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Dari: sumardi adi (sumardi\_bio@yahoo.co.id)

Kepada: ijfr.forda@gmail.com

Tanggal: Kamis, 25 Maret 2021 07.32 WIB

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----- Pesan yang Diteruskan -----

**Dari:** sumardi adi <sumardi\_bio@yahoo.co.id>

**Kepada:** Dian Anggraini, S.Hut., MM. <publikasi.litbang@gmail.com>

**Terkirim:** Selasa, 23 Maret 2021 08.37.03 WIB

**Judul:** Re: [IJFR] Editor Decision

Dear Dian Anggraini, S.Hut, MM

This is manuscript revision

regards

Sumardi

Pada Jumat, 12 Februari 2021 20.02.18 WIB, Dian Anggraini, S.Hut., MM. <publikasi.litbang@gmail.com> menulis:

Dear

Universitas Lampung Sumardi Sumardi:

Based on the review, your manuscript, titled:

"MANGROVE-PROTEOLYTIC *Bacillus* sp. AND ITS POTENCY AS PROBIOTIC CANDIDATE".

need further revision to be published in Indonesian Journal of Forestry Research (IJFR)

kind regards,

Dian Anggraini, S.Hut., MM.

Forest Products Research & Development Center

[dian\\_forester@yahoo.com](mailto:dian_forester@yahoo.com)

Dian Anggraini, S.Hut., MM

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Reviewer A:

The originality of the subject matter, and whether the manuscript would be of interest to the international, academic and practitioner readership of the IJFR:

This paper is original and there is still some information about bioprospecting in mangrove forest

Does the manuscript draws on an appropriate range and depth of literature?:

No, there is still gap in Introduction. The authors should add state of the art of this research. What the researchers in the world have done for microbes investigation in mangrove forests.

Does the methods employed are rigorous, ethical, and suitable for the topic under investigation?:

The method is ok.

Do the findings presented are subjected to suitable analysis and sound conclusions drawn?:

Analysis and discussion is still poor. The authors should read many papers in international journal, and answer the question in Introduction

Does the paper clearly identify any implications for future research? Are these implications consistent with the findings and conclusions of the manuscript?:

The manuscript has implication for future research, but they must make complete it with many references from recent international papers.

Please indicate decision and write your detailed comments to the author:

Major revisions required

One sentence to summarise your decision:

MAJOR REVISIONS

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## [IJFR] Editor Decision

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Dari: Dian Anggraini, S.Hut., MM. (publikasi.litbang@gmail.com)

Kepada: sumardi\_bio@yahoo.co.id

Tanggal: Jumat, 12 Februari 2021 20.02 WIB

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Major revisions required

One sentence to summarise your decision:

MAJOR REVISIONS

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## Re: [IJFR] Editor Decision

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Dari: sumardi adi (sumardi\_bio@yahoo.co.id)

Kepada: publikasi.litbang@gmail.com

Tanggal: Minggu, 21 Juni 2020 22.46 WIB

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Dear  
Dian Anggraini, S.Hut., MM.

Thank you, my manuscript had been reviewed. I will correct immediately.

Regards  
Sumardi

Pada Sabtu, 20 Juni 2020 16.29.36 WIB, Dian Anggraini, S.Hut., MM. <publikasi.litbang@gmail.com> menulis:



Dian Anggraini, S.Hut., MM. (publikasi.litbang@gmail.com) is not on [your Guest List](#) | [Approve sender](#) | [Approve domain](#)

Dear  
Universitas Lampung Sumardi Sumardi:

Based on the review, your manuscript, titled:

"PROTEOLYTIC Bacillus sp. FROM MANGROVE AT MARGASARI " EAST LAMPUNG".

need further revision to be published in Indonesian Journal of Forestry Research (IJFR)

kind regards,

Dian Anggraini, S.Hut., MM.  
Forest Products Research & Development Center  
[dian\\_forester@yahoo.com](mailto:dian_forester@yahoo.com)  
Dian Anggraini, S.Hut., MM

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2. Apakah naskah sudah sesuai cakupan jurnal IJFR ?:  
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d. Apakah abstrak Bahasa Inggris tersedia tidak lebih dari 250 kata ?:

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e. Apakah ukuran dan tipe font garamond, 12pts, 1,5 spasi, 1 kolom ?:

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Catatan ::

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## PROBIOTIC CANDIDATE PROTEOLYTIC *Bacillus* sp. COLLECTED FROM MANGROVE OF MARGASARI –LAMPUNG

Received: ..... Revised: ..... Accepted: ..... (Filled by IJFR)

### PROBIOTIC CANDIDATE PROTEOLYTIC *Bacillus* sp. COLLECTED FROM MANGROVE OF MARGASARI –LAMPUNG

Intensive shrimp culture has encountered with many problems such as declining in water quality through disease caused by pathogenic microbes which affected in mortality. The aim of the study was to determine any potential probiotic from *Bacillus* sp. collected from mangrove in East Lampung in which then could be used to improve proteolytic and probiotic activity of the cultured shrimps. This is descriptive research with sampling and data collection bacteria from many of sample mangrove. The study found 128 isolate Bacillus from which then come to 5 potential probiotic Bacillus sp. From the study 5 Bacillus sp has been isolated and have potential properties for probiotic, they were KPP212, IP121, UJ131, UJ132 and SB141, with each isolate has characteristics with proteolytic property, growth in wide range pH (4 – 10) and osmotic stress (0 – 6% NaCl), non-pathogenic, ability for glucose fermentation, non-motile and has negative catalase activity. The fives potential Bacillus sp. can be used as probiotic for shrimp farming.

Key words: characterization, *Bacillus* sp., mangrove, probiotic

### KANDIDAT PROBIOTIK PADA *Bacillus* sp. PROTEOLITIK DIKOLEKSI DARI HUTAN MANGROVE DI MARGASARI - LAMPUNG.

Budidaya udang dengan cara intensif telah menyebabkan banyak masalah seperti penurunan kualitas air yang mengakibatkan munculnya mikroba patogen sehingga mempengaruhi kematian. Tujuan dari penelitian ini adalah untuk menemukan *Bacillus* sp. yang diisolasi dari beberapa sampel di mangrove dan mengkarakterisasi mikroba terisolasi tersebut untuk penggunaan probiotik. Penelitian ini merupakan penelitian diskriptif dan koleksi data bakteri dari berbagai jenis sampel mangrove. –Dari penelitian ditemukan 5 *Bacillus* sp. yang terisolasi dan memiliki sifat potensial untuk probiotik, yaitu KPP212, IP121, UJ131, UJ132 dan SB141, dengan masing-masing isolat memiliki karakteristik bersifat proteolitik, pertumbuhan dalam rentang pH yang luas (4 - 10) dan tahan pada tekanan osmotik (0 - 6% NaCl), tidak patogen, kemampuan untuk fermentasi glukosa, tidak motil, serta tidak memiliki aktivitas katalase. Kelima bakteri Bacillus sp. tersebut dapat digunakan untuk probiotik pada budidaya udang.

Kata kunci : karakterisasi, *Bacillus* sp., mangrove, probiotik

## I. INTRODUCTION

Mangrove of Margasari village in district of Labuhan Maringgai – East Lampung regency laid in S: 5°51'84" - E: 105° 64'84" covers for about 700 hectares which is accounted for 6.65% of total mangrove in Lampung Province. Mangrove has ecologically and economically functions, such as protecting coastal abrasion, brackish water quality control, habitat for many organism, medicines, and paper pulp. Ecological activities of mangrove are influenced by presence of waste, decomposition by microorganisms, mineral taking by plants and other biological activities, so that the mangrove ecosystem keeps equilibrium (Kementerian Kehutanan, 2014; Kariada and Andin, 2014). Furthermore, it is necessary to rehabilitate mangrove for further conservation of coastal area.

Many researchers in various places have also isolated the bacteria potential from mangrove. Study of Deivanai et al. (2014) isolated *Pantoea ananatis* (1MSE1) and *Bacillus amyloliquefaciens* (3MPE1) bacteria from mangrove interacted positively with rice seedlings which resulted in the significant increase in root and shoot length, fresh weight, and chlorophyll content. The study of Castro et al. (2018) proved that *Enterobacter* sp. MCR1.48 from mangrove endophyte that this strain effectively promotes the *Acacia*

**Commented [MT1]:** There is still missing some description of methods briefly, please add 3-4 statements and also concluding remark in this abstract.

I introduction

M methods ????? 3-4 statements

R Results

A and

D Discussion/Concluding Remarks? Please one statement is important for bioprospecting in this research

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Add: state of the art of past or current researchs, and please citation new publication ; i.e .

-Castro, R.A., Quecine, M.C., Lacava, P.T. et al. Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. *SpringerPlus* 3, 382 (2014). <https://doi.org/10.1186/2193-1801-3-382>

- Hamzah T, Lee S, Hidayat A, Terhem R, Faridah-Hanum I and Mohamed R (2018) Diversity and Characterization of Endophytic Fungi Isolated From the Tropical Mangrove Species, *Rhizophora mucronata*, and Identification of Potential Antagonists Against the Soil-Borne Fungus, *Fusarium solani*. *Front. Microbiol.* 9:1707. doi: 10.3389/fmicb.2018.01707

-Subramanian Deivanai, Amrithagata Santhanam Bindusara, [...], and Subhash Janardhan Bhore. 2014. *Culturable bacterial endophytes isolated from Mangrove tree (Rhizophora apiculata Blume) enhance seedling growth in Rice.* *J Nat Sci Biol Med.* 2014 Jul-Dec; 5(2): 437–444. doi: 10.4103/0976-9668.136233

B I G Maulani, D A C Rasmi and L Zulkifli\* . Isolation and characterization of endophytic bacteria from mangrove *Rhizophora mucronata* Lam. and antibacterial activity test against some pathogenic bacteria 1402 (2019) 033038 IOP Publishing doi:10.1088/1742-6596/1402/3/033038

RiccardoSoldan<sup>a12</sup>FrancescaMapelli<sup>a1</sup>ElenaCrotti<sup>a</sup>SylviaSc hnell<sup>a</sup>DanieleDaffonchio<sup>a</sup>RamonaMarasco<sup>a</sup>MarcoFusi<sup>a3</sup>Sar aBorin<sup>a</sup>MassimilianoCardinale<sup>a4</sup>. 2019. Bacterial endophytes of mangrove propagules elicit early establishment of the natural host and promote growth of cereal crops under salt stress. *Volumes 223–225, June–August 2019, Pages 33–43.* <https://doi.org/10.1016/j.micres.2019.03.008>

Renata AssisCastro<sup>ab</sup>Manuella NóbregaDourado<sup>a</sup>Jaqueline Raquel deAlmeida<sup>a</sup>Paulo TeixeiraLacava<sup>a</sup>AndréNave<sup>a</sup>Itamar Soares deMelo<sup>a</sup>João Lucio deAzevedo<sup>ab</sup>Maria CarolinaQuecine<sup>a</sup>. Mangrove endophyte promotes reforestation tree (*Acacia polyphylla*) growth. *Brazilian Journal of Microbiology*. *Volume 49, Issue 1, January–March 2018, Pages 59–66* <https://doi.org/10.1016/j.bjm.2017.04.002>

**please open this link :**  
<https://iopscience.iop.org/article/10.1088/1755-1315/236/1/012060/pdf>

Please browsing many references in google, with many keywords: i.e mangrove, endophytic, isolation

i.e.

