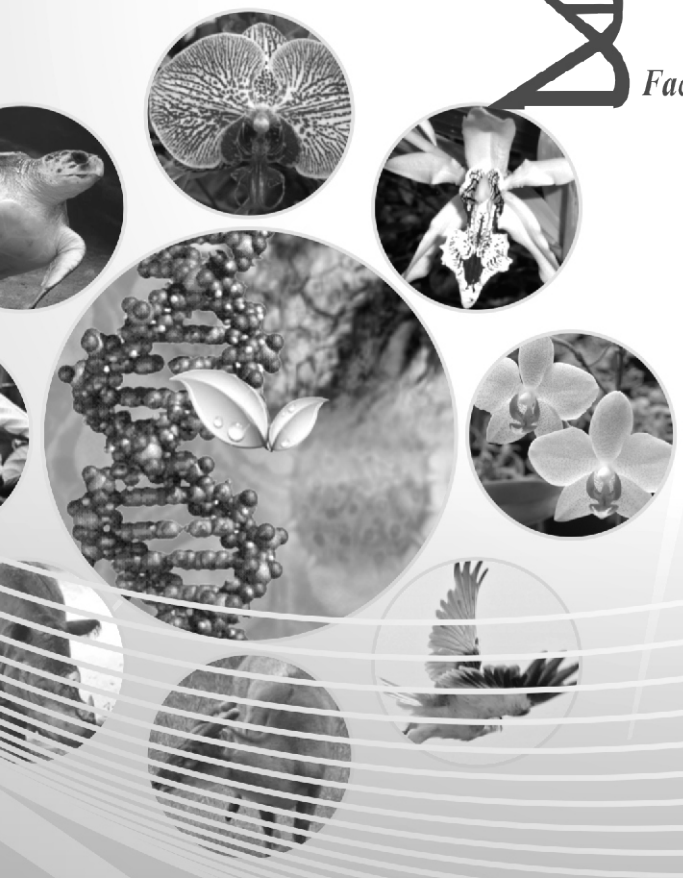
POSTER - TOPIC 5

Biomedics (O-BM)

ICBS 2011

Faculty of Biology Universitas Gadjah Mada



FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

P-BM01

EFFECT OF DRYING TEMPERATURE ON THE ANTIOXIDATIVE ACTIVITIES AND ACCEPTABILITY OF *ALOE VERA* (*Aloe vera var. chinensis*) POWDER

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P-BM0

Antibacterial activity of petroleum ether-hexane extract of *Artemisia cina* Berg. ex Poljakov

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ABSTRACT

The paradigm of bacteria resistence raises alternative ways to use other compounds that have antibacterial activity. Society tends use plants as alternative medicine because it's better advantage considered relatively safer than synthetic drugs, in terms of cost is relatively cheaper and environmentally friendly. The aims of this research was to compare the antibacterial activity of extracts of *Artemisia cina* Berg. ex Poljakov against *Escherichia coli* and *Staphylococcus aureus*, and to determine qualitatively the various compounds in the extract. The study design was based on completely randomized design with four times repititions. Concentration extract at 0, 50, 75, 100, 150, 200 mg/l and tetracycline 10 mg/l as a control standard antibiotics. A solvent extraction plant Artemisia using petroleum ether-hevane mixture (1:1). Antibacterial activity performed by agar diffusion method. Petri dish containing Nutrien Agar medium were spread with tested bacterial. Paper disc containing extract were put in the petri disc. Antibacterial activity is determined by measuring the zone of inhibition on agar after 24 hours incubation at 37°C. Quantitative data obtained were statistically analyzed with one way of ANOVA. Phytochemical screening was performed following procedures described by Harborne. The growth of both bacteria were inhibited by the extract with zone of inhibition ranged from 2-15 mm, but smaller than the standard antibiotic tertacycline. Inhibitory power were at strong category at 75-150 mg/l concentrations against *E. coli* and at 75-200 mg/l against *S. aureus.* Extract were consist of flavonoid, alkaloid, saponin, sterol and triterpen, hidrolized tannin, and especially essential oil and coumarin.

Keywords: *Artemisia cina* ex Poljakov Berg, antibacterial activity, *Escherichia coli*, *Staphylococcus aureus*

Introduction

The infection disease usually carried out using antibiotics. Because of the infection caused by bacterial action, antibiotics called as antibacterial. The used of antibiotics at a long time can bring about the resistance properties of microorganisms to antibiotics used(1). These paradigm raises alternative way to find other compounds that have antibacterial activity, likely from plants. Society tends to take of plants as alternative medicine because it's better advantage considered relatively safer than synthetic drugs, in terms of cost is relatively cheaper and environmentally friendly.

One of the types of plants which have antibacterial activity are Artemisia. Artemisia plants have ±400 species in the world(2), but only several species found in Indonesia. *Artemisia anua* L., *Artemisia cina* Berg. ex Poljakov, *Artemisia vulgaris* Linn. dan *Artemisia sacrorum* Ledeb were found in Wamena (Irian Jaya), Tawangmangu, Kopeng (Central Java), Bandung dan Sukabumi (West Java)(3). Many researcher found that all the part of Artemisia plants shown anteibacterial activity. This research were done to compare the antibacterial activity of extracts of Indonesian Artemisia that is *Artemisia cina* Berg. ex

Poljakov. *Escherichia coli* and *Staphylococcus aureus* were used as tested bacteria. Beside that, qualitative analysis were done to determine the various compounds in the extract which have role in the antibacterial activity.

Materials and methods

A whole part of the plants were oven at 40°C until became dry before used. The dry plants were blended until to be powder. The powder were maseration with hexane:petroleum eter (1:1) for 24 h. The filtrates were filtered using Whatman No. 1 filter paper and then dried using rotary evaporator (Quickfit j.Bibby/34912). The dried extract stored in the refrigerator. Redissolved extract using ethanol and aquabides until the concentration of treatment at 0, 50, 75, 100, 150, and 200 mg/l respectively before used.

Antibacterial activity of extracts against tested bacteria performed by agar diffusion method[4]. Two tested bacterial were *Escherichia coli* (gram negative) and *Staphylococcus aureus* (gram-positive bacteria). Tetracycline were used as the standard of antibiotic. Antibacterial activity is determined by measuring the diameter of zone of inhibition (in milimeters, excluded paper disc diameter) on agar after 24 hours incubation at 37°C. The treatment were performed in four times repititions and the means of result were statistically analysis with values of $p<0.05$.

Phytochemical screening was performed following procedures described by Harborne[5]. These assay used as qualitative analysis of secondary metabolites such as flavonoids, alkaloids, essential oils, saponins, sterols and triterpene, tannin, and coumarin.

Result and discussion

The ability of the extract to inhibit the growth of bacteria indicated by the bright zones around the paper disc. That is show the sensitivity of tested bacteria against the antibacterial agents which used. Extract hexane-petroleum eter of *A. cina* varied in their antibacterial activity (Table 1). The growth of *S. aureus* were inhibit by *A. annua*[6] but the research didn't studi on *A. cina*. On other hand, antibacterial activity of *A. asiatica Nakai* were studied by[7] toward *S. aureus, E. Coli.*

The effect of extract to both tested bacterial were significantly since 50 mg/l. The pattern of properties antibacterial showed the kuadratic curve. The higher effect were at 100 mg/l in *E. coli* and at 150 mg/l in *S. aureus*. At the all antibacterial activity, the strengh were less than standart antibiotic tetracycline were used. The strengh of antibacterial activity were on strong category at 75-150 mg/ml concentrations against *E. coli*, while against *S. aureus* at 75-200 mg/ml concentration.

Table 1. Antibacterial activity of extract hexane-petroleum eter (1:1) of *A. cina* toward *E. coli* and *S. aureus* after 24 hour incubation at 37°C.

Exctract concentration (mg/l)	Diameter of zone of inhibition (mm±SD)[4] and the strengh of antibacterial activity[8] on tested bacterial			
	E. coli		*S. aureus*	
0	2.0± 1.7 ^{A}	W	0.8± 1.0 ^{A}	W
50	6.0± 0.0 ^{AB}	I	9.0±2.4 ^{B}	I
75	10.5± 6.7 ^{BC}	S	13.5±2.4 ^{BC}	S
100	14.8± 5.9 ^{C}	S	10.3±2.6 ^{BC}	S
150	10.3± 4.2 ^{BC}	S	15.0±4.2 ^{C}	S
200	8.5± 3.1 ^{B}	I	11.0±1.2 ^{BC}	S
Tetracycline	20.3±11.5 ^{D}	VS	42.5±9.6 ^{D}	VS

- The same abjad behind the values means there are not significantly different effect between concentration of extract at the same tested bacterial.
- The strengt category of antibacterial activity based on diameter of zone of inhibition (x mm) excluded diamter of paper disc (Stout (1997) loc cit [7]): Weak (x<5 mm); Intermediate (5<x<10 mm); Strong (10<x<20 mm); Very Strong (x>20 mm)

Phytochemical screening shown that extract hexane-petroleum eter of *A. cina* consist of flavonoid, alkaloid, saponin, sterol and triterpen, hidrolized tannin, and expecially essential oil and coumarin at the hard positive result (Table 2). These result were appropiated with[9, 10] that the chemical compounds in *A. cina* included flavonoid, phenolic compounds, coumarin, alkanoid, and essential oils, altought at different place of plant growth. These compounds could be together as active component to inhibit the growth of tested bacterial. Some compounds of secondary metabolites such as phenolic compounds, polyphenols, quinone, flavones, flavonoids, tannins, coumarin, terpenoids, alkanoid, lectin, and polypeptides suggested as antibacterial agent[11].

