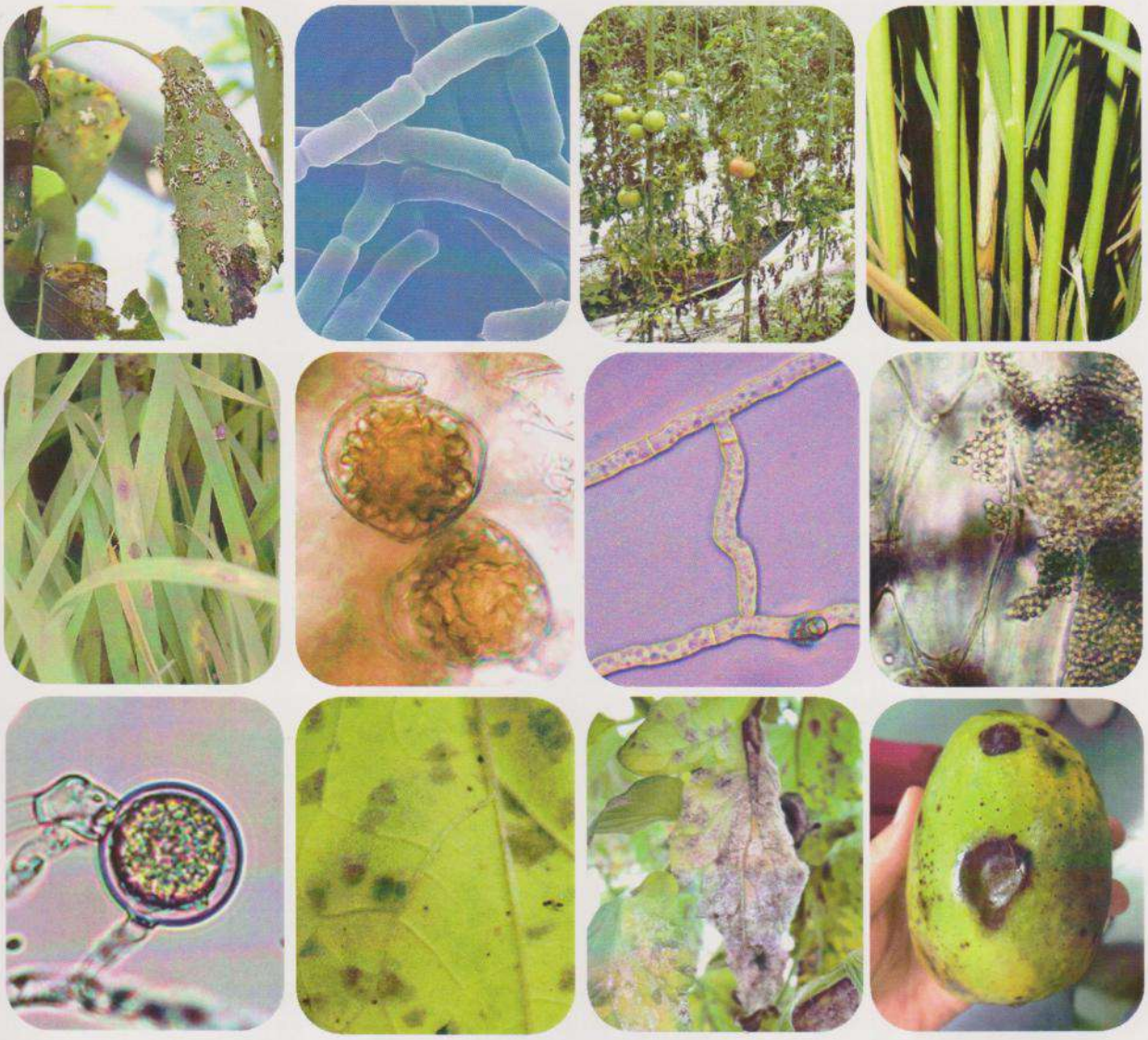
Proceedings of International Symposium on Innovative Crop Protection for Sustainable Agriculture 2018

Date: March 7-8, 2018

Venue: 6th Floor, UGSAS Building, Gifu University, Japan



The United Graduate School of Agricultural Science, Gifu University

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* This symposium is supported by IC-GU12.