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polyphylla growth and fitness. The bacteria can be used in the seedling production of the tree. Other researcher, Maulani et al. (2019) was successfully isolated eighteen endophytic bacteria from mangrove *Rhizophora mucronata* Lam from Gili Sulat, East Lombok. The 18 isolates of endophytic consist of 15 isolates were Gram positive bacteria and 3 isolates were Gram negative bacteria. The endophytic bacteria isolate that had antibiotic activity to against *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli*. On the other hand, the potential fungi from Mangrove also discover. Hamzah et el. (2018). Isolated endophytic fungi from mangrove *Rhizophora mucronata* in Malaysia. From the study obtained several fungi i.e. *Alternaria*, *Fusarium*, *Nigrospora*, *Pestalotiopsis*, *Phoma*, and *Xylaria*. After their culture assay for their antagonism activities with phytopathogenic fungus *Fusarium solani* between 45–66%. Of the six isolates, only *Fusarium lateritium* and *Xylaria* sp. showed antibacterial activities against the pathogenic bacteria, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

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Mangrove plays important role on the coastal water food web and habitat supporting many different biota, such fish, crabs, shrimps and molluscs (Scharler, 2011). Around of the Margasari mangrove, there are shrimp farmings. The mangrove has many wastes. It is good nutrition for various of bacterial growth. In nature, the bacteria can pass into the digestive tract of animals. Several types of bacteria found in the digestive tract of animals had role important to improve the utilization of food and fish healthy (Sarastiti et al., 2020). *Bacillus* sp. be one of the bacteria that can increase the digestibility of fish or shrimp and it has the potential as a probiotic (Anggriani et al., 2012). Lack of information has been done related to isolation of *Bacillus* sp. from Mangrove of Margasari – East Lampung, especially related to those used for probiotics.

Mangrove plays important role on the coastal water food web and habitat supporting many different biota, such fish, crabs, shrimps and molluscs (Scharler, 2011). Around of the Margasari mangrove, there are shrimp farmings. The mangrove has many wastes. It is good nutrition for various of bacterial growth. In nature, the bacteria can pass into the digestive tract of animals. Several types of bacteria found in the digestive tract of animals had role important to improve the utilization of food and fish healthy (Sarastiti et al., 2020). *Bacillus* sp. be one of the bacteria that can increase the digestibility of fish or shrimp and it has the potential as a probiotic (Anggriani et al., 2012). Lack of information has been done related to isolation of *Bacillus* sp. from Mangrove of Margasari – East Lampung, especially related to those used for probiotics.

The advantage of the probiotic technology has process of natural and safe. Probiotics had a healthy effect which includes interacting directly with commensal and pathogenic microorganisms. The probiotics were used many functions i.e. (1) to prevent and treat infections, (2) to improve the balance microorganisms in the small intestine, (3) produce extracellular enzymes, and (4) produce beneficial compounds such as vitamins and short chain fatty acids (Tensiska, 2008).

Isolation and selection of proteolytic bacteria is very potential to be used as probiotics. Character of the bacteria is needed to increase of feed efficiency. Study reported that shrimp and fish feed contained as much as 55.51% - 67.68% protein (Handayani, 2011). Other studies have also shown that some microorganisms as probiotic candidate, they were *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas putida*, *Bacillus bataviensis*, and *Caulobacter* sp. (Rahmawan et al., 2014 ; Seprianto et al., 2017).

Shrimp cultures have been widely growing in Lampung Province, in which intensive shrimp culture deliberates some diseases related to its cultures (Taukhid et al., 2008). In order to prevent or regulate the disease therefore development of probiotic is necessary established (Samosir et al., 2017), one of which by developing probiotic from surrounding ecosystem. Many isolated bacteria which potential

sources of probiotic can be collected from mangrove communities, such as from mangrove plant (its root and bark), fishes like milky fish, cuttlefish, mangrove shrimps, mollusks, crabs and other fishes as well as from the mud, water and other abiotic of mangrove ecosystem (Pratiwi *et al.*, 2013 ; Seprianto *et al.*, 2017 ; Muliani *et al.*, 2017). Therefore, it is necessary to figure out any potential of proteolytic *Bacillus* sp. as local probiotic collected from mangrove of Margasari from which then it possibly could be applied into the aquaculture related to digestion and disease problems especially in shrimp and fish cultures encountered most in Lampung Province.

## II. MATERIAL AND METHOD

### A. Sources of isolated bacteria

Sampel used to isolate bacteria was collected from mangrove communities in Margasari village – Labuhan Maringgai – East Lampung Regency. Abiotic and biotic samples were collected from water and mud, fishes, shrimps, crabs, mollusk, cuttlefish found in mangrove ecosystem, as well as the *Rhizophora* sp' bark and roots.

### B. Isolation of *Bacillus* sp.

Isolation of *Bacillus* sp. was done from different sample mass, 10 g of mud, 1 g mangrove root and skin, 1 ml of water, 1 ml intestinal suspension of shrimp, crab, mollusk, fish, etc. (Figure 1). Sample suspension then was made by adding 90 ml physiological salt for mud and 9 ml for water, mangrove root and bark, shrimp, mollusk, crab and fish. All samples were homogenized by using *vortex mixer* at 80°C for 15 minutes. Dilution was made for each sample in series of  $10^{-1}$  and  $10^{-2}$ . 1 ml of each diluted sample suspension was spread into skim milk agar media modification of *Sea Water Complete* (SWC), followed by incubation for 24 hours at 37°C (Hamtni, 2014). The isolate then was purified by *quadrant streak* into the SWC Agar media.

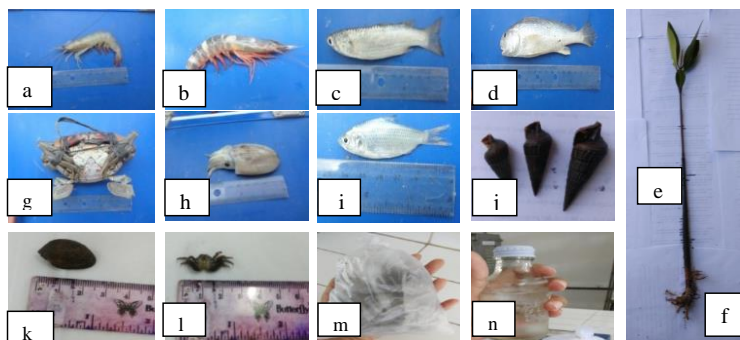


Figure 1. Collected biotic samples a. *Penaeus merguensis* (Udang Jerbung), b. *Litopenaeus vannamei* (Udang Kucing) c. *Mugil* sp (Ikan Belanak) d. *Mallotus villosus* (Ikan Kepala batu) e. Epidermis of *Rhizophora*

sp. (Kulit bakau), f. Root of *Rhizophora* sp. (Akar bakau), g. *Scylla serrata* (Kepiting Bakau) h. *Sepia latimanus* (Sotong), i. *Tenualosa toli* (Ikan Pirit), j. *Telescopium telescopium* (Siput bakau) k. *Nerita violacea* (Keong bulat), and l. *Episesarma* sp (Kepiting pemanjat pohon) and abiotic samples (m. mud and n. water)

### C. Proteolytic Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into SWC media modified with skim milk. The culture then was incubated for 24 hours at 37°C. Observation was made by determining the proteolytic index formed (Hamtini, 2014., Hapsari et al., 2016., Sumardi et al., 2018)

### D. Osmotic/salinity stress Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into modified SWC media with NaCl concentration of 0%; 3%; and 6%. The culture then was incubated for 24 hours at 37°C. Observation was made by determining the number of growing colonies (Subagiyo et al., 2015).

### E. pH-stress Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into modified SWC media with pH 4, 7 and 10. The culture then was incubated for 24 hours at 37°C. Observation was made by determining the measurement of growing colonies (Kepel et al., 2020).

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into modified SWC media with pH 4, 7 and 10. The culture then was incubated for 24 hours at 37°C. Observation was made by determining the measurement of growing colonies (Kepel et al., 2020).

### F. Patogenetic Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into blood modified SWC media. The culture then was incubated for 24 hours at room temperature (Hamtini, 2014). Observation was made by determining the hemolytic ability of isolate from change in different color (Figure 2).

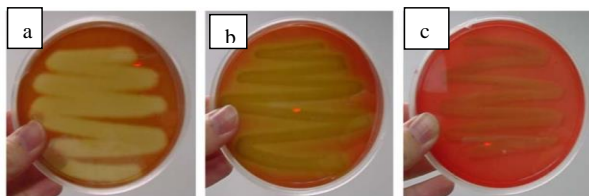


Figure 2. Hemolysis test in blood agar a. Beta hemolysis, b. Alpha Hemolysis and c. Gamma hemolysis (Aryal, 2015).

### G. Characteristic Test

Characterization of isolate bacteria presumably *Bacillus* sp. was done in 2 steps, as follows:

- a. Morphological characterization of colony and cell. Colony characterization was done by observation on the colony formed while cell morphology was made on gram smear (Yulvizar, 2013).
- b. Biochemical test  
Biochemical test as colony characterization was conducted in different tests, such as tests on catalase, mortality and glucose fermentation.
  - b.1. Catalase test

2 drops of H<sub>2</sub>O<sub>2</sub> was placed in sterile glass. Then, 1 ose needle picked of isolate *Bacillus* sp. was mixed into H<sub>2</sub>O<sub>2</sub> in object glass (Yulvizar, 2013).

b.2. Motility test

As much as 1 ose needle isolat *Bacillus* sp. was placed into SWC agar media SWC. The culture then was incubated for 24 hours at 37°C temperature (Samosir et al., 2017).

b.3. Glucose fermentation test

As much as 1 ose needle isolat *Bacillus* sp. was inoculated into liquid SWC agar media and added with 1% sugars (glucose, lactose, manitol, succrose and mannose). The culture then was incubated for 24 hours at 37°C temperature (Samosir et al., 2017).

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III. RESULT AND DISCUSSION

A. Margasari Mangrove

Margasari mangrove is very important for coastal area of East Lampung. Most of the shrimp or fish cultures/ponds found in mangrove belt of East Lampung. It is known that mangrove is able to support many biota, including fish, shrimps, mollusks (Fig.1). Margasari mangrove, as well other mangrove ecosystems, provides food for many aquatic biota and contributes to biological cycle in coastal water. With special structure of mangrove plants, such as *Rhizophora mucronata*, *Avicennia marina*, *Sonneratia alba* and other ground floor of the mangrove as well as swamp and water flow within the mangrove connecting to each other provides shelter for many larvae of mangrove biota. Spawning, nursery, shelter of many biotas which provided by mangrove is not away from the activity of microbes as decomposer. These microbes possible believed have beneficial uses such as for probiotic, antibiotic and bioactive products and else (Subagiyo et al., 2017). Recent study also indicated that some of the mangrove shrubs, such as *Acanthus illicifolius* contains antioxidant (Widiastuti et al., 2019). Other studies proved that *Bacillus* sp. as probiotic bacteria have been found in Mangrove Lampung (Sumardi et al., 2019, and Sumardi et al., 2020)

B. Isolation of *Bacillus* sp.

Five (5) isolate *Bacillus* sp. was found and predicted potentially used for probiotic from mangrove samples. They were from climbing crab intestine, fish intestine, 2 isolat from shrimp and one from mollusk intestine. Those five isolates *Bacillus* sp. were collected after undergoing selection tests for potential/candidate probiotic and can be seen at Table 1.