Table 2. The result of phytochemical screening of hexane:petroleum eter (1:1) extract of *Artemisia cina* Berg. ex Poljakov using Harborne method

No.	Substance	Result
1	Flavonoid	+
2	Alkaloid	+
3	Essential oil	++
4	Saponin	+
5	Sterol and triterpene	+
6	Hidrolized tannin	+
7	Coumarin	++

+ : light positive test; ++: hard positive test

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P-BM03

SECONDARY METABOLITES OF *Sargassum duplicatum* CHLOROFORM EXTRACT AND IN VIVO BIOACTIVITY AS ANTI-PARASITE TOWARD *Trypanosoma evansi* Steel, 1885

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ABSTRACT

Various primary and secondary metabolites produced by algae have a pharmacologic action which often called the bioactive substance. This active substance can be used as an alternative medicine for trypanosomiasis that has shown resistance to existing drugs. The purpose of this research was to determine the content of secondary metabolites from chloroform extract of *Sargassum duplicatum* and its bioactivity towards the level of parasitemia *Trypanosoma evansi* after in vivo treatment. This research used several methods includes the maceration extraction process generated an chloroform extract of *Sargassum duplicatum*, identification of secondary metabolites using TLC (*Thin Layer Chromatography*), infection of *Trypanosoma evansi* in mice Balb/C and several variation of treatment using chloroform extract of *Sargassum duplicatum* orally for 4 days, making the blood smear and the calculation of the level of parasitemia. In vivo bioactivities assay of chloroform extract of *Sargassum duplicatum* for bioactivity against trypanosomiasis revealed that several group of secondary metabolites were detected and showed an significant effect against *Trypanosoma evansi*, the etiological agent of Trypanosomiasis. This algae extraction resulted in the identification of four secondary metabolites acted as Bioactive substance. They were identified by *Thin Layer Chromatography* Method means as flavonoid, terpenoid, alkaloid and fenol. All isolated compund were previously known from other brown algae but most notably, this is the first report on their occurrence in *S. duplicatum* and as well on their anti-parasite activity. Bioactivities of chloroform extract of *Sargassum duplicatum* were examined and showed its optimum activity on decreasing the level of parasitemia *Trypanosoma evansi* up to 96.9% with a dose of 800mg/kg body weight.

Keywords: trypanosomiasis, secondary metabolites, *Sargassum duplicatum*, In vivo

Introduction

Indonesia is known as a country either rich in population or biodiversities of brown algae. *Sargassum duplicatum* (J.Agardh) J. Agardh. is one of the species that potentially can be used as a source of alginophyte, especially the utilization of its chemical compund to biomedical application (1). In addition to alginate content, there are secondary metabolites that have potential as bioactive substance. Plant secondary metabolites are often classified as: alkaloids, terpenoids, flavonoids anda phenols. Some secondary metabolites have begun to study but not specific for *S. duplicatum* (J.Agardh) J. Agardh. specifically steroids and phenols as antibacterial compounds (2). Trypanosomiasis or often we called as *Surra* disease, is one of infectious animal disease that can be either chronic or acute ones. This disease is caused by one of blood's protozoa called as *Trypanosoma evansi* and acted as vectors are fies: *Tabanus* sp. and *Stomoxys* sp. After infection, *Trypanosoma* will spread out in perifer blood periodically and cause fever. Besides, parasitemia also will be shown as

a result of infection. For medicinal purpose, there are so many alternative medicines made from arsenic i.e: *diminazene, suramin* and *quinapyramine*, but trypanosomiasis have shown resistance to these existing medicines due to overdose and long time treatment (3).

Another isolated compound of secondary metabolites from another brown algaes were previously known, i.e : *Euchema cottonii, Eucheuma spinosum, Gracilaria verrucosa, G. convervoides, Gelidium* sp., and *Dictyota* sp. some research also reported that the extract of those brown algaes had bioactivities as antivirus and antitumour (1). Some secondary metabolites have begun to study but not specific for *S. duplicatum* specifically steroids and phenols as antibacterial compounds. The purpose of this research was to determine the content of secondary metabolites from chloroform extract of *S. duplicatum* and as well on their anti-parasite activity.

Materials and Methods

This research used several methods includes :

Sampling of *Sargassum duplicatum* was done in Karimun Java National Park, Jepara, Central Java. Sample of *S. duplicatum* was carried by cruise method along Nirwana Island.

Extraction of *Sargassum duplicatum* was processed by maceration extraction. Samples that collected then chopped and placed in a closed vessel with chloroform solvent for 24 hours, then filtered. The dregs then macerated twice and filtrat generated then evaporated to eliminate the solvent. After that, so we get the chlorofom extract of *Sargassum duplicatum.*

Identification of secondary metabolites was processed using Thin Layer Chromatography (TLC). Extracts that have been dried then dissolved in a chloroform solvent. The stationary phase used is silica gel GF 254 type and the mobile phases used are: n-hexane : ethyl acetate (3:1 v/v). Extract solution was then spotted on TLC plates using capillary tube 0,1 mm with a distance of 1 cm from the top edge. The spots then sprayed by colours reagent : ammonia vapour, iron (III) chloride, dragendorff and cerium sulfate. Coloured spots then detected by UV with 254 nm and 365 nm wavelength.

Preparation and in vivo treatment. Preparation for mice (*Mus musculus* L.) that was used is the female Balb/C strain, 2-3 months old. Infection of *Trypanosoma evansi* injected by 0,2 ml of blood containing *T. evansi* 10^6 intra perotoneal. Treatment with the chloroform extract of *S. duplicatum* and DMSO orally was given after 24 hrs after *T. evansi* infection. The concentration of extract used in this research were : 50 ; 100; 200; 400 ; 800 (mg/kgs weight).

Analysis of parasitemia was processed by blood smear preparation using giemsa solvent and methanol as a fixative. Calculation of parasite was observed with the aid of microscope at 1000x magnification and helped with the counting chamber. Calculation of percentage of inhibition was calculated using this formula (K + = positive control) :

$$\% \text{ inhibition} = \frac{\% \text{ parasitemia K (+) - \% parasitemia } s.\ duplicatum \text{ x } 100\%}{\% \text{ parasitemia K (+)}} \quad (4)$$

Results and Discussion

The results showed that secondary metabolites that were identified by TLC method means as alkaloids, terpenoids, flavonoids and phenols. Rf values are 0,525; 0,803; 0,541; 0,883 respectively.

Table 1. Identification of secondary metabolites uding TLC methods

Class of compounds	Colour reagents	Visible colour	UV colour detected	Rf values
Terpenoids	Cerium Sulfate	Green	-	0, 803
Flavonoids	Amonia Vapour	Yellow	Bright yellow	0, 541
Alkaloids	Dragendorff	Orange	Blue	0, 525
Phenols	$FeCl_3$	Dark purple	Yellow	0, 883

All isolated compund were previously known from other brown algae but most notably, this is the first report on their occurrence in *S. duplicatum* and as well on their anti-parasite activity. Bioactivities of chloroform extract of *Sargassum duplicatum* were examined and showed its optimum activity on decreasing the level of parasitemia *Trypanosoma evansi* up to 96.9% with a dose of 800mg/kg body weight.

Table 2. The inhibition percentage of *Sargassum duplicatum* chloroform extract against number of *T. evansi.*

Treatment Dose	% inhibition
50mg/kg weight	24,78 %
100mg/kg weight	90,9 %
200mg/kg weight	6,19 %
400mg/kg weight	53,54 %
800mg/kg weight	**96,9 %**

From the table 2. we can conclude that secondary metabolites contained in *Sargassum duplicatum* chloroform extract were acted as bioactive substances and showed its optimum acitivity on decreasing the level of parasitemia *Trypanosoma evansi* up to 96.9% with a dose of 800mg/kg body weight treatment.

Secondary metabolites of these compounds are consistent with existing theory that the activity of the alkaloid in inhibiting cell wall synthesis, nucleic acids, and the performance of enzymes in the body of parasites, because the alkaloid interacts with a specific enzyme as a target molecule causes a biochemical reaction of *T . evansi* is catalyzed will be inhibited. Then, flavonoids are polyphenols, these compounds reducing and may inhibit oxidation reactions, as well as upsetting the balance of the body that inhibit the growth and development of the parasite. While the class of terpenoids in the extract of *Sargassum duplicatum* role interfere with the formation of ATP in mitochondria at the *T. evansi* (5). So that the process of energy metabolism and the formation of disrupted parasites and *T. evansi* will die. Mice were infected *T. evansi* showed the symptoms of neurological disorders such as staggering, circling, and convulsions. We need further studies to prove this symptoms specifically (6).