Daily schedule

March 7th (Wed)	9:30–10:00	Registration
	10:00–10:05	Opening remarks
		Dr. Masateru Senge (Dean of UGSAS, Gifu University)
	10:05–10:10	Welcome speech
		Dr. Fumiaki Suzuki (Executive Director and Vice President of Gifu University)
	10:10–10:30	Special guest speech
		Dr. Shirley C. Agrupis (President of Mariano Marcos State University)
	10:30–10:40	Photo session
	10:40–11:40	Poster presentation & free discussion
	11:40–12:10	Plenary lecture 1
	12:10–13:30	Lunch break & poster viewing
	13:30–14:00	Plenary lecture 2
	14:00–15:15	Oral session 1
	15:15–15:30	Coffee break& poster presentation
	15:30–17:10	Oral session 2
	17:40–19:00	Dinner meeting
March 8th (Thu)	9:30–10:00	Registration
	10:00–10:30	Plenary lecture 3
	10:30–11:45	Oral session 3
	11:45–12:45	Lunch break & poster viewing
	12:45–13:15	Plenary lecture 4
	13:15–14:55	Oral session 4
	14:55–15:10	Coffee break & poster presentation
	15:10–16:50	Oral session 5
	16:50–17:00	Closing remarks
		Dr. Kohei Nakano (Gifu University)

Oral sessions –Day 1– (Main seminar room, 6th floor of UGSAS-GU Building)

OS I : Current status and management of crop diseases in Indonesia

March 7th, 14:00–15:15

Chair: Dr. Yuyun Fitriana (Lampung Univ.)

OS I-1 (14:00–14:25)	**Dr. Achmadi Priyatmojo** (Gadjah Mada University) "Current status and management of *Rhizoctonia solani,* the causal pathogen of sheath blight disease on rice and maize in Indonesia"
OS I-2 (14:25–14:50)	**Ms. Hanifah Ihsaniyati** (Sebelas Maret University) "Indonesian farmers problems in implementing integrated pest management (IPM)"
OS I-3 (14:50–15:15)	**Ms. Dwiwiyati Nurul Septariani** (Sebelas Maret University) "Taxonomical studies of blood disease bacterium of banana"

OS II : Plant probiotic bacteria

March 7th, 15:30–17:10

Chair: Dr. Md. Motaher Hossain (BSMRA Univ.)

OS II-1 (15:30–15:55)	**Dr. Tri Joko** (Gadjah Mada University) "Bacterial endophytes isolated from orchids and their influence on plant health"
OS II-2 (15:55–16:20)	**Dr. Radix Suharjo** (Lampung University) "Potential of endophytic bacteria as plant growth promoter and antagonist against pineapple-fungal plant pathogen in Indonesia"
OS II-3 (16:20–16:45)	**Dr. Hadiwiyono** (Sebelas Maret University) "Endophytic Bacillus as biological control agent of banana wilt"
OS II-4 (16:45–17:10)	**Dr. Md. Rashidul Islam** (Bangladesh Agricultural University) "Molecular based identification and formulation of cyanogenic *Pseudomonas* spp. controlling *Phytophthora infestans*"

Oral sessions –Day 2– (Main seminar room, 6th floor of UGSAS-GU Building)

OS III : Natural product-based pesticides and physical control measures

March 8th, 10:30–11:45
Chair: Dr. Tri Joko (Gadjah Mada Univ.)

OS III-1 (10:30–10:55)
Dr. Siti Subandiyah (Gadjah Mada University)
"Utilization of chitosan and glukomanan for fruit coating of chili againts antrachnose disease"

OS III-2 (10:55–11:20)
Dr. Pongphen Jitreerat (King Mongkut's University of Technology Thonburi)
"Antifungal effects of ethanolic shellac - Modified coconut oil (ES-MCO) combined with physical treatments against postharvest diseases of mango and mangosteen"

OS III-3 (11:20–11:45)
Dr. Kanlaya Sripong (King Mongkut's University of Technology Thonburi)
"Enhancing plant defense in mango fruit by hot water and UV-C treatments"

OS IV : Plant probiotic fungi

March 8th, 13:15–14:55
Chair: Dr. Achmadi Priyatmojo (Gadjah Mada Univ.)