Table 1. Number of *Bacillus* sp. from isolation and selection tests

Table 1. Number of *Bacillus* sp. from isolation and selection tests

Source	Type of Isolant	Protease Test		Osmotic Stress Test		Pathogen Test	
		Non Proteolytic	Proteolytic	No growth on pH and	Growth on pH and salinity stress tests	Pathogen	Non Pathogen

	salinity stress tests						
Water	12	1	11	7	4	4	0
Mud	22	4	18	16	2	2	0
<i>Sepia latimanus</i>	14	9	5	4	1	1	0
<i>Episesarma</i> sp.	11	5	6	3	3	2	1
<i>Scylla serrata</i>	13	2	11	9	2	2	0
<i>Tenualosa toli</i>	8	2	6	4	2	1	1
<i>Penaeus merguensis</i>	8	2	6	3	3	1	2
<i>Telescopium telescopium</i>	7	1	6	2	4	3	1
<i>Nerita violacea</i>	7	0	7	5	2	2	0
<i>Litopenaeus vannamei</i>	5	2	3	2	1	1	0
<i>Rhizopora</i> sp. root	5	2	3	2	1	1	0
<i>Rhizopora</i> sp. bark	6	1	5	4	1	1	0
<i>Mallotus villosus</i>	7	3	4	3	1	1	0
<i>Mugil</i> sp.	3	0	3	3	0	0	0
<b>Number</b>	<b>128</b>	<b>34</b>	<b>94</b>	<b>67</b>	<b>27</b>	<b>22</b>	<b>5</b>

Initially, the number of isolated and purified bacteria from collected samples were 128 isolated, from which the highest isolated was from the mud (Table 1). The color of mud was dark black, indicating it contained high concentration of organic molecules (Suhendar *et al.*, 2019). It could be used as an indicator that there was some bacterial colony which could found. Other study also found that there were 7 isolated bacteria collected from mangrove mud of Wonorejo, Rungkut-Surabaya (Pratiwi *et al.*, 2013). This diverse in bacterial colonies found was possible since the mangrove area was fully covered by the plant debris that can be degraded and used by microorganism as source of energy (Sinatryani *et al.*, 2014).

Fourteen bacterial isolation was found from squids which was the highest number of bacterial isolations found among others. Some study also indicated 4 different probiotic isolation found from carpio fish intestine (Samosir *et al.*, 2017), while other study was able to isolate 16 probiotic colonies from shrimp intestine (Febrianti, 2011). Isolation of this probiotic colony mostly was from the intestine/gut of the animal samples, in where most of these variety microorganism plays important role in digestive system like produced enzymes (Sarastiti *et al.*, 2020). In addition to it, the existing of these variety microorganism was also able to compete with the growth of pathogenic bacteria and presumably increase in the animal immunity. With normal digestive process affected the growth and development of animal which as the bacterial hosts (Samosir *et al.*, 2017).

While those in plant parts, such as from roots and skin of mangrove plants, each 5 and 6 isolated bacteria was found. The roots and skins of the mangrove plants were used since these parts of mangrove

plants had contact with mud and water, which presumably also contained of bacteria which can be isolated and had different characteristics with other bacterial colony found from animal samples, yet they had potential characteristic as probiotic candidate.

### C. Cell morphology

The morphology of isolate is correct bacteria had similarity yet only the edge of isolate colony had different shape (Table 2), while the cell morphology indicated same shaped and Gram stain, bacillus and positive Gram (Table 2 and Figure 3).

Table 2. Morphology of isolat *Bacillus* sp. for probiotic candidate

Isolat	Colony				Cell Morphology	
	Form	Edges	Elevation	Colors	Gram	Form
KPP212	Circular	Raised	Entire	White	+	Basil
IP121	Circular	Convex	Entire	White	+	Basil
UJ131	Circular	Flat	Entire	White	+	Basil
UJ132	Circular	Convex	Entire	White	+	Basil
SB141	Irregular	Raised	Lobate	White	+	Basil

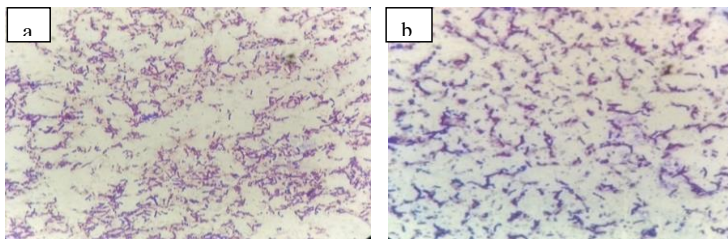


Figure 3. Cell morphology of isolat bacteria a. UJ132 dan b. KPP212

Positive Gram stain was indicated by violet color of the bacterial cell. The crystal violet was trapped in thick cellular wall of bacteria with one layer membrane in which made bacteria undergone dehydration and shrink after expose to 96% alcohol (Samosir et al., 2017).

### D. Proteolytic test

Many different tests given to the isolated bacteria colony caused drastically selection among them, from which prior the selection given, 128 isolated bacteria were found then with proteolytic selection 94 isolates was collected. Further selection was made based on ability to deal with salinity and pH, the *Bacillus* sp. isolates reduced to 27 isolates. Final selection was made for their ability to be pathogenic, in order to ensure that the bacterial colony was no harm if they were given as probiotics, and left for 5 isolates of *Bacillus* sp. (which their characteristics can be seen in Table 3).

Table 3. Proteolytic Index of isolat *Bacillus* sp.

Isolat	Area of Colony (cm <sup>2</sup> )	Area of clear zone (cm <sup>2</sup> )	Proteolytic Index
KPP212	0.50	1.22	2.44
IP121	0.94	2.11	2.24
UJ131	0.22	1.17	5.26

UJ132	0.56	1.67	3.00
SB141	0.72	1.94	2.69

Protein contained in shrimp feeds is approximately 30-40%, therefore proteolytic test was necessary performed. All the isolate probiotic candidates indicated their ability to degrade casein from media, indicated by clear zone as hydrolysis process surrounding the isolated colony (Fig. 3). This ability was also indicated by those colonies of probiotic candidate obtained other study (Samosir *et al.*, 2017). Proteolytic ability of *Bacillus* sp. occurred since the bacteria produced protease (Hamtni, 2014). Protease as extracellular enzyme of *Bacillus* sp. able to break the peptide bond of protein into oligopeptida and amino acids (Ilmiah *et al.*, 2018).

Study on the potential of proteolytic bacteria from mangroves also has been observed from other researchers. Utomo *et al.* (2019) succeeded in observing the protease enzyme-producing bacteria from mangrove of Gunung Anyar, Surabaya. There are two species of bacteria obtained, namely *Yersinia enterocolitica* and *Enterobacter agglomerans*. The bacteria have been characterized of proteolytic enzyme. Other researchers, Castro *et al.* (2014) isolated endophytic microorganisms from two mangrove species, *Rhizophora mangle* and *Avicennia nitida*. They found that mangrove microorganisms demonstrated a diverse range of enzymatic activities. The isolates produced enzymes of amylase, esterase, lipase, protease, and endoglucanase. The difference between our study and other researchers that we observed *Bacillus* sp. of proteolytic and non-pathogenic as a potential probiotic.

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All the collected isolate probiotic candidate showed different in proteolytic index. The isolate bacteria which had the highest proteolytic index was in code of SB141, while the lowest was in code of IP121. This different in proteolytic index can be seen in clear zone of each isolate bacteria (Figure 4).

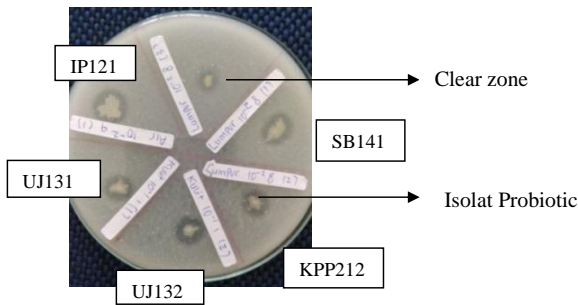


Figure 4. Illustration of proteolytic index of *Bacillus* sp.

Salinity and pH tests was conducted to elucidate the ability of selected isolate probiotic candidate from different salinity and pH stresses of media. Isolate with codes of KPP212, IP121, UJ131, UJ132 and SB141 survived and growth in salinity of 0%, 3% and 6% and with pH of 4, 7 and 10.

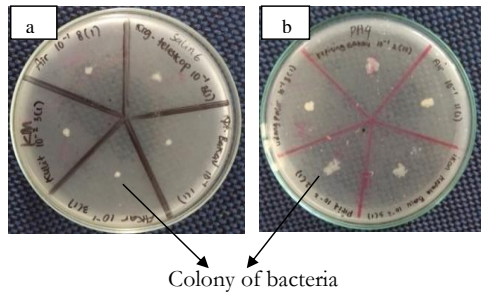


Figure 5. Colony of bacteria under saline 6% (a) and pH 4 (b)

### E. Bacteria Characterization

The five isolated bacteria produced protease and survived in different range of pH (4 - 10) and different salinity (0% - 6%) (Fig. 5), meanwhile their hemolysis activity was in gamma hemolysis or non-pathogenic activity. From biochemical tests, all of the isolated also indicated non motile, and only isolate with code of UJ132 had positive catalase activity, while in sugar fermentation test, all of isolate had positive result in all different type of sugar used for the test.

The ideal of probiotic is survive in many different stress conditions, therefore survival test was conducted. Isolates of KPP212, IPI21, UJ131, UJ132 and SB141 indicated that they able to survive and grew very well in pH and salinity stress (Fig. 4).

Table 4. Bacteria isolated characterization

Isolat	Pr	Stress on pH			Stress on Salinity (%)			Mo	K	Sugar fermentation					Pa Hemolysis		
		4	7	10	0	3	6			Ms	G	S	L	Mt	$\alpha$	$\beta$	$\gamma$
KPP212	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
IP121	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
UJ131	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
UJ132	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+
SB141	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+

Note:

-	= no reaction/no growth	$\alpha$	= partial hemolysis
+	= reaction/ growth	$\beta$	= total hemolysis
G	= Glucose	$\gamma$	= no hemolysis
K	= Catalase	L	= Lactose
Mo	= Motility	Ms	= Mannose
Mt	= Manitol	Pa	= Pathogenic test
Pr	= Protease	S	= Sukrose

This ability to survive was similar to those found by other study (Triyanto *et al.*, 2009) which isolated from the mud of mangrove. The survival of this bacteria colony from different stress of pH and salinity

presumably that this colony used to with the environment stress, unstable, which very common in estuary ecosystem (Hutabarat, 2000).

Pathogenic or virulent of isolates was determined by the degree of clear zone media produced by the isolates. All the isolates probiotic candidates had  $\gamma$  (Gamma) hemolysis characteristic (Table 4). Blood agar as differentiate media was used to determine the ability of bacteria to lyse red blood cells (RBCs) (Hamtini, 2014). The ability of bacteria to lyse RBCs was done by extracellular protein produce called haemolysin (Khusnan *et al.*, 2018). Pathogenety in RBCs was defined into 3 levels, alpha hemolysis, betha hemolysis and gamma hemolysis. Alpha hemolysis occurred when partly RBCs and hemoglobin were lyzed, betha hemolysis occurred when all of RBCs and hemoglobin were lyzed causing the surrounding media clear, and gamma hemolysis occurred when there was no lysis at all for both RBCs and hemoglobin, causing no color change in media (Hamtini, 2014).

In sugar fermentation test, all of the isolates colony indicated positive fermentation test for sugars such as lactosa, mannososa, manitol, glucosa and sucrosa (Table 4), indicated by formation of yellow color media. The change in media color occurred since fermentation caused acidity of the media in which by using *phenol red* as indicator it turned to yellow. Acids released in media was produced from breaking down of sugars by bacteria. In motility test, all isolates indicated negative, shown by undispersed growth of bacteria colony in their media (Damayanti *et al.*, 2018).