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P-BM04

Effects of Toxic Compound of *Barugum* Local Varieties of *Buah Merah* (*Pandanus conoideus* Lamk.) on Proliferation and Apoptosis of Colon Cancer Cells WiDr

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ABSTRACT

Colon cancer is a major health problem worldwide. Significant improvement has been made in the management of this disease mainly through the introduction of herbal therapy agents such as *buah merah*. Thus, the objective of this study is to investigate the anti-proliferative and apoptotic effect of the toxic compound of *buah merah* local variety *Barugum* on colon cancer cells WiDr. *Buah merah* local variety *Barugum* were collected from Sentani, Papua. *Buah merah* were extracted using chloroform, methanol and water. Cytotoxicity of those extracts were determined by MTT assay. The potential extract was fractionated by Vacuum Column Chromatography and the bioactive compound monitored by Thin Layer Chromatography (TLC). The fractions that had similar pattern were combined and the toxicity were determined. Preparative TLC was performed to separate the bioactive compound on the most potential fraction. The toxicity of each part was then examined. The most toxic part was analyzed by TLC with various spray reagents for the compound identification. Doubling time assay was used to examine cell proliferation kinetics. Apoptotic study was determined by double staining methods followed by immunocytochemistry analysis for the protein expressions detection. The results revealed that the highest toxicity effect on WiDr cells was metanol extract (IC_{50} 121.55 µg/ml). The most toxic fraction collected by preparative TLC indicated that the lower part of *buah merah* Barugum had the highest toxicity among the other (IC_{50} 96.13 µg/ml). Analysis of toxic compound showed that the toxic compound was terpenoid. No significant different was found between the effect of *buah merah* extract and the most toxic fraction of TLC preparative on proliferation of WiDr. However, apoptosis was induced by both, *buah merah* extract and the most toxic fraction of preparative TLC.

Keywords : *buah merah*, MTT assay, immunocytochemistry, apoptotic

1. INTRODUCTION

Colon cancer is the second most frequent malignant neoplasm in both genders, worldwide. Treatment of colon cancer is using conventional therapies, including chemotherapy, radiotherapy, surgery, and the combination of those therapies [1]. Now, people tend to move using natural product, as the conventional therapies have some side effects and they also less expensive and is relatively safe. *Buah merah* (*Pandanus conoideus* Lamk.) is one of natural products which have been extensively used to treat cancer. In the previous study, extracts of *buah merah* have ability to inhibit colon cancer cells (CC531) proliferation [2]. The methanol extracts of *buah merah* Barugum had the toxicity effects against breast cancer cells, T47D. The bioactive compounds obtained that

have cytotoxic activity on T47D cells in methanol extract of Barugum was terpenes [3]. *Buah merah* extracts was also able to induce apoptosis on T47D cells through caspase-3 activation [4]. In this study, the proliferative and apoptotic effect of the toxic compound of Barugum local varieties of *buah merah* on colon cancer cells WiDr were investigated.

2. MATERIAL AND METHODS

Buah merah local variety Barugum collected from Sentani, Papua, Indonesia were used in this study. The colon cancer cell line used was WiDr.

Extraction of samples

All samples were extracted using three solvents (chloroform, methanol, water) (Merck, Germany). Continued extraction was done using Soxhlet apparatus.

Cytotoxicity Assay

A hundred micro litres of monolayer culture was incubated in microtitration plate for 24 hours, at 37˚C, 5% CO_2. Extracts of *buah merah* Barugum at different concentrations (as needed) were added and then incubated for 24 hours at the same condition. The extracts were then removed and 110 µl MTT (MP Biomedical, USA) was added. The plate was incubated in the dark for 4 hours. A hundred micro litres of stop solution (10% SDS stopper in 0.1 N HCL) (Sigma Aldrich, USA) was added and again then incubated overnight. The absorbance was recorded in an ELISA plate reader at 595 nm. IC_{50} was determined by linear regression analysis.

Fractination

The toxic extracts were fractionated by vacuum column chromatography using 16 eluents respectively. TLC was performed to monitor bioactive compounds. The fractions with the same profiles were combined. The combined fractions were again monitored by TLC followed by cytotoxicity assay.

Bioactive Compound Isolation by Preparative TLC

Preparative TLC was performed with the same procedure as TLC performed before, except the plates of preparative TLC using silica gel PF_{254} (Merck, Germany) as stationary phase and developed by suitable mobile phase. The separated fraction obtained from the preparative TLC plate was subjected to cytotoxicity assay.

Identification of the Toxic Compound Classes

The most toxic fraction obtained from preparative TLC was analyzed by TLC visualized by many kinds of reagents to identify the compound.

Proliferation Assay

Proliferation assay was observed by doubling time method using MTT assay at 0, -24^{th}, -48^{th}, and -72^{th} hours, in order to know the proliferation activity of WiDr cells. Concentration of extracts and the part of the most toxic fraction were IC_{50} concentration.

Apoptosis Assay

WiDr cells were treated with *buah merah* extract and the most toxic part of the potential fraction, at IC_{50} concentration. After incubation, cells were stained using *ethidium bromide – acridine orange* staining solution and viewed immediately by fluorescence microscope. Apoptotic cells which had lost their membrane integrity appeared orange and showed morphological features of apoptosis, including formation of apoptotic bodies, condensation and fragmentation of chromatin.

Immunocytochemistry assay

WiDr cells were seeded into coverslip in 24 wells-plate. Following 24 hours incubation, cells were treated with *buah merah* extract and the most toxic part of the potential fraction, at IC_{50} concentration. The cells were treated with immunocytochemistry reagent according to the manufacturer's protocol (Starr Trek HRP *universal kit*, Biocare USA).

3. RESULT AND DISCUSSIONS

The results of *buah merah* Barugum extraction followed by TLC for monitoring of bioactive compound showed that there is no bioactive compound in water extract, therefore water extract did not studied in the next step. Cytotoxicity assay of two extracts, chlorofom and methanol, showed that the lowest IC_{50} were methanol extracts (IC_{50} 121.55 µg/ml). Fractionation was done using 16 eluent and the fractions with the same TLC profiles were combined. Six combined fractions were obtained (**Figure 1**). Cytotoxicity assay of fractions of the methanol extract showed that the combined fraction II was the most toxic fraction could kill almost 100% cells.

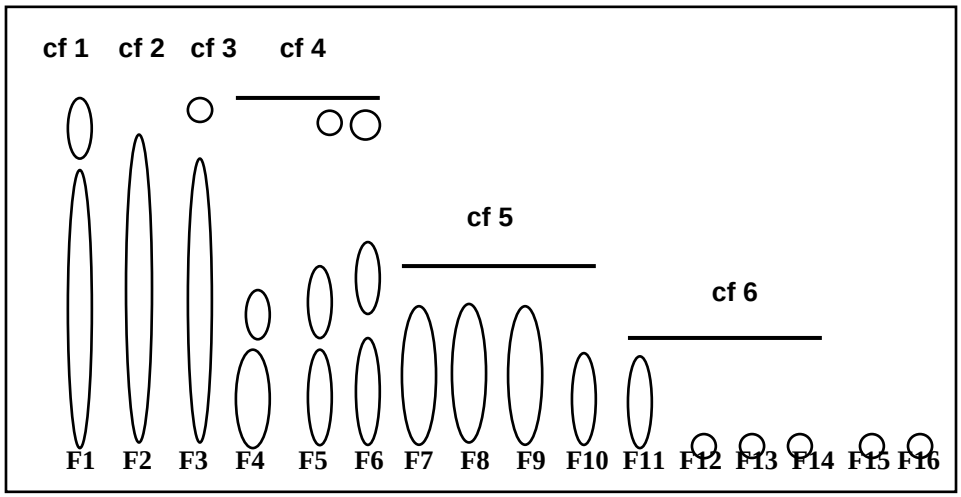

Figure 1. TLC profile of Barugum variety (cf: combined fraction)

Based on the chromatogram profile of preparative TLC, chromatogram was divided into three portions, upper, middle, and lower. Each portion was dissolved in chloroform : methanol = 9 : 1 $^{v}/_{v}$. Then cytotoxicity assay was done on WiDr using these solutions. Result showed that the lower portion was better than upper and middle portion (IC_{50} 96.13 µg/ml). Solution of lower portion of preparative TLC Barugum varieties was analyzed further for identification of toxic compound class using TLC by spraying various reagents. TLC analysis showed that the toxic compounds of methanol extract of Barugum were terpenes. Terpenes are naturally occurring substances produced by a wide variety of plants. It has been shown that terpenes induces apoptosis in colon cancer cells both *in vitro* and *in vivo* [5].

No significant different was found between the effect of buah merah extract and the most toxic fraction on proliferation assay. The toxic compounds in *buah merah* is estimated have lack ability to inhibit the activity of proliferation regulatory protein. However, apoptosis was induced by both, *buah merah* extract and the most toxic fraction of TLC preparative. Apoptosis is a specific process that leads to programmed cell death through the activation of an evolutionary conserved intracellular pathway leading to cellular changes. Abnormalities in apoptotic function have been identified as contributing events in the pathogenesis of colon cancer [6]. Consequently, apoptosis based therapeutics has emerged as an important area.

Extract of *buah merah* and the most toxic fraction were capable of inducing apoptosis at inhibitory concentration. Apoptotic cells showed the occurrence of chromatin condensation and the orange apoptotic bodies. The most toxic fraction of Barugum appeared to have the strongest apoptotic effect. To understand the further mechanism of apoptosis induction, immunocytochemical assay was done to determine several protein expression, including p53, p21, BCl-2, caspase-3, caspase-9, and COX-2. The result showed that *buah merah* extract and the most toxic fraction induced p53, p21, caspase-3, and caspase-9. In contrast, *buah merah* extract and the most toxic fraction could suppress antiapoptosis protein expression, Bcl-2 and proinflammatory protein, COX-2.