OS IV-1 (13:15–13:40)
Dr. Moslama Aktar Maya (British American Tobacco Bangladesh Limited)
"Management of fusarium wilt in cyclamen plants using multiple soil microbes (AMF and *Piriformospora indica*)"

OS IV-2 (13:40–14:05)
Dr. Maria Viva Rini (Lampung University)
"Mycorrhizal oil palms seedlings response to different sources of *Ganoderma boninense* as the causal agent of basal stem rot disease"

OS IV-3 (14:05–14:30)
Dr. Purnomo (Lampung University)
"Potency of watery extract compost plus *Beauveria* sp. after storage for controlling planthopper and rice bug"

OS IV-4 (14:30–14:55)
Dr. Yuyun Fitriana (Lampung University)
"Low pH-tolerant mutant of *Trichoderma* spp. induced by EMS, gamma rays and UV irradiation"

Low pH-tolerant mutant of *Trichoderma* spp. induced by EMS, gamma rays and UV irradiation

Yuyun FITRIANA, Radix SUHARJO, Maria Viva RINI, Kuswanta Futas HIDAYAT

(Faculty of Agriculture, Lampung University)

SUMMARY

Basal stem rot caused by *Ganoderma boninense* is one of the most important problems causing severe economic losses in many oil palm industries, including in Indonesia. Recently, *Trichoderma* spp. has been widely used as biological control agent of *G. boninense*, however, this fungus was negatively affected by low pH condition. In Indonesia, oil palm commonly cultivated in marginal soil such us ultisol and peat soil with low pH (acid). In this study, three mutagens (EMS, gamma rays and UV irradiation) were used to generate low pH-tolerant mutant of *Trichoderma* spp. to increase its survival and effectiveness when it is applied in the previous mentioned field circumstance. Four Trichoderma isolates were used as the wild type. The mutant isolates were able to grow and produce spores in the pH 2-Potato Dextrose medium but not for the wild type. Only 1 out of 109 potentially low pH-tolerant mutant of Trichoderma showed better colony growth, sporulation, viability and antagonist to *G. boninense* than the wild type. These results showed that EMS, gamma rays and UV irradiation can be used to improve low-pH tolerant of *Trichoderma* spp.. However, the mutants must be carefully evaluated for any possibility of its negative impacts.

Introduction

Basal stem rot caused by *Ganoderma boninense* is one of the most important problems causing severe economic losses in many oil palm industries, including in Indonesia. Initially, this fungi attacked the roots of oil palm. Over time, it attacks continue to grow toward the base of the stem (Rini, 2003). At the beginning of the attack, the symptoms cannot be seen in the plant canopy. However, when the symptoms have appeared in the plant canopy (unopened shoots, pale green leaves, and old leaves begin to droop, fruiting bodies appear at the base of the stem), the fungal attack on the root and base of the stem is severe. Almost 50% of the base of the stem is damaged (Gurmit, 1991). Therefore, for controlling the *Ganoderma* is more difficult, because early symptoms is occur within the ground that are difficult to detect.

Trichoderma is a fungi that can act as a biological agent and can increase plant growth (Tsvetkov et al., 2014). In Indonesia, most of oil palm is grown on marginal land such as Ultisol and peat soil with low soil pH. In addition, intensive use of chemical fertilizers can further decrease soil pH (Firmansyah and Sumarni, 2013) and decrease the population and microbial effectiveness in soils, including beneficial microbes for plants (Rousk et al., 2009) including Trichoderma.

In this study, four isolates were used as a material for mutation activity to obtain mutant Trichoderma that were resistant to low pH. The mutation techniques used were Gamma rays irradiation, Ultraviolet irradiation (UV), and Ethyl Methane Sulfonate (EMS).

Material and Method

Fungal preparation. Four isolates of wild-type *Trichoderma* spp. (T1, T2, T3 and T4) that had been screened was cultured in Petri dishes (90 mm diameter) containing Potato's dextrose agar (PDA) for 2 weeks. Conidial suspensions were prepared by scraping the conidia/mycelia into sterile 0.1% Tween 80 and then filtering the mixture through sterile cloth (0.2 mm in mesh size) to provide a suspension of conidia.