Catalase test was done in order to determine the ability of isolates colony to produce catalase enzyme. Positive result was shown from UJ132 isolate, while KPP212, IP121, UJ131 and SB141 isolates indicated negative results (Table 4). Positive result was indicated by oxygen bulb from mixing of  $H_2O_2$  with isolate bacteria, indicating that catalase enzyme was produced by bacteria and used to break hydrogen peroxide in water and oxygen. Hydrogen peroxide was compound that interfere with intracellular enzyme activity (Yulvizar, 2013).

#### IV. CONCLUSION

From this study, about 128 isolated *Bacillus* sp. was found from intestinal of mangrove biota in Lampung Mangrove Center, such as shrimp, mollusk, fish and crabs, from which 94 isolated *Bacillus* sp. had proteolytic character and 5 of them very potential as probiotic candidate. The five of probiotic were *Bacillus* sp. KPP212 collected from climbing crab, *Bacillus* sp. IP121 collected from fish, *Bacillus* sp. SB141 collected from mollusk, *Bacillus* sp. UJ131 and *Bacillus* sp. UJ132 collected from shrimp. Diversity of *Bacillus* sp. and biota are found in various type. Therefore, mangrove of Margasari of Lampung is very important to be conserved.

#### ACKNOWLEDGEMENT

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#### REFERENCES

- Anggriani, R., Iskandar, and Ankiq, T. (2012). Efektivitas Penambahan *Bacillus* Sp. Hasil Isolasi dari Saluran Pencernaan Ikan Patin pada Pakan Komersial terhadap Kelangsungan Hidup dan Pertumbuhan Benih Ikan Nila Merah (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan*. 3 (3) : 75-83.
- Aryal S. (2015). Haemolysis of *Streptococci* and Its Types with Examples. <https://microbiologyinfo.com/haemolysis-of-streptococci-and-its-types-with-examples>. Manuscript accessed on Apryl 9<sup>th</sup> 2018, at 08.29 am.

Castro R A, Dourado M N, de Almeida J R, Lacava P T, Nave A, de Melo I S, de Azevedo J L, and Quecine M C. (2018). Mangrove endophyte promotes reforestation tree (*Acacia polyphylla*) growth. *Braz J Microbiol*, 49 (1): 59-66 DOI: 10.1016/j.bjm.2017.04.002

Castro R A, Quecine M C, Lacava P T, Batista B D, Luvizotto D M, Marcon J, Ferreira A, Melo I S, and Azevedo J L. (2014). Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. *SpringerPlus* 3: 382 <http://www.springerplus.com/content/3/1/382>

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Damayanti, S. S., Oom, K., and Effendi, E. M. 2018. Identifikasi Bakteri Dari Pupuk Organik Cair Isi Rumen Sapi. *Ekologia : Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 18 (2) : 63-71.

Deivanai S, Bindusara A S, Prabhakaran G, and S J Bhore (2014). Culturable bacterial endophytes isolated from Mangrove tree (*Rhizophora apiculata* Blume) enhance seedling growth in Rice. *J Nat Sci Biol Med*. 5(2): 437–444. doi: 10.4103/0976-9668.136233

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Febrianti, D. (2011). Efektivitas Probiotik Asal Usus Udang dalam Menghambat Pertumbuhan *Vibrio harveyi* pada Larva Udang Vaname (*Litopenaeus vannamei*). *Skrripsi*. Institut Pertanian Bogor. Bogor.

Hamtini. (2014). Isolasi dan Karakterisasi *Bacillus* sp. dari Ikan Lele (*Clarias* sp.) serta Potensinya sebagai Probiotik. *Tesis*. Institut Pertanian Bogor. Bogor.

Hamzah TNT, Lee SY, Hidayat A, Terhem R, Hanum IF, and Mohamed R. 2018. Diversity and Characterization of Endophytic Fungi Isolated From the Tropical Mangrove Species, *Rhizophora mucronata*, and Identification of Potential Antagonists Against the Soil-Borne Fungus, *Fusarium solani*. *Front. Microbiol*. <https://doi.org/10.3389/fmicb.2018.01707>

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Handayani, H. (2011). Optimalisasi Substitusi Tepung Azolla Terfermentasi Pada Pakan Ikan Untuk Meningkatkan Produktivitas Ikan Nila Gift. *Jurnal Teknik Industri*. 12 (2) : 177–181 DOI: <https://doi.org/10.22219/JTIUMM.Vol12.No2.177-181>

Hapsari, T., W. Tjahjaningsih, M. A. Alamsjah, and H. Pramono. (2016). Aktivitas Enzimatis Bakteri Proteolitik Asal Gastrointestinal Udang Vannamei (*Litopenaeus vannamei*). *Journal of Marine and Coastal Science*. 5 (3) : 109. [https://journal.unair.ac.id/MARINE/enzymatis-activity-of-isolate-proteolytic-bacteria-from-gastrointestinal-of-white-shrimp-\(litopenaeus-vannamei\)-article-10527-media-76-category-5.html](https://journal.unair.ac.id/MARINE/enzymatis-activity-of-isolate-proteolytic-bacteria-from-gastrointestinal-of-white-shrimp-(litopenaeus-vannamei)-article-10527-media-76-category-5.html)

Hutabarat, S. (2000). *Produktivitas Perairan dan Plakton Telaah terhadap Ilmu Perikanan dan Kelautan*. Badan Penerbit Universitas Diponegoro. Semarang.

Ilmiah, S. N., Nisa, R. M., and Budiasih, W. (2018). Characterization of Protease from *Bacillus licheniformis* F11.1 as a Bio-Detergent Agent. *Makara Journal of Science*. 22 (3): 105-112.

Kariada, N. and Andin, I. (2014). Peranan Mangrove Sebagai Biofilter Pencemaran Air Wilayah Tambak Bandeng Tapak, Semarang. *Jurnal Manusia dan Lingkungan*. 21 (2) : 188-194.

Kementrian Kehutanan. 2014. *Pengelolaan Hutan Mangrove dan Ekosistem Pantai*. Sintesis Hasil Litbang 2010-2014. Badan Penelitian dan Pengembangan Kehutanan. Pusat Penelitian dan Pengembangan Konservasi dan Rehabilitasi.

Kepel, B. J., Widdhi, B., and Fatimawali. (2020). Pengaruh pH dan Suhu terhadap Aktivitas Pereduksi Merkuri Bakteri Resisten Merkuri Tinggi *Bacillus cereus* yang Diisolasi dari Urin Pasien dengan Amalgam Gigi. *e-GiGi*. 8 (1): 15-21.

Khusnan, Dwi, K., and Agus, P. (2018). Deteksi Hemaglutinin, Hemolisin dan Koagulase Secara Fenotipik dan Genotipik pada *Staphylococcus aureus* Isolat Asal Broiler. *Jurnal Sains Veteriner*. 36 (1): 103-114.

[Maulani B I G, Rasmi D A C, and L Zulkifli. \(2019\). Isolation and characterization of endophytic bacteria from mangrove \*Rhizophora mucronata\* Lam. and antibacterial activity test against some pathogenic bacteria. 4th Annual Applied Science and Engineering Conference Journal of Physics: Conference Series doi:10.1088/1742-6596/1402/3/033038](#)

Muliani, M., Nurbaya, N., Arifudin, T., and Muharijadi, A. (2017). Eksplorasi Bakteri Filosfer dari Tanaman Mangrove sebagai Bakteri Probiotik pada Budidaya Udang Windu, *Penaes monodon*. *Jurnal Penelitian Perikanan Indonesia*. 10 (2) : 47.

Pratiwi, I., Rahayu, K., and Wahju, T. (2013). Eksplorasi Bakteri Kandidat Probiotik di Lumpur Hutan Mangrove Wonorejo. *Jurnal Ilmiah Perikanan dan Kelautan*. 5 (2) : 187-192.

Rahmawan, Mohamad, E. A., Suminto, and Vivi Endar Herawati. (2014). Penggunaan Bakteri Kandidat Probiotik pada Pakan Buatan terhadap Efisiensi Pemanfaatan Pakan, Pertumbuhan dan Kelulushidupan Lele Dumbo (*Clarias gariepinus*). *Journal of Aquaculture Management and Technology*. 3(4) : 257-264.

Samosir, M. F., D. Suryanto, and Desrita. (2017). Isolasi dan Identifikasi Bakteri Potensial Probiotik pada Saluran Pencernaan Ikan Mas (*Cyprinus caprio*). *Jurnal aquacoastmarine*. 5(1): 1-14  
<https://jurnal.usu.ac.id/index.php/aquacoastmarine/article/view/17263/7258>

Sarastiti, S., Suminto, and Sarjito. (2020). Identifikasi Molekuler Spesies Bakteri Kandidat Probiotik yang Diisolasi dari Usus Udang Vaname (*Litopenaeus vannamei*) Koleksi dari Kabupaten Subang, Jawa Barat. *Jurnal Pasir Laut*. 4: 1 – 9.

Scharler, U. M. (2011). Whole Food-Web Studies: Mangroves. *Treatise on Estuarine and Coastal Science*. 6 : 271-286.

Seprianto, Feliatra, and Nugroho, T.T. (2017). Isolasi dan Identifikasi Bakteri Probiotik dari Usus Udang Windu (*Penaes monodon*) berdasarkan Sekuens Gen 16S rDNA. *Biogenesis*. 5(2):83-92.

Sinatryani, D., Moch, A. A., Sudarno, and Kustiawan, T. P. (2014). Kelimpahan Bakteri Selulolitik di Muara Sungai Gunung Anyar Surabaya dan Bancaran Bangkalan. *Jurnal Ilmiah Perikanan dan Kelautan*. 6 (2): 143-148.

Subagiyo, Sebastian, M., Triyanto, and Wilis, A. S. (2015). Pengaruh pH, Suhu dan Salinitas terhadap Pertumbuhan dan Produksi Asam Organik Bakteri Asam Laktat yang Diisolasi dari Intestinum Udang Penaeid. *Ilmu Kelautan*. 20 (4) : 187-194.

Subagiyo, Muhammad, S. R. D., and Wilis, A. S. (2017). Potensi Ekosistem Mangrove Sebagai Sumber Bakteri Untuk Produksi Protease, Amilase, dan Selulase. *Jurnal Kelautan Tropis*. 20 (2) : 106-111.

Suhendar, D., Esti, S., and Asep, S. (2019). Lumpur Hitam Tanah Rawa Hutan Mangrove Karangsong (Kabupaten Indramayu): Komposisi Kimia dan Transformasi Fasa yang Dihasilkan Melalui Penanganan Secara Termal. *Jurnal Riset Geologi dan Pertambangan*. 29 (2) : 127-139.