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P-BM05

Synthesis of Silver Nanoparticles Using Medicinal Fruit Water Extract of *Phaleria macrocarpa* (Scheff) Boerl.) and Its Characterization by UV-Vis Spectrophotometry

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ABSTRACT

Silver nanoparticles (AgNPs) are proven to be broad-spectrum antimicrobial agent. Preparation of silver nanoparticles (AgNPs) catalyzed by medicinal fruit water extract of *Phaleria macrocarpa* (Scheff) Boerl. is outlined. The fruit was peeled to remove seeds and boiled for 1 hour. Concentrated extract was diluted into five concentration sets (w/v), e.g. P5 (0.5 g/ml), P4 (0.4 g/ml), P3 (0.3 g/ml), P2 (0.2 g/ml), and P1 (0.1 g/ml). Ten milliliter solution of each concentration was taken into a glassware and added by 90 milliliter of 1mM $AgNO_3$. Each set was done up to 3 multiplications and then incubated in room temperature. Optical density of each set was determined in a period of 24 hours for 5 days by spectrophotometry using wavelength of 370-500 nm to determine AgNPs formation. Result showed that a dark-brown solution had already formed in the first 24-hours period, indicating AgNPs formation. A peak at 400 nm from absorption curve of P3-P5 was noticed, confirming AgNPs formation with an estimation size of 10-20 nm. The spectrophotometry curve also showed that a peak was formed within range 465 nm from absorption curve of P1-P2, indicating formation of AgNPs with an estimation size of 60-70 nm.

Keywords : silver nanoparticles (AgNPs), fruit water extract, *Phaleria macrocarpa*, UV-Vis Spectrophotometry

INTRODUCTION

Silver has been used since long time ago, to treat chronic wound caused by bacterial infection. Improving the healing capacity of silver has been conducted by reducing the dimensional size of its particle into nanometer scale. Silver in nanometer-sized particle has more surface area. Therefore, increasing its surface contact and antimicrobial activity, respectively.

Silver nanoparticles (AgNPs) were first synthesized using reducing agent, such as hydrazine and sodium citrate [1]. These chemicals are known for their environmentally hazardous properties. Therefore, based on the green chemistry principle, the synthesis of AgNPs has been re-routed into environmentally low-risk pathway using 'green' catalyst.

In this research, we describe the potency of medicinal fruit water extract of *Phaleria macrocarpa* (Scheff) Boerl. as catalyst in the synthesis of AgNPs. Fruit of *P. macrocarpa* contains secondary metabolites, e.g. saponin and flavonoid [2]. These secondary metabolites are proven to be reducing agent [3].

MATERIALS AND METHODS

Preparation of fruit water extract of *Phaleria macrocarpa*

Fruit of *Phaleria macrocarpa* are peeled to remove seeds and chopped into small pieces. The fruit was weighed to make 0.5 g/ml (w/v) fruit water extract of *P. macrocarpa.* The mixture was refluxed using water as solvent. The concentrated mixture was diluted into five concentration sets, e.g. 0(0.5 g/ml), P4 (0.4 g/ml), P3 (0.3 g/ml), P2 (0.2 g/ml), and P1 (0.1 g/ml) (w/v).

Silver Nanoparticles synthesis

$AgNO_3$ powder was weighed and put into double-distilled water (ddH_2O) to make 1 mM of $AgNO_3$ solution. $AgNO_3$ solution were taken out by the volume of 90 ml and were placed into Erlenmeyer flask which containing 10 ml of fruit water extract of *Phaleria macrocarpa.* For each concentration set was done similarly, with three replication. The flasks were kept under room temperature.

Characterization using UV-Vis Spectrophotometry

Characterization was conducted by UV-Vis Spetrometry using Genesys-20 Spectrophotometer. Measurements were taken daily for 5 days using wavelength of 370-500 nm. The solution from each flask was taken out by volume 1 ml and placed into cuvette. The spectrophotometer was calibrated using ddH_2O as a blank solution. The sample was placed into the chamber inside the spectrophotometer and the absorbance was read. Measurements were conducted three times for each concentration set.

RESULTS AND DISCUSSION

The result shows that AgNPs were already formed in the 24-hours period. The positive result of AgNPs formation was indicated by the brown solution (Figure 1). This result is in line with previous study [3], that browning of solution is one of the indication of AgNPs formation.

Figure 1. Browning color of solution indicating formation of AgNPs

Size estimation of AgNPs formation was done by spectrophotometry. A peak was noticed at about 400 nm from the absorption curve of P3-P5 (0.3-0.5 g/ml). Another peak was also noticed within range 465 from the absorption curve of P1-P2 (0.1-0.2 g/ml).

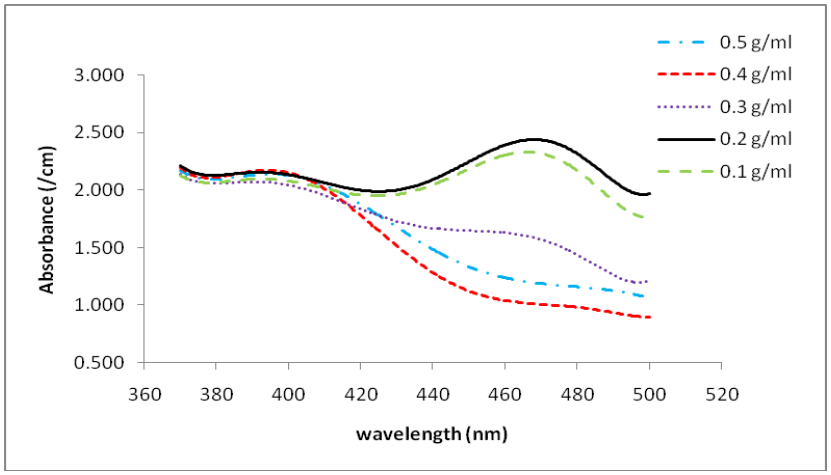

Figure 2. Formation of AgNPs detection using UV-Vis Spectrophotometry.

AgNPs have specific interaction with light, so that light beam in certain wavelength can create oscillation on their surface electron [4]. This oscillation phenomenon is called Surface Plasmon Resonance (SPR), which resulted in specific absorption properties. Strong SPR indicated by a peak of wavelength can be used to determine the size of AgNPs. The peak at about 400 nm in absoption curve indicates that AgNPs was formed with an estimation size of 10-20 nm. Meanwhile, the peak within range of 465 nm also indicates AgNPs formation with an estimation size of 60 nm.

This research is successfully shows that concentration of fruit water extract of *P. macrocarpa* affects the dimensional size of AgNPs being synthesized. An advance investigation to fully determine the dimension of AgNPs shall be done thoroughly. Following research are still on going, to investigate AgNPs size using XRD (X-ray Diffraction) and TEM (Transmission Electron Microscope).

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P-BM06

THE EFFECT OF *BUAH MERAH* ACTIVE FRACTION (*Pandanus conoideus* Lamk.) ON MACROPHAGES PHAGOCYTOSIS ACTIVITY OF C3H MICE BREAST CANCER MODEL

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ABSTRACT

Breast cancer is the second leading cause of cancer deaths after cervical in women today and the most common cancer among women in Indonesia. The use of natural products for cancer treatment is currently more attractive than chemotherapy. This is because natural products are cheap and low side effects. One of natural products that are trusted by the people as an anticancer is *buah merah* (*Pandanus conoideus* Lamk.). Biological therapy using natural products can increase the body's resistance of cancer cells through cellular immunity involving macrophages. The objective of this research was to determine the effect of *buah merah* active fraction on macrophage phagocytosis activity of C3H mice breast cancer model. *Buah merah* Maler from Cycloups Sentani Papua was extracted using chloroform, then the extract was fractionated by vacuum liquid chromatography (VLC). Eighteen female C3H mice aged 2 months were divided into 3 groups of 6 : control, the fraction was given at the end of latent period, and the fraction was given for 2 weeks before cancer transplantation and continued for 3 weeks after cancer transplantation. The active fraction was given orally 0.2 ml/day (0.557 mg/day) for 21 days. Furthermore, each mice was terminated then peritonial macrophages were isolated for examinating the macrophages phagocytosis activity. The result showed that macrophages phagocytosis activity of the group that was given fraction before and after transplantion was the highest (p=0.160) and significantly different to the control group (p=0.096) and the group treated at the end of latent period (p=0.073). It could be concluded that giving active fraction of *buah merah* before and after cancer transplantation showed the best of macrophages phagocytosis activity.

Keywords : *buah merah*, macrophages phagocytosis, breast cancer, C3H mice

INTRODUCTION

In Indonesia, breast cancer is still took second place as the deadly disease (1). The prevention or treatment of cancer is becoming increasingly important given the higher incidence rate. Up to now, breast cancer therapy is often done with conventional chemotherapy, radiation therapy (radiation), surgery, and the combination of all three. Chemotherapy is very expensive and have many weaknesses. For example, cancer treatment with chemotherapy has many side effects that healthy cells die, the skin becomes wrinkled, hair loss, decreased appetite, nausea and cause pain in the patient's body so strong that not all cancer patients undergoing chemotherapy cycles (2).

Therefore, scientists continue to search for anticancer drugs, mainly from natural products, for examples from sponge animals and various plants. The use of natural products

for cancer treatment is currently more attractive than chemotherapy. This is because natural products are cheap and low side effects (3). One of the plants that are trusted by the community as an anticancer drug is *Buah Merah* (*Pandanus conoideus* Lamk.). *Buah Merah* comes from Papua. Scientific research on the potential of *Buah Merah* as a cure for cancer has been investigated by Moeljopawiro over the last five years. The results showed that the red juice has a cytotoxic potential against breast cancer cells (T47D).