Gamma rays and UV irradiation. Conidia of the wild-type isolates were irradiated with gamma rays at Badan Tenaga Nuklir Nasional (BATAN), Serpong. UV irradiation and EMS was done at Biotechnology Laboratory, Faculty of Agriculture, Lampung University. Twenty mL of the conidial suspension was injected on membrane filter and then was placed in each replicate plastic Petri dish (60 mm diameter). The dishes were irradiated at a range of doses 0, 30, 100, 300, 1000 and 3000 Gy (Gamma ray). For UV irradiation, wave length that was used was 245 nm with 10, 20 30, 40 and 50 minutes irradiation. All dishes were incubated at 20°C for 24 hours then moved into 3 mL *Potato Dextrose* (PD) liquid medium.

EMS solution. Ten mL of the conidial suspension was centrifused for 10 minutes at 3000 rpm. The pellet was soaked on 1.5 and 2% of EMS suspension for 30 and 50 minutes. Then, it was recentrifuged for 10 minutes at 3000 rpm. The pellet was incubated for 10 minutes at 0°C. Ten mL of *Phosphate buffer salin* steril was added to the pellet then it was centrifuged for 10 minutes at 3000 rpm. This step was replicated twice. The pellet was incubated at 20°C selama 24 jam.

Inoculation of *Trichoderma* isolates that have been treated on medium with low pH conditions. Each of treated isolates grown on 4 mL of PD Broth (liquid medium) at pH 2. Twenty mL of the incubated conidial suspension were entered into each medium. Observation were made daily for 7 days on the growth of fungus, the rate of growth and the formation of conidia. The wild-type were also included as a control.

The growth of colonies diameter, sporulation, viabilitity dan antagonism of *Trichoderma* Mutant Suspects to *Ganoderma* sp. This procedurs were conducted to know whether the mutants have the same ability as its wild type. Measurement methods of growth, sporulation, viability and ability of mutant antagonists were performed according to the same measurement method used in the screening stage to obtain selected Trichoderma fungi. Isolate to be tested previously moved from liquid medium to solid PDA media.

Result and Discussions

Isolates of the Trichoderma Putative Mutants. A total of 109 mutant suspects that capable of growing at pH 2 were obtained in this study. The mutant suspected isolates were obtained from gamma ray irradiation (54 isolates), UV irradiation (8 isolates) and EMS (47 isolates). The first step of determining the mutant was seen from the growing ability and spore production of each isolate that has been treated on each type of media compared to the wild type. If the treated isolate has a different appearance than the wild type, the isolate was determined as putative mutant. On the medium of pH 2, the putative mutant isolates resulting from gamma ray irradiation were able to form spores (marked with green colony color) but not for wild type. Wild type was still able to grow, however it did not produce spores (marked with colony color that remains white). This also occured in UV irradiation.

The growth of colony diameter of Low pH putative mutants.

Gamma rays irradiation. A total of 54 isolate mutants of low pH (pH 2) obtained in this irradiation. Ten mutants of T1 have larger colonies diameter than wild-type and 9 the other putative mutants had smaller colonies diameter than wild-type. The colonies diameter of the mutant resuted from T1 ranges from 4.6 to 7.4 cm. From the isolate T2, 6 mutants had larger colonies diameter than wild-type, while the other 12 isolates have smaller colonies diameter than wild-type. The diameter of mutant isolates from T2 ranges from 4.68 to 8.00 cm. T3 isolate, produced 1 mutant isolate, and has colonies diameter larger than wild type (6.61 cm). For T4 isolates, 14 mutant isolates had larger colonies diameter than wild-type and 2 isolates had colonies diameter smaller than wild-type. The diameter of mutant isolates from T4 ranged from 3.73 to 7.50 cm (Fig. 1).