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- Sumardi, Farisi S., Ekowati C. N., Arifiyanto A, and Rahmawati, D. E. (2020). Halotolerant *Bacillus* sp. for Mannan Degradation Isolated from Mangrove Ecosystem at Hanura Beach Lampung. al. | J Pure Appl Microbiol. 14(2): 1237-1244.
- Sumardi, Farisi S., Ekowati C. N., and Hairisah S. F. (2019). Karakterisasi Enzim Xilanase dari Isolat *Bacillus* sp. UJ131 di Hutan Mangrove Margasari Lampung Timur sebagai Kandidat Probiotik. seanologi dan Limnologi di Indonesia 2019 4(3): 167-174. DOI: 10.14203/oldi.2019.v4i3.201
- Sumardi, Agustrina R, Ekowati C N, and Pasaribu Y S. (2018). Characterization of protease from *Bacillus* sp. on medium containing FeCl<sub>3</sub> exposed to magnetic field 0.2 mt. IOP Conf. Series: Earth and Environmental Science, 130. 012046 doi:10.1088/1755-1315/130/1 /012046.
- Taukhd, Supriyadi, H., and Koesharyani, I. (2008). Survey of Viral Diseases of Pacific white shrimp, *Litopenaeus vannamei* in indonesia. *Indonesian Aquaculture Journal*. 3(1) : 59-68 <http://ejournal-balitbang.kkp.go.id/index.php/iaj/article/view/1618/1263>
- Tensiska. 2008. *Probiotik dan Prebiotik Sebagai Pangan Fungsional*. Unpad. Bandung.
- Triyanto, A. Isnansetyo, I. D. Prijambada, J. Widada., and A. Tarmiawati. (2009). Isolasi, Karakterisasi dan Uji Infeksi Bakteri Proteolitik dari Lumpur Kawasan Hutan Bakau. *Jurnal Perikanan*. 11 (1) : 13-18.
- [Utomo P B, Sudarno and B S Rahardja. \(2019\). Identification of Proteolytic Bacterial Isolates in Sediment Ecosystem of Gunung Anyar Mangrove Forest, Surabaya. The 1st International Conference on Fisheries and Marine Science. IOP Conf. Series: Earth and Environmental Science. IOP Publishing doi:10.1088/1755-1315/236/1/012060](#)
- Widiastuti, E.L., Arifianti, R, Khairani, I.A., Christianto Y., Ara N.F., and Maharani, H.W. (2019). Antioxidant Effect of *Clerodendrum* sp and *Acanthus illicifolius* Methanol Extraction on Blood Profile of Male Mice Induced by Benzo(a)pyrene. IOP Conf. Series: Earth and Environmental Science, 305 <https://iopscience.iop.org/article/10.1088/1755-1315/305/1/012011>
- Yulvizar, C. (2013). Isolasi dan Identifikasi Bakteri Probiotik pada *Rastrelliger* sp. *Biospecies*. 6 (2):1-7. <https://online-journal.unja.ac.id/index.php/biospecies/article/view/884>

## PROBIOTIC CANDIDATE PROTEOLYTIC *Bacillus* sp. COLLECTED FROM MANGROVE OF MARGASARI –LAMPUNG

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### PROBIOTIC CANDIDATE PROTEOLYTIC *Bacillus* sp. COLLECTED FROM MANGROVE OF MARGASARI –LAMPUNG

Intensive shrimp culture has encountered with many problems such as declining ~~in~~ water quality through disease caused by pathogenic microbes which affected ~~the~~ mortality. The aim of the study was to determine any potential probiotic from *Bacillus* sp. collected from mangrove in East Lampung ~~in~~ which then could be used to improve proteolytic and probiotic activity of the cultured shrimps. This is a descriptive research with sampling and data collection of bacteria from many ~~of~~ samples of mangrove. The study found 128 isolate *Bacillus* from which then it has arrived at ~~come to~~ 5 potential probiotic *Bacillus* sp. From the study 5 *Bacillus* sp. has been isolated which ~~and~~ have potential properties for probiotic, they were KPP212, IP121, UJ131, UJ132 and SB141, ~~with~~ each isolate has characteristics with proteolytic property, growth in a wide range of pH (4 – 10) and osmotic stress (0 – 6% NaCl), non-pathogenic, ability for glucose fermentation, non-motile and has negative catalase activity. The ~~five~~s potential *Bacillus* sp. can be used as probiotic for shrimp farming.

Key words: characterization, *Bacillus* sp., mangrove, probiotic

### KANDIDAT PROBIOTIK PADA *Bacillus* sp. PROTEOLITIK DIKOLEKSI DARI HUTAN MANGROVE DI MARGASARI - LAMPUNG.

Budidaya udang dengan cara intensif telah menyebabkan banyak masalah seperti penurunan kualitas air yang mengakibatkan munculnya mikroba patogen sehingga mempengaruhi kematian. Tujuan dari penelitian ini adalah untuk menemukan *Bacillus* sp. yang diisolasi dari beberapa sampel di mangrove dan mengkarakterisasi mikroba terisolasi tersebut untuk penggunaan probiotik. Penelitian ini merupakan penelitian diskriptif dan koleksi data bakteri dari berbagai jenis sampel mangrove. Dari penelitian ditemukan 5 *Bacillus* sp. yang terisolasi dan memiliki sifat potensial untuk probiotik, yaitu KPP212, IP121, UJ131, UJ132 dan SB141, dengan masing-masing isolat memiliki karakteristik bersifat proteolitik, pertumbuhan dalam rentang pH yang luas (4 - 10) dan tahan pada tekanan osmotik (0 - 6% NaCl), tidak patogen, kemampuan untuk fermentasi glukosa, tidak motil, serta tidak memiliki aktivitas katalase. Kelima bakteri *Bacillus* sp. tersebut dapat digunakan untuk probiotik pada budidaya udang.

Kata kunci : karakterisasi, *Bacillus* sp., mangrove, probiotik

## I. INTRODUCTION

Mangrove of Margasari village in the district of Labuhan Maringgai – East Lampung regency is situated ~~in~~ in coordinates S: 5°51'84" - E: 105° 64'84" covers ~~for~~ about 700 hectares which is accounted ~~for~~ 6.65% of the total mangrove in Lampung Province. Mangrove has functions ecologically and economically ~~functions~~, such as protecting coastal abrasion, brackish water quality control, habitat for many organisms, medicines, and paper pulp. Ecological activities of mangrove are influenced by presence of waste, decomposition by microorganisms, mineral uptaking by plants and other biological activities, so that the mangrove ecosystem keeps the equilibrium (Kementerian Kehutanan, 2014; Kariada and Andin, 2014). Furthermore, it is necessary to rehabilitate mangrove for further conservation of coastal areas.

Many researchers in various places have also isolated the bacteria potential from mangrove. Study of Deivanai *et al.* (2014) isolated *Pantoea ananatis* (1MSE1) and *Bacillus amyloliquefaciens* (3MPE1) bacteria from mangrove which interacted positively with rice seedlings which resulted in the significant increase in root and shoot length, fresh weight, and chlorophyll content. The study of Castro *et al.* (2018) proved that *Enterobacter* sp. MCR1.48 strain from mangrove endophyte ~~that this strain~~ effectively promotes the *Acacia polyphylla* growth and fitness. The bacteria can be used in the seedling production of the tree. Other researcher, Maulani *et al.* (2019) ~~wh~~as successfully isolated eighteen endophytic bacteria from mangrove *Rhizophora mucronata* Lam from Gili Sulat, East Lombok. The 18 isolates of endophytic consist of 15 isolates which were Gram positive bacteria and 3 isolates were Gram negative bacteria. The endophytic bacteria isolate that had antibiotic activity ~~to against were~~ *B. cereus*, *P. aeruginosa*, *S. aureus* and *E. coli*. On the other hand, the potential fungi from Mangrove was also discovered. Hamzah *et al.* (2018) in Malaysia. ~~Isolated endophytic fungi from mangrove Rhizophora mucronata in Malaysia. From~~ ~~the study obtained found~~ several fungi i.e. *Alternaria*, *Fusarium*, *Nigrospora*, *Pestalotiopsis*, *Phoma*, and *Xylaria*. ~~After their culture assay for their antagonism activities with phytopathogenic fungus Fusarium solani between 45–66%. this sentence is not clear. What are the % numbers refer to?~~ Of the six isolates, only *Fusarium lateritium* and *Xylaria* sp. showed antibacterial activities against the pathogenic bacteria, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Mangrove plays important role on the coastal waters, food web and habitat supporting many different biota, such as fish, crabs, shrimps and molluscs (Scharler, 2011). Around ~~of~~ the Margasari mangrove, there are shrimp farmings. The mangrove has many wastes. It is a good nutrition for various ~~of~~ bacterial growths. In nature, the bacteria can pass into the digestive tract of animals. Several types of bacteria found in the digestive tract of animals had ~~role~~ important role to improve the healthy utilization of food and fish healthy (Sarastiti *et al.*, 2020). *Bacillus* sp. can be one of the bacteria that can increase the digestibility of fish or shrimp and it has the potential as a probiotic (Anggriani *et al.*, 2012). ~~There was no lack of information has been done~~ related to isolation of *Bacillus* sp. from Mangrove of Margasari – East Lampung, especially related to those used for probiotics.

The advantage of the probiotic technology is that the ~~has~~ process ~~is of~~ natural and safe. Probiotics had a healthy effect which includes interacting directly with commensal and pathogenic microorganisms. The probiotics were used for many functions i.e. (1) to prevent and treat infections, (2) to improve the balance of microorganisms in the small intestine, (3) produce extracellular enzymes, and (4) produce beneficial compounds such as vitamins and short chain fatty acids (Tensiska, 2008).

Isolation and selection of proteolytic bacteria has ~~is~~ very good ~~very~~ potential to be used as probiotics. Character of the bacteria is needed to increase of feed efficiency. Study reported that shrimp and fish feed contained as much as 55.51% - 67.68% protein (Handayani, 2011). Other studies have also shown ~~that some~~ other microorganisms as probiotic candidates, they were *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas putida*, *Bacillus bataviensis*, and *Caulobacter* sp. (Rahmawan *et al.*, 2014; Seprianto *et al.*, 2017).

Shrimp cultures have been widely growing in Lampung Province, in which intensive shrimp culture deliberates some diseases related to its cultures (Taukhid *et al.*, 2008). In order to prevent or regulate the disease therefore development of probiotic is necessary. ~~established~~ (Samosir *et al.*, 2017), established ~~one of which~~ by developing probiotic from surrounding ecosystem. Many isolated bacteria with ~~high~~ potential sources of probiotic can be collected from mangrove communities, such as from mangrove plant (its root and bark), fishes like milky fish, cuttlefish, mangrove shrimps, mollusks, crabs and other fishes as well as from the mud, water and other abiotic factors of the mangrove ecosystem

(Pratiwi *et al.*, 2013–; Seprianto *et al.*, 2017–; Muliani *et al.*, 2017). Therefore, it is necessary to figure out any potential of the proteolytic *Bacillus* sp. as local probiotic collected from mangrove of Margasari from which then it possibly could be applied into the aquaculture related to digestion and disease problems especially in shrimp and fish cultures encountered most in Lampung Province.

## III.II. MATERIAL AND METHOD

### A. Sources of isolated bacteria

Sample used to isolate bacteria was collected from mangrove communities in Margasari village – Labuhan Maringgai – East Lampung Regency. Abiotic and biotic samples were collected from water, and mud, fishes, shrimps, crabs, mollusk, and cuttlefish found in the mangrove ecosystem, as well as the *Rhizophora* sp.'s bark and roots.

### B. Isolation of *Bacillus* sp.

Isolation of *Bacillus* sp. was done from different sample mass, 10 g of mud, 1 g mangrove root and skin, 1 ml of water, 1 ml intestinal suspension of shrimp, crab, mollusk, fish, etc. (Figure 1). Sample suspension then was made by adding 90 ml physiological salt for mud and 9 ml for water, mangrove root and bark, shrimp, mollusk, crab and fish. -All samples were homogenized by using *vortex mixer* at 80°C for 15 minutes. Dilution was made for each sample in series of  $10^{-1}$  and  $10^{-2}$  is it really minus “-”? 1 ml of each diluted sample suspension was spread into skim milk agar media modification of *Sea Water Complete* (SWC), followed by incubation for 24 hours at 37°C (Hamtni, 2014). The isolate then was purified by *quadrant streak* into the SWC Agar media.