Immunity against cancer cells can be enhanced by cellular immune system approach through the activity of macrophages. Macrophages are the majority terminally differentiated cells of the mononuclear phagocyte system (4). Macrophages are versatile cells with capability to adapt their metabolism, phenotype and functional capacities to their mecroenvironment. As a consequence of this flexibility, macrophages survive and function under adverse circumstances, including healing wounds and hypoxic areas within tumors (5). Tumors grow through signals elicited from cells in their Microenvironment. One type of immune cell, the macrophage, plays an important role in normal breast tissue development. Macrophage activity, stimulated by macrophage colony-stimulating factor (M-CSF), is essential for normal breast development (6). In breast tumors, macrophages constitute up to 35% of the infiltrating inflammatory cells (7). Thus, our research objective was to determine the effect of *buah merah* active fraction on macrophage phagocytosis activity of C3H mice breast cancer model

MATERIALS AND METHODS

Tumor Transplantation and Treatment

This study was an experimental laboratory research with the post test only control group design. The object of the study were 18 female C3H mice, 16 weeks old. Mammary cancer induction was conducted by injecting subcutaneously of 0.2 ml (6 x 10^6/ml) tumor cell suspension in the armpit area of the recipient mice. Mice were randomly divided into 3 groups of six. First group was used as a control, second group the *buah merah* fraction was given at the end of latent period (Kuratif), and the last group was used as a preventive group (the fraction was given for 2 weeks before tumor injection and continue after tumor injection for 3 weeks) (Preventif). The active fraction were given orally 0,2 ml/day for 21 days (8).

Isolating and Culturing Peritoneal Macrophages

At the end of this study, each mice was euthanized. After ± 3 minutes, pelt was opened and cleaned with Alcohol 70%. Subsequently 10 ml of cold RPMI medium injected into the peritoneum cavity of mice. Peritoneum gently massaged for ± 3 minutes to get a lot of macrophages. Furthermore peritoneal fluid aspirated (sucked back). Fluid accommodated in

the conical tube and centrifuged 600 rpm at a temperature of 400 ^{0}C for 10 minutes. Supernatant was removed and supplemented with complete RPMI medium consisting of RPMI, Fetal Bovine Serum (FBS), fungizon, and Pen Strep. Then cell suspension was calculated by haemocytometer with desired cell density of 2.5 x 106 cells / ml. Furthermore, cell suspension were cultured on microplate 24 wells that have been given a coverslip. Each wells contained 200 mL cell suspension with a density of 5 x 105 cells / ml. Cell suspension was then incubated in CO_2 incubator at 37 ^{0}C for 30 minutes. Later each wells added with 0.5 ml of complete RPMI medium and incubated in 5% CO2 incubator at 37 ^{0}C for 24 hours.

Test of Latex Phagocytic Activity of Peritoneal Macrophages

Macrophages that have been cultured 24 hours were washed with RPMI medium 2 times. Subsequently 200 mL of latex beads was mixed and incubated in CO2 incubator at 37 ^{0}C for 30 minutes. Then washed with PBS 3 times to remove particles that are not difagosit. Absolute methanol fixation for 30 minutes. Furthermore, methanol was removed and culture wells dried at room temperature. Once dry daubed with 20% Giemsa dye for 20 minutes. Then washed with aquades and dried at room temperature.

Analysis of Phagocytic Activity of Peritoneal Macrophages

To analyse the phagocytic activity of peritoneal macrophages used this formula below :

$$\% \text{ Phagocytic index} = \frac{\Sigma \text{ of macrophages that phagocyte latex} \times \Sigma \text{ latex in the macrophages}}{300 \text{ macrophages}} \quad (9)$$

Statistic analysis

Shapiro-wilk test was used to know the distribution of the data. Furthermore, *one way* ANOVA test (analysis of variance) was used to compare the mean values of phaocytic activity obtained data between the three experimental groups. The significance level was set at $p \leq 0,05$.

RESULT AND DISCUSSION

Phagocytic activity of macrophages, was calculated in the form of macrophage phagocytosis index. The results of macrophage phagocytosis index of each group can be seen in Table 1:

Table 1. phagocytosis index of peritoneal macrophages

Groups	Phagocytosis Index
Control	0,096 ± 0,147
Kuratif	0,073 ± 0,147
Preventif	0,160 ± 0,147*)

*) The mean difference is significant at the 0,05 level

Phagocytosis is a multistep process by which phagocytic cells (macrophages) engulf and destroy infectious agents (10). According to the immunosurveillance hypothesis, tumors expressing antigens are regarded as “nonself” by the immune system, and a major function of the immune system is to survey the body for the development of malignancy and to eliminate tumor cells as they arise (11). But it sometimes happens cancer cells escape our immune system surveillance. The ability of tumors to evade immune surveillance plays a central role in tumor progression (12,13).

Based on the Table 1, we can see that phagocytosis index of preventif groups was significantly higher than control and kuratif group. It means that given active fraction of *buah merah* before and after cancer transplantation showed the best of macrophages phagocytosis activity. In this group, *buah merah* has an ability to increas our immunity According to (14), b*uah merah* contains of alkaloid, flavonoids, and antrakuinon. Flavonoids acts as an immunostimulant in our body (15).

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P-BM07

In vivo Antiplasmodial Activity of Moss *Marchantia polymorpha* L. Chloroform Extract Against *Plasmodium berghei* in Mice

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Introduction

Malaria is one of major parasitic infectious disease in the world. Total of 3,3 billion people living in malaria endemic areas in 109 countries. There are about 107 million people living in malaria endemic areas in Indonesia (CDC, 2010) The prevention for a long time affect resistance mechanism to Anopheles population as vector and to *Plasmodium* as its parasite (Groth *et al*., 2001). Alkaloids, Flavonoids and Terpene are three compounds that have biological activity as growth inhibitor to *Plasmodium* (Nogueira and Lopes, 2011; Goulart *et al*., 2004). Alkaloids is one of compound group that is used as an antimalarial treatment widely. Quinine, one of the first anti-malarial compound, is a member of Alkaloids (Saxena, 2003). It has known that the class of Hepaticopsida moss (it is called Lumut Hati in Indonesia) is containing lipophilic mono-, sesqui- and diterpenes, as well as acetogenins aromatic compounds incorporated in the oil bodies (Asakawa, 2007). Other studies have also shown that one species from Hepaticopsida, *Marchantia convoluta* contains triterpene, flavonoids, steroid and marchantin (Pryce, 1971; Yan *et al*., 2008). Another species of moss that is abundant in Indonesia is *Marchantia polymorpha*. Moss *M. polymorpha* widely spread in upland areas, but until now, bioactive compound of this species is unknown. According to the fact, it is necessary to explore and utilize bioactive compounds moss of *M. polymorpha* as an alternative antimalarial drugs in Indonesia. The purpose of this study to identify the class of bioactive compounds contained in *M*. *polymorpha* chloroform extract, and study influence of it to the level of *Plasmodium berghei* parasitemia in vivo.

Materials and Methods

Plant material. Fresh moss of *M. polymorpha* were collected from Kaliurang, Kalikuning, and Tempel Village, Sleman, Yogyakarta between February 2011 and April 2011. The specimen has been identified in Laboratory of Plant Taxonomy, Faculty of Biology, Gadjah Mada University.

Preparation of moss extract. The moss were cleaned from debris or soil, air-dried at room temperature for 10 days and crushed into powder. Chloroform extract was obtained by maceration method (Suyitno, 1989).

Experimental animals. 35 of male Swiss albino mice weighing between 23-30 g were used for the experiments. The standard condition of humidity, temperature and 12 h light/12 h darkness cycle were maintained. They were fed with standard diet and had free access to water *ad libitum*.

Phytochemical tests. The chloroform extract of *M. polymorpha* was subjected to qualitative phytochemical investigation for various plant constituent according to Thin Layer Chromatography (TLC) method (Harborne, 1987).

Parasite inoculation. The *Plasmodium berghei* was obtained from Department of Parasitology, Medical Faculty, Gadjah Mada University, which parasites are maintained through weekly passage in mice. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor mice and diluting the blood with RPMI medium in proportions indicated by both determinations. Each mouse was inoculated with 0.2 mL of infected blood containing about 1×10^7 *P. berghei* parasitized red blood cells on day 0 intraperitoneally.

Chemosupressive test. The antiplasmodial test of the extract was evaluated using Peter's Suppresive Test (Peter, 1970). The animals were divided into seven groups of five mice each and administered shortly after inoculation, with 5.75, 12.5, 25, 50, and 100 mg/BW doses of the *M. polymorpha* chloroform extract, chloroquine 5 mg/BW (positive control) and 0,3% Tween (negative control) for four days (day 0 to day 3) orally. On the fifth day (day 4), thin films were made from the tail blood of each mouse with stained Giemsa. Parasitemia was determined microscopically, and the difference between the mean value of the control group (taken as 100%) and the experimental groups was calculated and expressed as percentage inhibition.

$$\text{Growth inhibition (\%)} = \frac{Parasitemia\ in\ negative\ control - parasitemia\ in\ study\ group}{Parasitemia\ in\ negative\ control} x100\%$$

Statistical Analysis. Statistical analysis was made by Probit analyze to determine Effectivity Dose 50% (ED_{50}). The ED_{50} values were calculated by SPSS 17 (Finney, 1971).