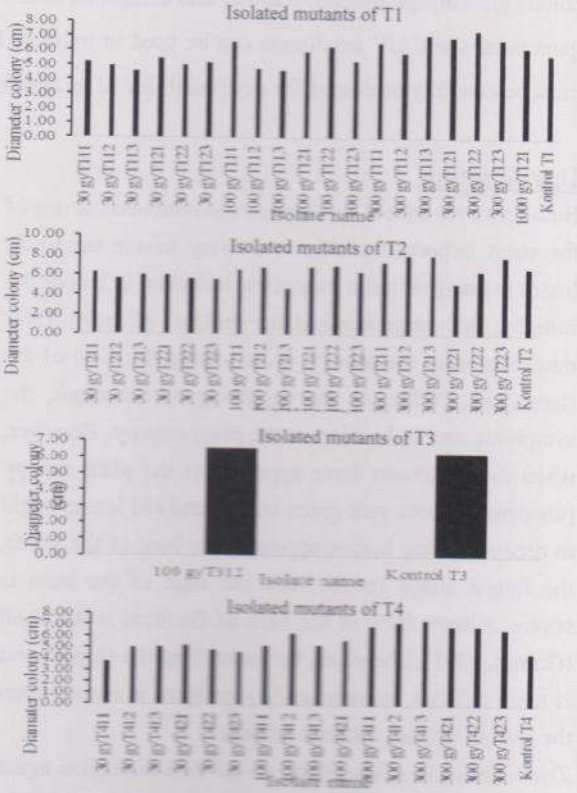

Fig.1 Colonies diameter of low pH mutant irridiated from gamma rays irradiation

UV irradiation. There were 8 isolates of low pH mutants (pH 2), consisting of 6 isolates of T2 and 2 isolates of T3. Two mutants of T2 resulted smaller colonies diameter than wild-type and 6 isolates mutants larger than wild-type. While 2 mutants of T3 had larger colonies diameter than wild-type (Fig. 2).

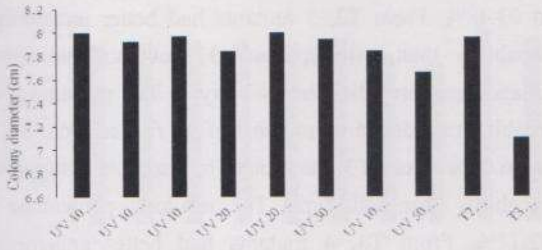

Fig.2 Colonies diameter of low pH mutant irridiated from UV irradiation

EMS solution. Total mutants isolates of low pH of 47 isolates consisted of 12 mutants of T1, 12 mutants of T2, 11 mutants of T3 and 12 mutants of T4. At 2 days after inoculation, 2 mutants of T1 produced larger colonies growth than wild-type meanwhile 10 mutants of T2 produced lower colony diameter than wild-type. Six mutant of T2 had same colonies diameter as wild-type and the other 6 mutants is lower. Five mutants of T3 had same colonies diameter as wild-type but the other 6 mutants were smaller. Only 1 mutant of T4 had larger colonies diameter than wild-type, the other 11 mutants were smaller.

Sporulation.

Gamma rays irradiation. Of all the isolates obtained, 2 mutants of T1 produced spore higher than wild-type and the other 16 mutants have spores lower than wild-type. Spores production of mutants T1 had range from 2.25 to 16.88 x 10^8 spores/ml. One mutant of T2 had spore production higher than wild-type, while 17 mutants had lower spore production than wild-type. Spores production from mutants of T2 ranged from 1.38 to 18.75 x 10^8 spores/ml. The mutant isolates derived from T3 isolates are currently still in the process of analysis. All of the mutants of T4 had lower spore production than wild-type. Spores production of mutants T4 ranged from 2.19 to 13.75 x 10^8 spores/ml (Fig. 3).

UV irradiation. Sporulation of 5 mutants of T2 were smaller than wild-type (15.63 x 10^8 spore/ml) and only 1 mutants equal to wild-type. While 2 mutants of T3, 1 mutant was larger than wild-type (8.13 x 10^8 spores/ml), but 1 mutant had smaller than wild type (Fig.4).

EMS solution. The highest sporulation produced by T2 isolate (6.88 x 10^8 spores/ml) and the lowest was T3 isolate (3.38 x 10^8 spores/ml). Three mutants of T1 produced spore higher than wild-type (> 3.75 x 10^8 spores/ml), while the other 8 mutants have lower spore than wild-type. T2 isolate produced 6 mutants that have higher sporulation than wild-type (> 6.88 x 10^8 spore/ml), while the other 6 mutants had sporulation lower than wild-type. T3 isolate produced 3 mutants that had sporulation smaller than wild-type (> 3.38 x 10^8 spore/ml), while the other 9 mutants were larger than wild-type. T4 isolate produced only 2 mutants that had higher sporulation than wild-type (> 6.25 x 10^8 spore/ml), while the other 10 mutants were smaller than wild-type (Fig.5).