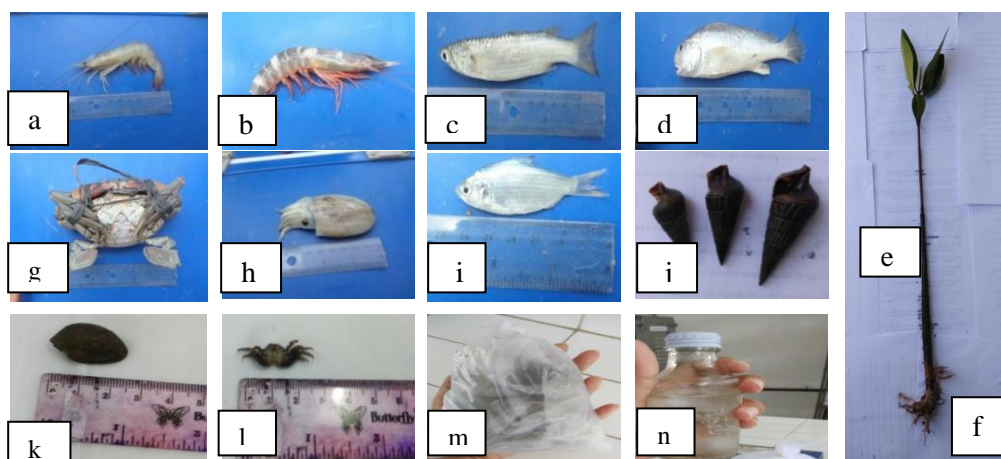


Figure 1. Collected biotic samples a. *Penaeus merguensis* (Udang Jerbung), b. *Litopenaeus vannamei* (Udang Kucing) c. *Mugil* sp (Ikan Belanak) d. *Mallotus villosus* (Ikan Kepala batu) e. Epidermis of *Rhizophora* sp.(Kulit bakau), f. Root of *Rhizophora* sp. (Akar bakau), g. *Scylla serrata* (Kepiting Bakau) h. *Sepia latimanus* (Sotong), i. *Tenulosa toli* (Ikan Pirit), j. *Telescopium telescopium* (Siput bakau) k. *Nerita violacea* (Keong bulat), and l. *Episesarma* sp (Kepiting pemanjat pohon) and abiotic samples (m. mud and n. water)

### C. Proteolytic Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into SWC media modified with skim milk. The culture then was incubated for 24 hours at 37°C. Observation was made by

determining the formed proteolytic index ~~formed~~ (Hamtni, 2014, Hapsari et al., 2016, Sumardi et al., 2018)

#### D. Osmotic/salinity stress Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into modified SWC media with NaCl concentration of 0%; 3%; and 6%. —The culture then was incubated for 24 hours at 37°C. Observation was made by determining the number of growing colonies (Subagiyo et al., 2015).

#### E. pH-stress Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into modified SWC media with pH 4, 7 and 10. The culture then was incubated for 24 hours at 37°C. Observation was made by determining the measurement of growing colonies (Kepel *et al.*, 2020).

#### F. Patogenetic Test

*Bacillus* sp. isolate was picked by using sterile ose needle and inoculated into blood modified SWC media. —The culture then was incubated for 24 hours at room temperature (Hamtni, 2014). Observation was made by determining the hemolytic ability of isolate from change ~~in different of~~ colors (Figure 2).

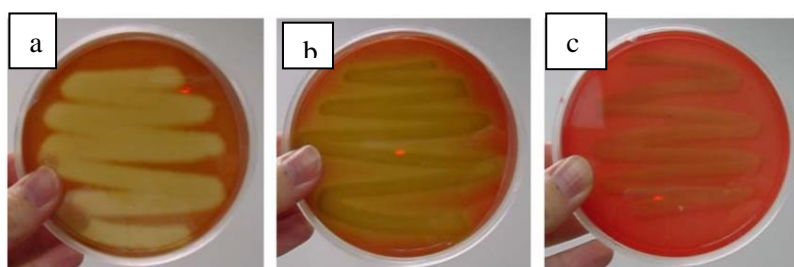


Figure 2. Hemolysis test in blood agar a. Beta hemolysis, b. Alpha ~~H~~hemolysis and c. Gamma hemolysis (Aryal, 2015).

#### G. Characteristic Test

Characterization of isolate bacteria presumably *Bacillus* sp. was done in 2 steps, as follows:

- a. Morphological characterization of colony and cell. Colony characterization was done by observation on the colony formed while cell morphology was made on gram smear (Yulvizar, 2013).
- b. Biochemical test  
Biochemical test as ~~colony~~ characterization of colony was conducted in different tests, such as tests on catalase, mortality and glucose fermentation.
  - b.1. Catalase test  
2 drops of H<sub>2</sub>O<sub>2</sub> was placed in sterile glass. Then, 1 ose needle picked of isolate *Bacillus* sp. was mixed into H<sub>2</sub>O<sub>2</sub> in object glass (Yulvizar, 2013).
  - b.2. Motility test  
As much as 1 ose needle isolat *Bacillus* sp. was placed into SWC agar media SWC. The culture then was incubated for 24 hours at 37°C temperature (Samosir et al., 2017).
  - b.3. Glucose fermentation test

As much as 1 ose needle isolat *Bacillus* sp. was inoculated into liquid SWC agar media and added with 1% sugars (glucose, lactose, manitol, succrose and mannose). The culture then was incubated for 24 hours at 37°C temperature (Samosir et al., 2017).

#### IV.III. RESULT AND DISCUSSION

##### A. Margasari Mangrove

Margasari mangrove is very important for the coastal area of East Lampung. Most of the shrimp or fish cultures/ponds are found in the mangrove belt of East Lampung. It is known that mangrove is able to support many biota, including fish, shrimps, mollusks (Fig.1). Margasari mangrove, as well as other mangrove ecosystems, provides food for many aquatic biotas and contributes to biological cycle in coastal waters. With special structures of mangrove plants, such as *Rhizophora mucronata*, *Avicennia marina*, *Sonneratia alba* and others the ground floor of the mangrove as well as swamp and water flow within the mangrove connecting to each other provides shelter for many larvae of mangrove biota. Spawning, nursery, shelter of many biotas which is provided by mangrove is not away. I don't understand it. Should it be "effected by"? from the activity of microbes as decomposer. These microbes possibly believed to have beneficial uses for such as for probiotic, antibiotic and bioactive products and else (Subagiyo et al., 2017). Recent study also indicated that some of the mangrove shrubs, such as *Acanthus illicifolius* contains antioxidant (Widiastuti et al., 2019). Other studies proved that *Bacillus* sp. as probiotic bacteria have been found in Mangrove in Lampung (Sumardi et al., 2019, and Sumardi et al., 2020)

##### B. Isolation of *Bacillus* sp.

Five (5) isolate *Bacillus* sp. isolate was found from mangrove samples and predicted to be used potentially used for probiotic from mangrove samples. They were from climbing crab intestine, fish intestine, 2 isolat from shrimp and one from mollusk intestine. Those five isolates *Bacillus* sp. were collected after undergoing selection tests for potential candidate for probiotic and can be seen in Table 1.

Table 1. Number of *Bacillus* sp. from isolation and selection tests

Source	Type of Isolat	Protease Test		Osmotic Stress Test		Pathogen Test	
		Non Proteolytic	Proteolytic	No growth on pH and salinity stress tests	Growth on pH and salinity stress tests	Pathogen	Non Pathogen
Water	12	1	11	7	4	4	0
Mud	22	4	18	16	2	2	0
<i>Sepia latimanus</i>	14	9	5	4	1	1	0
<i>Episesarma</i> sp.	11	5	6	3	3	2	1
<i>Scylla serrata</i>	13	2	11	9	2	2	0
<i>Tenuulosa toli</i>	8	2	6	4	2	1	1
<i>Penaeus</i>	8	2	6	3	3	1	2

<i>merguensis</i>							
<i>Telescopium</i>	7	1	6	2	4	3	1
<i>telescopium</i>							
<i>Nerita</i>	7	0	7	5	2	2	0
<i>violacea</i>							
<i>Litopenaeus</i>	5	2	3	2	1	1	0
<i>vannamei</i>							
<i>Rhizophora</i>	5	2	3	2	1	1	0
sp. root							
<i>Rhizophora</i>	6	1	5	4	1	1	0
sp. bark							
<i>Mallotus</i>	7	3	4	3	1	1	0
<i>villosus</i>							
<i>Mugil</i> sp.	3	0	3	3	0	0	0
<b>Number</b>	<b>128</b>	<b>34</b>	<b>94</b>	<b>67</b>	<b>27</b>	<b>22</b>	<b>5</b>

Initially, the number of isolated and purified bacteria from collected samples were 128-isolated, from which the highest isolated was from the mud (Table 1). The color of mud was dark black, indicating it contained high concentration of organic molecules (Suhendar *et al.*, 2019). It could be used as an indicator that there was some bacterial colony which could be found in the mud. Other study also found that there were 7 isolated bacteria collected from mangrove mud of Wonorejo, Rungkut-Surabaya (Pratiwi *et al.*, 2013). This diverse found in bacterial colonies found was possible since the mangrove area was fully covered by the plant debris that can be degraded and used by microorganism as source of energy (Sinatryani *et al.*, 2014).

Fourteen bacterial isolation was found from squids which whereas the highest number of bacterial isolations found among others. Some study also indicated that 4 different probiotic isolation was found from carpio fish intestine (Samosir *et al.*, 2017), while other study was able to isolate 16 probiotic colonies from shrimp intestine (Febrianti, 2011). Isolation of this probiotic colony mostly was from the intestine/gut of the animal samples, in where most of these variety microorganisms plays important role in the digestive system like produced enzymes (Sarastiti *et al.*, 2020). In addition to it, the existing of these variety microorganism was also able to compete with the growth of pathogenic bacteria and presumably increased in the animal immunity. With normal digestive process growth was affected the growth and the development of the animal which was the bacterial hosts (Samosir *et al.*, 2017).

While those in plant parts, such as from roots and skins of mangrove plants, each 5 and 6 isolated bacteria was found from each. The roots and skins of the mangrove plants were used since these parts of mangrove plants had contact with mud and water, which presumably also contained of bacteria which can be isolated, and had different characteristics with other bacterial colony found from animal samples, yet they had potential characteristic as probiotic candidates.

### C. Cell morphology

The morphology of isolate is correct bacteria, had similarity yet only the edge of isolate colony had different shape (Table 2), while the cell morphology indicated same shaped and Gram stain, bacillus and positive Gram (Table 2 and Figure 3).

Table 2. Morphology of isolat *Bacillus* sp. for probiotic candidate

Isolat	Colony				Cell Morphology	
	Form	Edges	Elevation	Colors	Gram	Form

KPP212	Circular	Raised	Entire	White	+	Basil
IP121	Circular	Convex	Entire	White	+	Basil
UJ131	Circular	Flat	Entire	White	+	Basil
UJ132	Circular	Convex	Entire	White	+	Basil
SB141	Irregular	Raised	Lobate	White	+	Basil

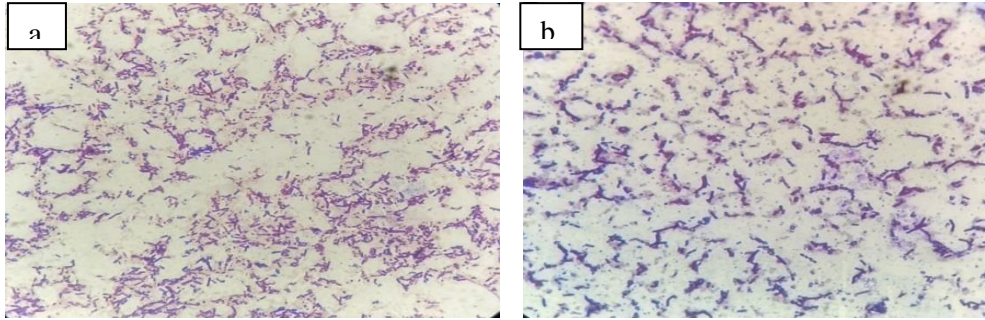


Figure 3. Cell morphology of isolat bacteria- a. UJ132 and b. KPP212

Positive Gram stain was indicated by violet color of the bacterial cell. The crystal violet was trapped in thick cellular wall of bacteria with one layer membrane in which bacteria undergone dehydration and shrink after exposure to 96% alcohol (Samosir et al., 2017).