Result and Discussion

Extraction and Phytochemical Screening

Phytochemical screening of the *M. polymorpha* chloroform extract gave positive indication containing secondary metabolites, alkaloids and flavonoids. The presence of these compounds may explain the medicinal use of this plant (Table 1).

Table 1. Chemical compound of *Marchantia polymorpha* chloroform extract

No.	Compound	Result
1.	Alkaloids	+
2.	Flavonoids	+
3.	Terpene	-
4.	Fenol	-

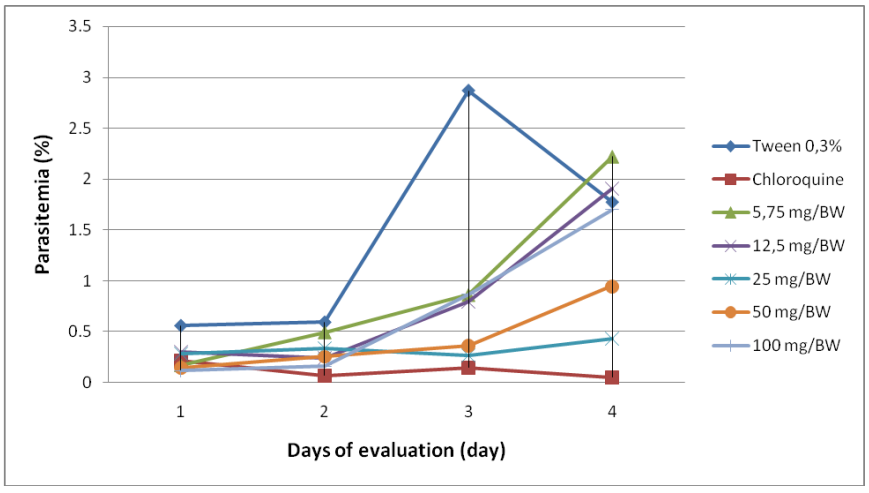

Fig.2. Antiplasmodial activity of *M. polymorpha* chloroform extract

Chemosupressive Test

Marchantia polymorpha exctract activity to inhibit the *P. berghei* activity was tested according to *Peter's Supressive Test*. From the five groups that were tested, 25 mg/BW dose was the most effective dose to inhibit *P. berghei* activity with 75,71% growth inhibition (fig. 2). Two groups with higher dose were less effective to inhibit *Plasmodium berghei* activity might be due to the alkaloids and flavonoids compound were too much that cause erythrocyte lysis. Erythrocyte lysis would make the parasitemia count unaccurate as reported by Widodo and Rahayu (2010). Probit analisys was used to analyze effectivity dose.

Effectivity Doses 50% (ED_{50}) according to the probit analisys was 48,669 mg/BW. But, There were increasing growth of *P. berghei* parasitemia from every extracts that tested. It might be caused antiplasmodial effect of *Marchantia polymorpha* chloroform extract was only short term.

Antimalarial activity by *Marchantia polymorpha* chloroform extract might caused by alkaloids and flavonoids compound. According to Nogueira and Lopes (2011) Alkaloids and flavonoids were effective to inhibit *Plasmodium* growth.

In conclusion, *M. polymorpha* chloroform extract could inhibit *P. berghei* growth indicating this moss contain some lead antiplasmodial compounds, they are alkaloids and flavonoids. However, there are increasing growth of *P. berghei* that could be attentioned.

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P-BM08

ULTRASTRUCTURAL OBSERVATION ON THE NEURON OF THE LAMINA MUSCULARIS MUCOSAE IN THE ILEUM OF SHEEP

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ABSTRACT

Observation on the neuron of the intestinal wall have mostly concentrated on the smooth muscle layer in the tunica muscularis. It has been established that the lamina muscularis mucosae is innervated but its role in intestinal activity has not yet been established. The study aims to provide morphological information on the neuron of the lamina muscularis mucosae. Two pieces of ileum of Merino sheep were cut into 1 mm^2 and fixed by 5% glutaraldehyde in Sorenson's phosphate buffer at pH 7.3. The tissue was dehydrated and cleared, then embedded in pure epoxy resin. The section stained with a saturated aqueous solution of uranyl acetat followed by lead citrate and examined in transmission electron microscope. The neuron within the ganglia classified into four types. Neuron type 1 had a dark perinuclear area due to the concentration of rough endoplasmic reticulum, mitochondria, Golgi complex and microtubules. Neuron type 2 appeared dense numerous ribosomal, stacks of rough ER and microtubules. Neuron type 3 had stacks of cysternae of rough ER, ribosomal rosettes, mitochondria and microtubules throughout the cytoplasm. Neuron type 4 had perinuclear arrays of neurotubules and neurofilaments, ribosomall rosettes and small dense-cored vesicles throughout the cytoplasm.

Keywords: ultrastructural, neuron, lamina muscularis mucosae, intestine

Introduction

Studies on the innervation of the intestinal wall have mostly concentrated on the smooth muscle layer in the tunica muscularis. Morphological aspects of neurons of the myenteric and submucosal plexuses on the intestines of several species have been described (1,2,3,4,5,6). In contrast, there have been few studies on the neuron of the thin layer of smooth muscle beneath the tunica mucosae, the lamina muscularis mucosae (7,8,9). It has been established that this layer is innervated but the function of this smooth muscle layer and its role in intestinal activity has not yet been established. The present research aims to provide basic morphological information on the neuron of the lamina muscularis mucosae.

Materials and methods

The adult 19 Merino sheep were euthanasia with an overdose of pentothal sodium injection. Two pieces of whole width of ileum were cut into 0.3 cm^2 and fixed by 5% glutaraldehyde in Sorenson's phosphate buffer at pH 7.3. After 15 minutes, the tissue was cut into 1 mm^2 and returned to the fixative for a minimum period of 2 hours. The tissue was washed with Sorenson's phosphate buffer for 10 minutes and post-fixed in Dalton's chrome osmic acid for 60-90 minutes at 4^oC. Dehydration in graded series of ethanol was followed by passage through propylene oxide and mixture of propylene oxide and epoxy resin., The tissue was embedded in pure epoxy resin. Five thin sections were taken from each single or group of neuron(s). The thin section were stained with a saturated aqueous solution of uranyl acetat followed by lead citrate and examined in a Phillips CM 100 BioTwin transmission electron microscope.

Result and discussion

The neurons (8-20µm) on the lamina muscularis mucosae had a large oval or round nucleus (4-10µm) which consisted mostly of euchromatin with up to 2 nucleoli, and some had peripheral heterochromatin. The neurons were surrounded by a basal lamina and covered by satellite cells cytoplasm, Schwann cell cytoplasm or axonal endings. However, in some areas the basal lamina was exposed to a thin layer of collagen fibres that separated the neuron from the adjacent muscle fibres. Based on the relationship with satellite cells, cell body profiles and the distribution of cytoplasmic organelles, these neurons were classified into 4 types. Neuron type 1 are single neurons had a dark perinuclear area due to the concentration of rough endoplasmic reticulum (ER), mitochondria, Golgi complex and microtubules. The peripheral cytoplasm was almost devoid of organelles except for numerous dense-core vesicles, ribosomal rosettes and microtubules. Satellite cell did not completely encircle these neurons whose profile was indented, in places, by axons. Neuron type 2 appeared dense due to numerous ribosomal rosettes, stacks of rough ER and microtubules. Most mitochondria and the Golgi complex were perinuclear. Small dense-cored vesicles were sometimes seen. The cell bodies were not ensheathed by satellite cell cytoplasm although this often completely covered the interneuronal surfaces. The cell body profiles was not smooth due to the indentation by axons and to spine-like processes which were especially prominent on surfaces adjacent to bundles of axons. Neuron type 3 have thin processes of satellite cell cytoplasm which surrounded the cell bodies of this type of neuron. Cell profiles were mostly smooth except for some probable dendritic processes. Stacks of cysternae of rough ER, ribosomal rosettes, small mitochondria and microtubules were distributed throughout the cytoplasm. The Golgi complex was prominent near the nucleus. These neuron type 4 were small polar neurons which were almost completely

surrounded by satellite cells. The cell body outline was not smooth and was indented by axon profiles especially in the polar region. Some of these neurons had perinuclear arrays of neurotubules and neurofilaments. Ribosomal rosettes and small dense-cored vesicles were obvious throughout the cytoplasm as were round mitochondria. Stacks of cysternae of rough ER were not present.