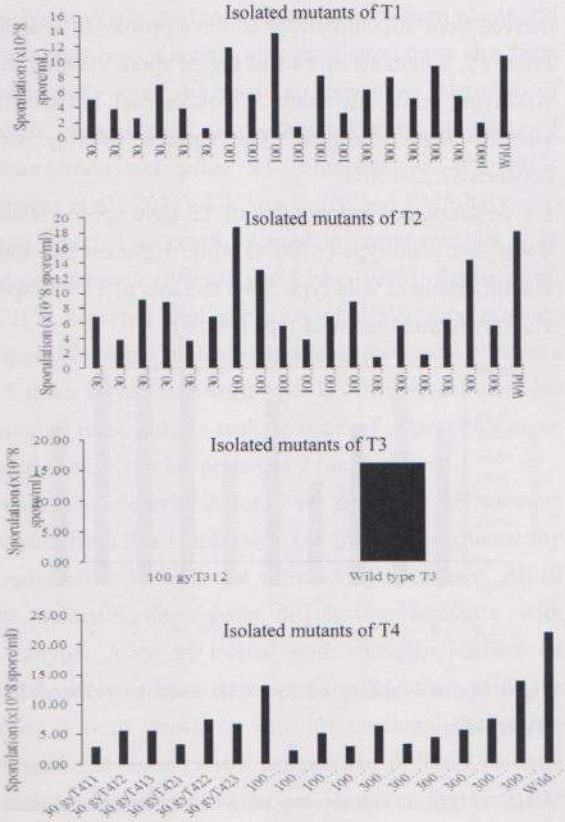

Fig.3 Sporulation of low pH mutant irridiated from gamma rays irradiation.

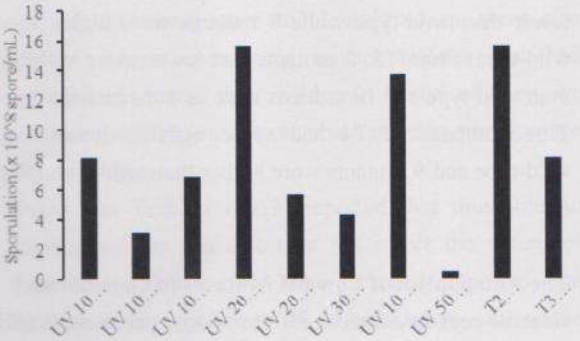

Fig.4 Produksi spora isolat terduga mutan pH rendah hasil iradiasi UV

Spore viability.

Gamma rays irradiation. Seven mutants of T1 produced spore viability higher than wild-type and the other 12 mutants had lower spore viability than wild-type. Spore's viability ranged between 94.17-100%. From T2, 15 mutants produced higher spore viability than wild-type, while the other 3 isolates had lower spore viability than wild-type. The spore's viability of mutants T2 ranged from 95.45-100%. For unexpected isolates of mutants derived from T3, currently is still in a process of analysis. From T4, 9 mutants of T4 had higher spore viability than wild-type, while the other 7 isolates had lower spore viability than wild-type. The spore's viability were between 82.32-100%.

UV irradiation. Five mutants of T2 have spore viability lower than wild-type (<100%) while 1 mutant had spore viability same as wild type. Two mutants of T3 had spore viability higher than wild-type (Fig. 5).

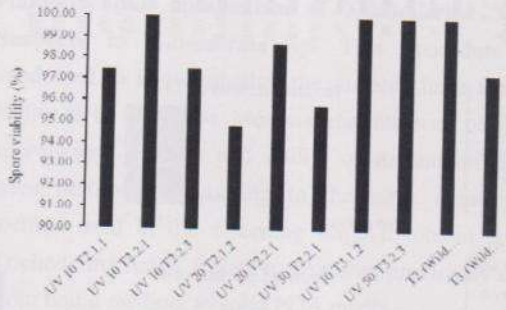

Fig.5 Spore viability of low pH mutant irridiated from UV irradiation

EMS solution. Immersion of EMS solution resulted 2 mutants of T1 that lower than wild-type (<98.52%); 1 mutant was equal to wild-type and 8 mutants were higher spore viability than wild-type. Two mutants of T2 were lower than wild-type while 8 mutants were higher than wild-type. From T3, 2 mutants had lower spore viability than wild-type and 10 mutants were as same as wild-type. Three mutants of T4 had spore viability lower than wild-type and 9 mutants were higher than wild-type (Fig. 6).