#### D. Proteolytic test

Many different tests were given to the isolated bacteria colony caused drastically selection among them, from which prior the selection given, 128 isolated bacteria were found then with proteolytic selection 94 isolates were collected. Further selection was made based on ability to deal with salinity and pH, the *Bacillus* sp. isolates were reduced to 27 isolates. Final selection was made for their ability to be pathogenic, in order to ensure that the bacterial colony caused no harm if they were given as probiotics, and that left for 5 isolates of *Bacillus* sp. (which their characteristics can be seen in Table 3).

Table 3. Proteolytic Index of isolat *Bacillus* sp.

Isolat	Area of Colony (cm <sup>2</sup> )	Area of clear zone (cm <sup>2</sup> )	Proteolytic Index
KPP212	0.50	1.22	2.44
IP121	0.94	2.11	2.24
UJ131	0.22	1.17	5.26
UJ132	0.56	1.67	3.00
SB141	0.72	1.94	2.69

Protein contained in shrimp feeds is approximately 30-40%, therefore proteolytic test was necessarily performed. All the isolate probiotic candidates indicated their ability to degrade casein from media, indicated by clear zone as hydrolysis process surrounding the isolated colony (Fig. 3). This ability was also indicated by those colonies of probiotic candidates which were obtained from other study (Samosir et al., 2017). Proteolytic ability of *Bacillus* sp. occurred since the bacteria produced protease (Hamtni, 2014). Protease as extracellular enzyme of *Bacillus* sp. is able to break the peptide bond of protein into oligopeptida and amino acids (Ilmiah et al., 2018).

Study on the potential of proteolytic bacteria from mangroves also has been observed ~~by~~<sup>from</sup> other researchers. Utomo *et al.* (2019) succeeded in observing the protease enzyme-producing bacteria from mangrove of Gunung Anyar, Surabaya. ~~There are~~<sup>Two</sup> species of bacteria ~~was~~<sup>obtained</sup>, namely *Yersinia enterocolitica* and *Enterobacter agglomerans*. The bacteria have been characterized ~~as~~<sup>of</sup> proteolytic enzyme. Other researchers, Castro *et al.* (2014) isolated endophytic microorganisms from two mangrove species, *Rhizophora mangle* and *Avicennia nitida*. They found that mangrove microorganisms demonstrated a diverse range of enzymatic activities. The isolates produced enzymes of amylase, esterase, lipase, protease, and endoglucanase. The difference between our study and other researchers ~~is~~<sup>that</sup> we observed *Bacillus* sp. of proteolytic and non-pathogenic as a potential probiotic.

All the collected isolate probiotic candidates showed different ~~in~~<sup>proteolytic</sup> index. The isolate bacteria which had the highest proteolytic index was in code ~~of~~<sup>SB141</sup>, while the lowest was in code ~~of~~<sup>IP121</sup>. This different ~~in~~<sup>proteolytic</sup> index can be seen in clear zone of each isolate bacteria (Figure 4).

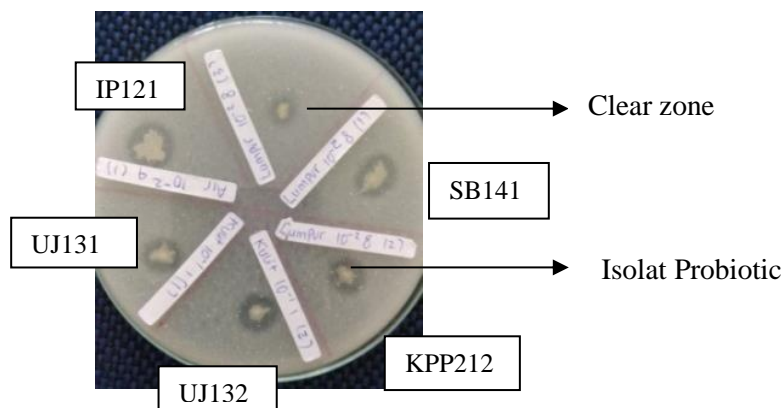


Figure 4. Illustration of proteolytic index of *Bacillus* sp.

Salinity and pH tests ~~were~~<sup>conducted</sup> to elucidate the ability of selected isolate probiotic candidates from different salinity and pH stresses of media. Isolate with codes of KPP212, IPI21, UJ131, UJ132 and SB141 survived and ~~growth~~<sup>in</sup> salinity of 0%, 3% and 6% and with pH of 4, 7 and 10.

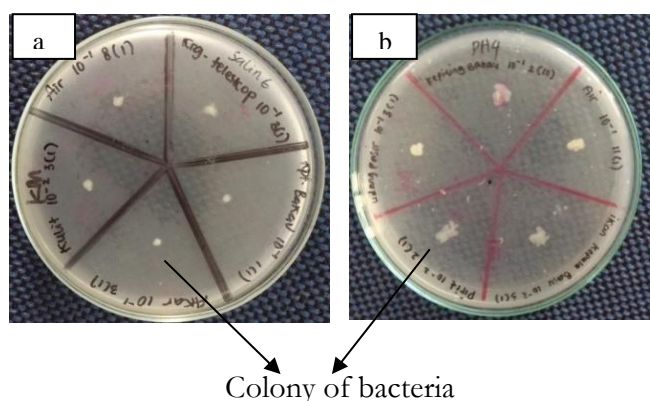


Figure 5. Colony of bacteria under saline 6% (a) and pH 4 (b)

### E. Bacteria Characterization

The five isolated bacteria produced protease and survived in different range of pH (4 - 10) and different salinity (0% - 6%) (Fig. 5), meanwhile their hemolysis activity was in gamma hemolysis or non-pathogenic activity. From biochemical tests, all of the isolated ones also indicated non motile, and only isolated with code of UJ132 had positive catalase activity, while in sugar fermentation test, all of isolated ones had positive result in all different type of sugar used for the test.

The ideal of probiotic is survive in many different stress conditions, therefore survival test was conducted. Isolates of KPP212, IPI21, UJ131, UJ132 and SB141 indicated that they are able to survive and grew very well in pH and salinity stress (Fig. 4).

Table 4. Bacteria isolated characterization

Isolat	Pr	Stress on pH			Stress on Salinity (%)			Mo	K	Sugar fermentation					Pa Hemolysis		
		4	7	10	0	3	6			Ms	G	S	L	Mt	$\alpha$	$\beta$	$\gamma$
KPP212	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
IP121	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
UJ131	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+
UJ132	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	-	+
SB141	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	+

Note:

-	= no reaction/no growth	$\alpha$	= partial hemolysis
+	= reaction/ growth	$\beta$	= total hemolysis
G	= Glucose	$\gamma$	= no hemolysis
K	= Catalase	L	= Lactose
Mo	= Motility	Ms	= Mannose
Mt	= Mannitol	Pa	= Pathogenic test
Pr	= Protease	S	= Sukrose

This ability to survive was similar to those found by other study (Triyanto *et al.*, 2009) which isolated from the mud of the mangrove. The survival of this bacteria colony from different stress of pH and

salinity presumably showed that this colony was used to ~~with the~~ environment stress, unstable condition, which is very common in estuary ecosystem (Hutabarat, 2000).

Pathogenic or virulent of isolates was determined by the degree of clear zone media produced by the isolates. All the isolates probiotic candidates had  $\gamma$  (Gamma) hemolysis characteristic (Table 4). Blood agar as differentiate media was used to determine the ability of bacteria to lyse red blood cells (RBCs) (Hamtini, 2014). The ability of bacteria to lyse RBCs was done by extracellular protein produce called haemolysin (Khusnan *et al.*, 2018). Pathogenety in RBCs was defined into 3 levels, alpha hemolysis, betha hemolysis and gamma hemolysis. Alpha hemolysis occurred when partly RBCs and hemoglobin partly were lyzed, betha hemolysis occurred when all of RBCs and hemoglobin were lyzed causing the surrounding media clear, and gamma hemolysis occurred when there was no lysis at all for both RBCs and hemoglobin, causing no color change in media (Hamtini, 2014).

In the sugar fermentation test, all of the isolates colony indicated positive fermentation test for sugars such as lactosa, mannos, manitol, glucosa and sucrosa (Table 4), indicated by formation of yellow color media. The change in media color occurred since fermentation caused acidity of the media in which, by using *phenol red* as indicator, it turned to yellow. Acids released in media was produced from breaking down of sugars by bacteria. In motility test, all isolates indicated negative result, shown by undispersed growth of bacteria colony in their media (Damayanti *et al.*, 2018).

Catalase test was done in order to determine the ability of isolates colony to produce catalase enzyme. Positive result was shown from UJ132 isolate, while KPP212, IP121, UJ131 and SB141 isolates indicated negative results (Table 4). Positive result was indicated by oxygen bulb from mixing of  $H_2O_2$  with isolate bacteria, indicating that catalase enzyme was produced by bacteria and used to break hydrogen peroxide in water and oxygen. Hydrogen peroxide was compound that interfere with intracellular enzyme activity (Yulvizar, 2013).

#### **V-IV. CONCLUSION**

From this study, about 128 isolated *Bacillus* sp. was found from intestinals of mangrove biota in Lampung Mangrove Center, such as shrimp, mollusk, fish and crabs, from which 94 isolated *Bacillus* sp. had proteolytic characters and 5 of them have very good potentials as probiotic candidate. The five ~~of~~ probiotic were: *Bacillus* sp. KPP212 collected from climbing crab, *Bacillus* sp. IP121 collected from fish, *Bacillus* sp. SB141 collected from mollusk, *Bacillus* sp. UJ131 and *Bacillus* sp. UJ132 collected from shrimp. Diversity of *Bacillus* sp. and biota are found in various types. Therefore, mangrove of Margasari of Lampung is very important to be conserved.

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#### **REFERENCES**

- Anggriani, R., Iskandar, and Ankiq, T. (2012). Efektivitas Penambahan *Bacillus* Sp. Hasil Isolasi dari Saluran Pencernaan Ikan Patin pada Pakan Komersial terhadap Kelangsungan Hidup dan Pertumbuhan Benih Ikan Nila Merah (*Oreochromis niloticus*). *Jurnal Perikanan dan Kelautan*. 3 (3) : 75-83.