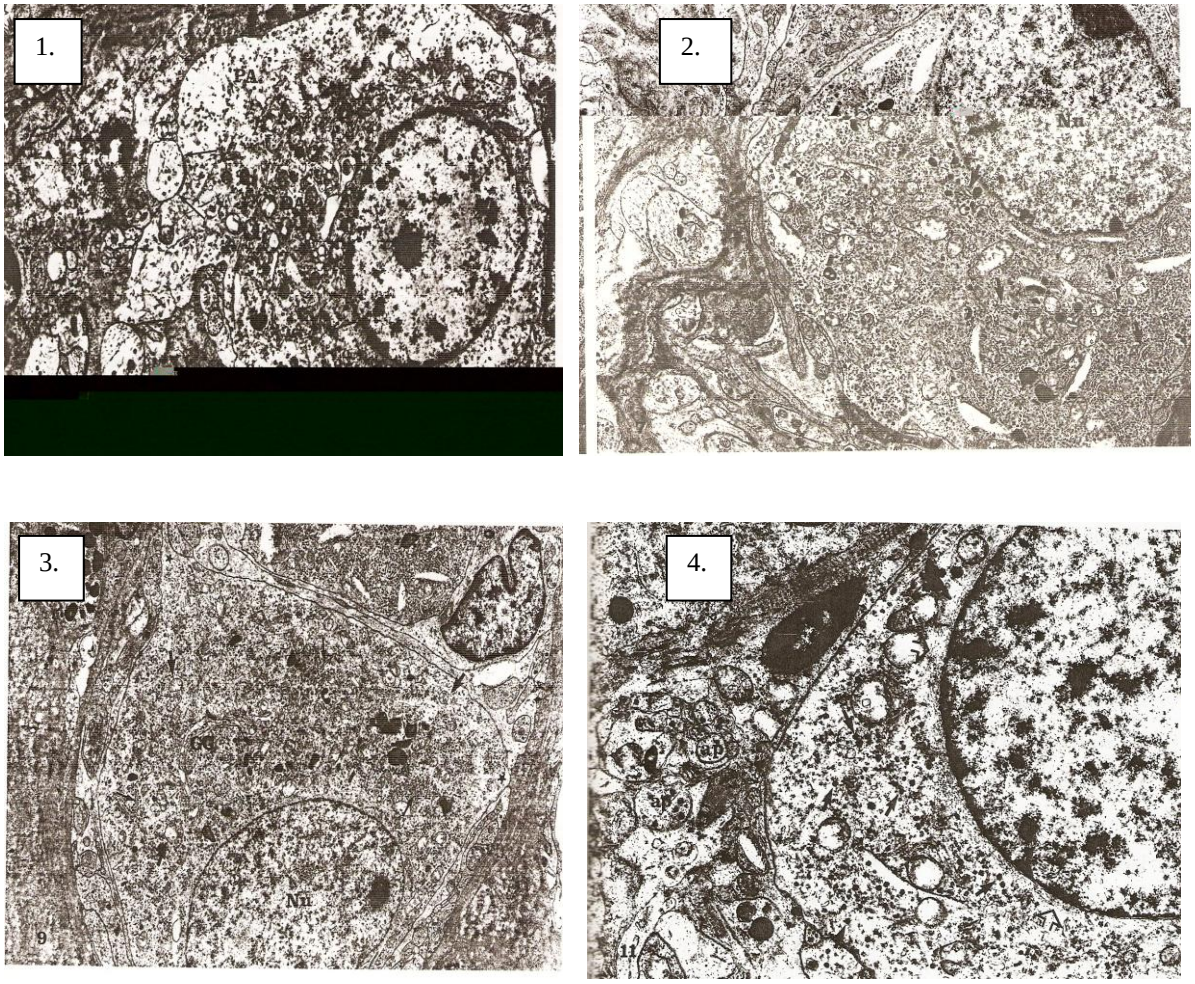

Fig. 1. Neuron type 1 showing the accumulation of cytoplasmic organelles in the perinuclear region and paler peripheral cytoplasmic region. x 9800.

Fig. 2. Neuron type 2 containing numerous ribosomal rosettes and rough ER. The nucleus contains relatively little heterochromatin. x 9300.

Fig. 3. Neuron type 3 contains numerous rough ER, mitochondria and prominent Golgi complex. x 7000.

Fig. 4. A portion of neuron type 4 showing the numerous dense-cored vesicles and ribosomal rosettes. Note the unusual perinuclear array of neurotubules and neurofilaments. x 13 300.

This study shows that the lamina muscularis mucosae has its own nerve plexus that consist of single or small clusters of neurons and associated unmyelinated axons. The general structure of these is similar to that of the myenteric and submucosal ganglia in that there are no blood vessels, intraganglionic connective tissue and small intensely fluorescent (SIF)

cells, an arrangement that is different to that seen in sympathetic ganglia (2, 3, 10). It is also shown that the enteric neurons are not completely surrounded by processes of glial cells (2,5) as seen with some neurons in this present study. It is unclear wether SIF cells are present in the intestine despite descriptions of nuerons containing numerous granular vesicles (4) and which had a similar ultrastructural appearance to small granule-containing or SIF cells. Type 4 neurons and some type 2 neurons in this present study did contain small dense-cored vesicles throughout their cytoplasm but these were not as numerous as previous descriptions. On the basis that it is believed that peptidergic profiles contain numerous large granular vesicles (LGVs) (6) it seem probable that the type 1 neurons, which contain numerous LGVs, may be peptidergic. This is supported by the fact that peptidergic vesicles are considered to be produced in nerve cell bodies and are then passed to the axonal endings by axonal transport (12, 13). Immunohistochemical studies have shown that there submucosal and myenteric ganglionic neurons which project to the mucosae (14, 15, 16) including the lamina muscularis mucosae (7, 8, 9). From these studies it is likely that some nerve fibres in the lamina muscularis mucosae originate in the submucosal and myenteric ganglia. However, since axonal endings in the lamina muscularis mucosae contain similar vesicles to the axonal endings within the ganglia, it is most probable that some of the nerve fibres in the smooth muscle layers are from the ganglia associated with them.

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P-BM09

Evaluation of Molecular Assays for The Detection of Dengue Virus Serotype In Dengue Fever Patients

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ABSTRACT

Dengue fever is one of the most important arthropod-borne diseases and it caused by four dengue virus serotypes (DENV-1, DENV-2, DENV-3, and DENV-4), belonging to the genus Flavivirus, family Flaviviridae. During the past decade, various forms of PCRs such as reverse transcription polymerase chain reaction (RT-PCR), Nested PCR and Multiplex PCR have been developed to address the need for rapid identification of viruses to serotype level with more accuracy.

In this research we studied the nested multiplex RT-PCR methods of Lanciotti *et al.* (1992) which is used by WHO and almost all country to detect Dengue Virus infection and one-step multiplex RT-PCR methods of Yong *et al* (2007). In the first method, primers oligonucleotide was designed against pre-M/C region for all primers and second method was designed against 5'UTR region as forward primer and 4 reverse primers were designed to target specific areas of the M and C proteins of the respective Dengue virus 1, 2, 3, and 4.

The first multiplex RT-PCR assay was able to detect only 8 samples especially for DENV-3 according to the size of PCR product estimation, whereas the second multiplex RT-PCR assay was able to detect all samples (30 samples). These results showed that there are polimorfism in primer target of DENV-1, DENV-2 and DENV-4 for Dengue Virus in Indonesian population. These results also showed that the second assays were 100 percent specific for detection of the Dengue Virus in Indonesia and could be use for the diagnosis of dengue and for epidemiologic surveillance in Indonesia.

Keywords: Dengue virus, serotyping, RT-PCR, UTR, pre-M/C.

P-BM10

Nutrient Content and Amino Acids composition of Gayam (*Inocarpus fagiferus* Fosb.) Tempeh

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ABSTRACT

Gayam tempeh is made of gayam (*Inocarpus fagiferus* Fosb.) seed fermented by using tempeh mold *Rhizopus* sp. The fermentation process eliminates flatulence property and increase the nutritional content in the gayam seed. Gayam tempeh has been produced in a small scale by some people of Pundong, Bantul, DI Yogyakarta, but the nutrients content have not been analyzed yet. The purpose of this study was to determine the nutritional content of gayam tempeh. Total protein, lipid, crude fiber, carbohydrates, water and ash were determined by proximate analysis. Moreover, amino acids and vitamin B2 of gayam tempeh were determined using HPLC (High Performance Liquid Chromatography) method. The minerals (Ca, P and Fe) were measured using AAS (Atomic Absorption Spectrophotometry).

The results showed that the content of total protein, lipid, crude fiber, carbohydrates, water, ash, minerals (Ca, P, Fe) and vitamin B2 in gayam tempeh were 4.57 %, 1.23 %, 3.64 %, 28.13 %, 65.49 %, 0.58 %, 0.04 %, 0.04 %, 0.01 % and 0.00913 % respectively. Moreover, gayam tempeh contained 7 essential amino acids (histidine, arginine, valine, phenylalanine, isoleucine, leucine and lysine) which are needed for human health. Therefore it can be concluded that gayam tempeh can be used as alternative food in addition to soybean tempeh.

Keywords: Gayam tempeh, *Inocarpus fagiferus* Fosb., nutrient content, amino acids

Introduction

Gayam tempeh is a traditional fermented gayam seed (*Inocarpus fagiferus* Fosb.) which is produced by some people in Pundong village, Bantul, Yogyakarta Province using tempeh mold *Rhizopus* sp. It is normally consumed as fried, boiled or steamed. The fermentation process eliminates flatulence property and increases the nutritional values in Gayam tempeh, such as development of vitamins, phytochemicals and antioxidative constituents. Moreover, the fermentation process reduces the phytic acid and increases the bioavailability of minerals including calcium, zinc and iron (Astuti and Dallas, 2000).

Gayam plant is found widespread throughout Indonesia, especially in Java, Sumatra and Kalimantan islands (Wardiyono, 2010). Gayam seed have flatted form, 4 -8 cm long, white endosperm and hard seed skin. Proximate nutrient content of Gayam seed were ash, water, protein, fat, carbohydrate and fiber (Epriliati et al., 2002). In some rural area, Gayam seed is consumed as salty chips and boiled seed. However it caused flatulency as low digestibility. Gayam tempeh has been produced in a small scale by some people of Pundong, Bantul, DI Yogyakarta, but the nutrients content have not been analyzed yet. The purpose of this study was to determine the nutrient content of gayam tempeh. In addition,

this study was conducted to help introduce nutritious fermented gayam tempeh in the rural areas and prepare products that can be used for supplementary feeding programmes.