- Aryal S. (2015). Haemolysis of *Streptococci* and Its Types with Examples. <https://microbiologyinfo.com/haemolysis-of-streptococci-and-its-types-with-examples>. Manuscript accessed on April 9<sup>th</sup> 2018, at 08.29 am.
- Castro R A, Dourado M N, de Almeida J R, Lacava P T, Nave A, de Melo I S, de Azevedo J L, and Quecine M C. (2018). Mangrove endophyte promotes reforestation tree (*Acacia polyphylla*) growth. *Braz J Microbiol*, 49 (1): 59-66 DOI: [10.1016/j.bjm.2017.04.002](https://doi.org/10.1016/j.bjm.2017.04.002)
- Castro R A, Quecine M C, Lacava P T, Batista B D, Luvizotto D M, Marcon J, Ferreira A, Melo I S, and Azevedo J L. (2014). Isolation and enzyme bioprospection of endophytic bacteria associated with plants of Brazilian mangrove ecosystem. *SpringerPlus* 3: 382 <http://www.springerplus.com/content/3/1/382>
- Damayanti, S. S., Oom, K., and Effendi, E. M. 2018. Identifikasi Bakteri Dari Pupuk Organik Cair Isi Rumen Sapi. *Ekologia : Jurnal Ilmiah Ilmu Dasar dan Lingkungan Hidup*. 18 (2) : 63-71.
- Deivanai S, Bindusara A S, Prabhakaran G, and S J Bhore (2014). Culturable bacterial endophytes isolated from Mangrove tree (*Rhizophora apiculata* Blume) enhance seedling growth in Rice. *J Nat Sci Biol Med*. 5(2): 437–444. doi: [10.4103/0976-9668.136233](https://doi.org/10.4103/0976-9668.136233)
- Febrianti, D. (2011). Efektivitas Probiotik Asal Usus Udang dalam Menghambat Pertumbuhan *Vibrio harveyi* pada Larva Udang Vaname (*Litopenaeus vannamei*). *Skripsi*. Institut Pertanian Bogor. Bogor.
- Hamtini. (2014). Isolasi dan Karakterisasi *Bacillus* sp. dari Ikan Lele (*Clarias* sp.) serta Potensinya sebagai Probiotik. *Tesis*. Institut Pertanian Bogor. Bogor.
- Hamzah TNT, Lee SY, Hidayat A, Terhem R, Hanum IF, and Mohamed R. 2018. Diversity and Characterization of Endophytic Fungi Isolated From the Tropical Mangrove Species, *Rhizophora mucronata*, and Identification of Potential Antagonists Against the Soil-Borne Fungus, *Fusarium solani*. *Front. Microbiol*. <https://doi.org/10.3389/fmicb.2018.01707>
- Handayani, H. (2011). Optimalisasi Substitusi Tepung Azolla Terfermentasi Pada Pakan Ikan Untuk Meningkatkan Produktivitas Ikan Nila Gift. *Jurnal Teknik Industri*. 12 (2) : 177–181 DOI: <https://doi.org/10.22219/JTIUMM.Vol12.No2.177-181>
- Hapsari, T., W. Tjahjaningsih, M. A. Alamsjah, and H. Pramono. (2016). Aktivitas Enzimatis Bakteri Proteolitik Asal Gastrointestinal Udang Vannamei (*Litopenaeus vannamei*). *Journal of Marine and Coastal Science*. 5 (3) : 109. [https://journal.unair.ac.id/MARINE@enzymatis-activity-of-isolate-proteolytic-bacteria-from-gastrointestinal-of-white-shrimp-\(litopenaeus-vannamei\)-article-10527-media-76-category-5.html](https://journal.unair.ac.id/MARINE@enzymatis-activity-of-isolate-proteolytic-bacteria-from-gastrointestinal-of-white-shrimp-(litopenaeus-vannamei)-article-10527-media-76-category-5.html)
- Hutabarat, S. (2000). *Produktivitas Perairan dan Plakton Telaah terhadap Ilmu Perikanan dan Kelautan*. Badan Penerbit Universitas Diponegoro. Semarang.
- Ilmiah, S. N., Nisa, R. M., and Budiasih, W. (2018). Characterization of Protease from *Bacillus licheniformis* F11.1 as a Bio-Detergent Agent. *Makara Journal of Science*. 22 (3): 105-112.
- Kariada, N. and Andin, I. (2014). Peranan Mangrove Sebagai Biofilter Pencemaran Air Wilayah Tambak Bandeng Tapak, Semarang. *Jurnal Manusia dan Lingkungan*. 21 (2) : 188-194.

- Kementrian Kehutanan. 2014. *Pengelolaan Hutan Mangrove dan Ekosistem Pantai*. Sintesis Hasil Litbang 2010-2014. Badan Penelitian dan Pengembangan Kehutanan. Pusat Penelitian dan Pengembangan Konservasi dan Rehabilitasi.
- Kepel, B. J., Widdhi, B., and Fatimawali. (2020). Pengaruh pH dan Suhu terhadap Aktivitas Pereduksi Merkuri Bakteri Resisten Merkuri Tinggi *Bacillus cereus* yang Diisolasi dari Urin Pasien dengan Amalgam Gigi. *e-GiGi*. 8 (1): 15-21.
- Khusnan, Dwi, K., and Agus, P. (2018). Deteksi Hemaglutinin, Hemolisin dan Koagulase Secara Fenotipik dan Genotipik pada *Staphylococcus aureus* Isolat Asal Broiler. *Jurnal Sains Veteriner*. 36 (1): 103-114.
- Maulani B I G, Rasmi D A C, and L Zulkifli. (2019). Isolation and characterization of endophytic bacteria from mangrove *Rhizophora mucronata* Lam. and antibacterial activity test against some pathogenic bacteria. *4th Annual Applied Science and Engineering Conference Journal of Physics: Conference Series* doi:10.1088/1742-6596/1402/3/033038
- Muliani, M., Nurbaya, N., Arifudin, T., and Muharijadi, A. (2017). Eksplorasi Bakteri Filosfer dari Tanaman Mangrove sebagai Bakteri Probiotik pada Budidaya Udang Windu, *Penaeus monodon*. *Jurnal Penelitian Perikanan Indonesia*. 10 (2) : 47.
- Pratiwi, I., Rahayu, K., and Wahju, T. (2013). Eksplorasi Bakteri Kandidat Probiotik di Lumpur Hutan Mangrove Wonorejo. *Jurnal Ilmiah Perikanan dan Kelautan*. 5 (2) : 187-192.
- Rahmawan, Mohamad, E. A., Suminto, and Vivi Endar Herawati. (2014). Penggunaan Bakteri Kandidat Probiotik pada Pakan Buatan terhadap Efisiensi Pemanfaatan Pakan, Pertumbuhan dan Kelulushidupan Lele Dumbo (*Clarias gariepinus*). *Journal of Aquaculture Management and Technology*. 3(4) : 257-264.
- Samosir, M. F., D. Suryanto, and Desrita. (2017). Isolasi dan Identifikasi Bakteri Potensial Probiotik pada Saluran Pencernaan Ikan Mas (*Cyprinus caprio*). *Jurnal aquacoastmarine*. 5(1): 1-14 <https://jurnal.usu.ac.id/index.php/aquacoastmarine/article/view/17263/7258>
- Sarastiti, S., Suminto, and Sarjito. (2020). Identifikasi Molekuler Spesies Bakteri Kandidat Probiotik yang Diisolasi dari Usus Udang Vaname (*Litopenaeus vannamei*) Koleksi dari Kabupaten Subang, Jawa Barat. *Jurnal Pasir Laut*. 4: 1 – 9.
- Scharler, U. M. (2011). Whole Food-Web Studies: Mangroves. *Treatise on Estuarine and Coastal Science*. 6 : 271-286.
- Seprianto, Feliatra, and Nugroho, T.T. (2017). Isolasi dan Identifikasi Bakteri Probiotik dari Usus Udang Windu (*Penaeus monodon*) berdasarkan Sekuens Gen 16S rDNA. *Biogenesis*. 5(2):83-92.
- Sinatryani, D., Moch, A. A., Sudarno, and Kustiawan, T. P. (2014). Kelimpahan Bakteri Selulolitik di Muara Sungai Gunung Anyar Surabaya dan Bancaran Bangkalan. *Jurnal Ilmiah Perikanan dan Kelautan*. 6 (2): 143-148.
- Subagiyo, Sebastian, M., Triyanto, and Wilis, A. S. (2015). Pengaruh pH, Suhu dan Salinitas terhadap Pertumbuhan dan Produksi Asam Organik Bakteri Asam Laktat yang Diisolasi dari Intestinum Udang Penaeid. *Ilmu Kelautan*. 20 (4) : 187-194.
- Subagiyo, Muhammad, S. R. D., and Wilis, A. S. (2017). Potensi Ekosistem Mangrove Sebagai Sumber Bakteri Untuk Produksi Protease, Amilase, dan Selulase. *Jurnal Kelautan Tropis*. 20 (2) : 106-111.

- Suhendar, D., Esti, S., and Asep, S. (2019). Lumpur Hitam Tanah Rawa Hutan Mangrove Karangsong (Kabupaten Indramayu): Komposisi Kimia dan Transformasi Fasa yang Dihasilkan Melalui Penanganan Secara Termal. *Jurnal Riset Geologi dan Pertambangan*. 29 (2) : 127-139.
- Sumardi, Farisi S., Ekowati C. N., Arifiyanto A, and Rahmawati, D. E. (2020). Halotolerant *Bacillus* sp. for Mannan Degradation Isolated from Mangrove Ecosystem at Hanura Beach Lampung. *al. | J Pure Appl Microbiol*. 14(2): 1237-1244.
- Sumardi, Farisi S., Ekowati C. N., and Hairisah S. F. (2019). Karakterisasi Enzim Xilanase dari Isolat *Bacillus* sp. UJ131 di Hutan Mangrove Margasari Lampung Timur sebagai Kandidat Probiotik. *seanologi dan Limnologi di Indonesia* 2019 4(3): 167-174. DOI: 10.14203/oldi.2019.v4i3.201
- Sumardi, Agustina R, Ekowati C N, and Pasaribu Y S. (2018). Characterization of protease from *Bacillus* sp. on medium containing FeCl<sub>3</sub> exposed to magnetic field 0.2 mt. *IOP Conf. Series: Earth and Environmental Science*, 130. 012046 doi:10.1088/1755-1315/130/1 /012046.
- Taukhid, Supriyadi, H., and Koesharyani, I. (2008). Survey of Viral Diseases of Pacific white shrimp, *Litopenaeus vannamei* in indonesia. *Indonesian Aquaculture Journal*. 3(1) : 59-68 <http://ejournal-balitbang.kkp.go.id/index.php/iaj/article/view/1618/1263>
- Tensiska. 2008. *Probiotik dan Prebiotik Sebagai Pangan Fungsional*. Unpad. Bandung.
- Triyanto, A. Isnansetyo, I. D. Prijambada, J. Widada., and A. Tarmiawati. (2009). Isolasi, Karakterisasi dan Uji Infeksi Bakteri Proteolitik dari Lumpur Kawasan Hutan Bakau. *Jurnal Perikanan*. 11 (1) : 13-18.
- Utomo P B, Sudarno and B S Rahardja. (2019). Identification of Proteolytic Bacterial Isolates in Sediment Ecosystem of Gunung Anyar Mangrove Forest, Surabaya. The 1st International Conference on Fisheries and Marine Science. *IOP Conf. Series: Earth and Environmental Science*. IOP Publishing doi:10.1088/1755-1315/236/1/012060
- Widiastuti, E.L., Arifianti, R, Khairani, I.A., Christianto' Y., Ara' N.F., and Maharani, H.W. (2019). Antioxidant Effect of *Clerodendrum* sp and *Acanthus illicifolius* Methanol Extraction on Blood Profile of Male Mice Induced by Benzo(α)pyrene. *IOP Conf. Series: Earth and Environmental Science*, 305 <https://iopscience.iop.org/article/10.1088/1755-1315/305/1/012011>
- Yulvizar, C. (2013). Isolasi dan Identifikasi Bakteri Probiotik pada *Rastrelliger* sp. *Biospecies*. 6 (2):1-7. <https://online-journal.unja.ac.id/index.php/biospecies/article/view/884>