Materials and Methods

1. Preparation of gayam tempeh

One kilogram of gayam seeds were harvested from Pundong, Bantul, Yogyakarta Province and boiled for 1 hour and peeled the skin. Boiled seeds were thinly sliced to about 1 cm thickness and steamed for 30 min. After cooling, sliced gayam seeds were mixed using 2 g mold *Rhizopus* sp (Raprima) which is purchased at traditional open market located at Pundong, Bantul. Gayam seeds were packed in banana leaf and stored for 36 hour at room temperature.

2. Proximate analysis of raw gayam tempeh

Determination of moisture (water content), crude fat, crude protein and total ash in raw gayam tempeh were carried out using AOAC (1990) method.

3. Determination of Ca, P, Fe and vitamin B2

The minerals (Ca, P and Fe) were determined using wet digestion methods which previously described (Gordon dan Robert (1977) in Moelyopawiro (1985) (Pratiwi, 1987) and measured by AAS (Atomic Absorption Spectrophotometry). Vitamin B2 of gayam tempeh were determined using HPLC (High Performance Liquid Chromatography) method.

4. Analysis of amino acids composition

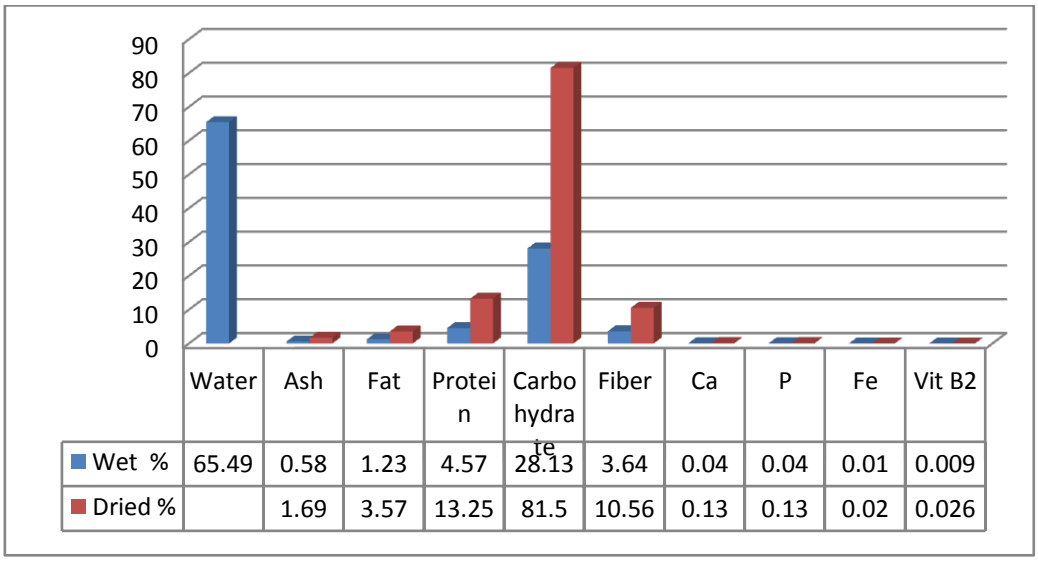
Sixty mg of ground raw gayam tempeh was added to 4 ml (6 N) hydrochloric acid and heated at $110^{o}C$ for 24 hour. After cooling, the mixture was neutralized (pH 7) using NaOH 6 N until up to 10 ml final volume and was passed through a Whatman paper (0,2 µ pores). One hundred µl of sample was diluted in OPA (Orthophalaldehyde) 100 µl and stirred for 5 min and then 20 µl of sample was injected into a reverse phase high performance liquid chromatography (HPLC). The composition of amino acids was determined using various concentrations of amino acids standard.

Results and Discussion

This research has been determined nutrient content of gayam tempeh per 100 g wet and dried basis (Fig. 1). The nutrient content of wet gayam tempeh less than dried gayam tempeh as water content of wet gayam tempeh is high (65,49%). Water content of gayam

seeds (Epriliati et al., 2002) compare to gayam tempeh is higher 15,45 % as a result of boiling process during gayam tempeh preparation.

Fermentation of gayam tempeh involves various of enzymes, such as lipase, amylase and protease which is produced by mold. Winarno (1995) explained that water in food effect enzymatic reaction. Enzyme or substrate is inhibited if free water content in food is low.



	Water	Ash	Fat	Protein	Carbohydrate	Fiber	Ca	P	Fe	Vit B2
Wet %	65.49	0.58	1.23	4.57	28.13	3.64	0.04	0.04	0.01	0.009
Dried %		1.69	3.57	13.25	81.5	10.56	0.13	0.13	0.02	0.026

Fig. 1. Proximate nutrient content of gayam tempeh per 100 g wet and dry-weight basis

The protein, fat and carbohydrate content of tempeh gayam were higher compare to gayam seed. Fermention of gayam tempeh hydrolyzes fat to fatty acid and glycerol by *Rhizopus* sp (Astuti *et al.* 2000). Moreover protease and amylase, selulase and xilase which are produced by mold hydrolyse protein and carbohydrate into amino acids and monosaccharide/oligosaccharides, respectively. Free amino acids were increased during fermentation. The results showed that tempeh gayam contained essensial amino acids including histidine, arginine, valine, phenylalanine, isoleucine, leucine and lysine (Table 1.)

Table 1. Amino acids composition of gayam tempeh

Essensial amino acids	Non essensial amino acids
Histidin	Asam aspartat
Arginin	Serin
Valin	Glisin
Fenilalanin	Alanin
Isoleusin	Tirosin
Leusin	
Lisin	

Mineral phospor and calcium are higher in gayam tempeh compare to unfermented gayam seeds, eventhough remain lower than soybean tempeh. However, the consumption of 200 g wet and dry gayam tempeh/day contribute 16% and 52% Ca need in diet for adult. Based on our results the consumption of 200 g wet and dry gayam tempeh/ day contribute 11.4% and 37.14 % of P, respectively. In addition, the consumption of 100 g wet gayam tempeh/ day is enough to cover Fe need for adult.

Nutrients contained in gayam seeds would increase by 2-fold after gayam tempeh turned into people food is very beneficial to the body other than easily digested by the body tempe also has many benefits. Contained fibers was also quite high, this fiber the body needs for healthy digestive tract. The fungus *Rhizopus oligosporus* also produce antibiotic substances that the body uses to fight harmful organisms for the human body.

In conclusion gayam tempeh was found to contain total protein, lipid, crude fiber, carbohydrates, water, ash, minerals (Ca, P, Fe) and vitamin B2 in gayam tempeh were 4.57 %, 1.23 %, 3.64 %, 28.13 %, 65.49 %, 0.58 %, 0.04 %, 0.04 %, 0.01 % and 0.00913 % respectively. Moreover, gayam tempeh contained 7 essential amino acids (histidine, arginine, valine, phenylalanine, isoleucine, leucine and lysine) which are needed for human health. Therefore it can be concluded that gayam tempeh can be used as alternative food in addition to soybean tempeh.

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ATTACHMENT

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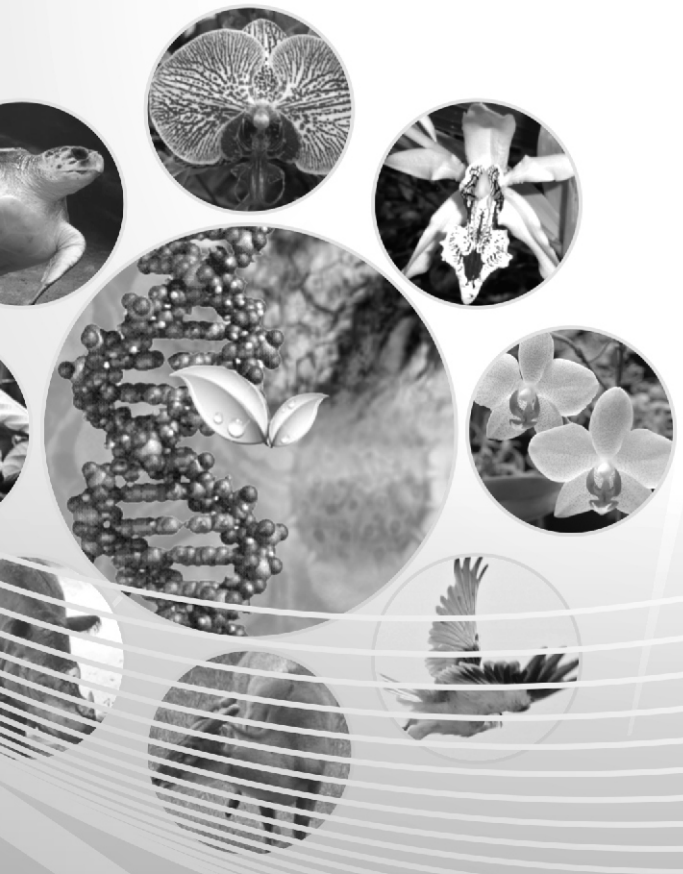

September 23rd-24th 2011

Yogyakarta, INDONESIA

FACULTY OF BIOLOGY
UNIVERSITAS GADJAH MADA

I-MHERE
PROJECT

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