The Antagonism of Low pH Mutants to Ganoderma

Gamma rays irradiation. Of the 54 low pH mutants (pH 2) obtained, 5 mutants of T1 had better antagonism capability than wild-type, 1 mutant had same antagonism capability as wild-type and 13 mutants had lower antagonism capability than wild-type. The percentage of inhibition produced by mutants of T1 ranged from 62.17 to 93.48%. From T2, 5 mutants had better antagonism capability than wild-type and 13 mutants had lower antagonism capability than wild-type. The percentage of inhibition produced by mutants of T2 ranged from 54.78 to 96.09%. From T3, the mutant had smaller antagonism capability than wild-type. The resulting percentage is 82.17%. From T4, 4 mutants had better antagonism capability than wild-type and 12 mutants had smaller antagonism capability than wild-type. The resulting percentage ranged from 73.91-94.35%.

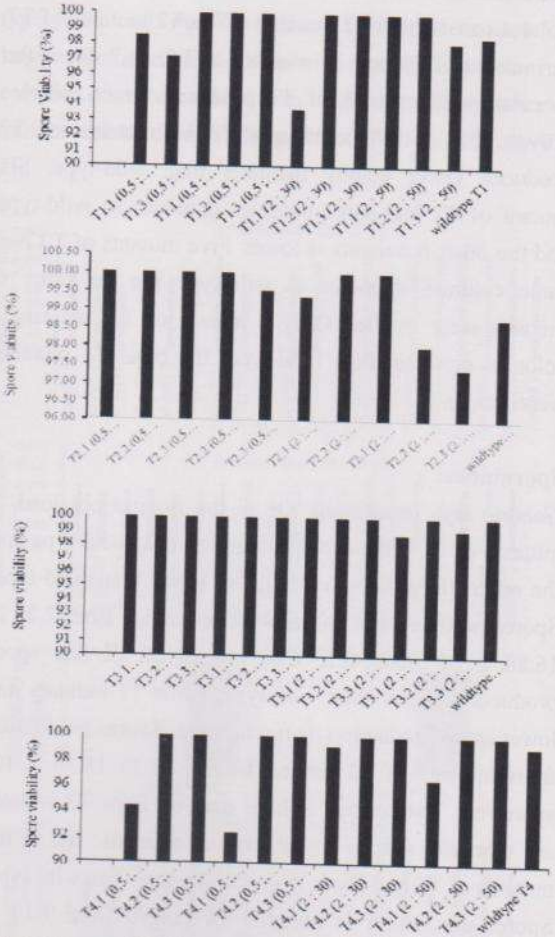

Fig.6 Spore viability of low pH mutant iridiated from EMS

UV irradiation. Six mutants of T2 were not better inhibition to wild-type (92.1%) meanwhile 2 mutants of T3 were better inhibition than wild-type (86.61%) (Fig. 7).

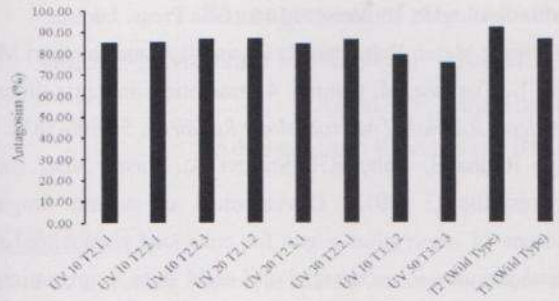

Fig.7 Antagonism to *G. boninense* of Low pH obtained from UV irradiation

EMS solution. Four wild-type produced various mutants. Mutants of T1 and T2 were not able to had better antagonism of wild-type (90.7% and 92.1%, respectively). Whereas 5 mutants of T3 had better antagonism ability than wild-type (86.61%) and the other 7 mutants were smaller than wild-type. Four mutants of T4 were better antagonism than wild-type but the other 8 mutants were smaller antagonism than wild-type (Fig.8).

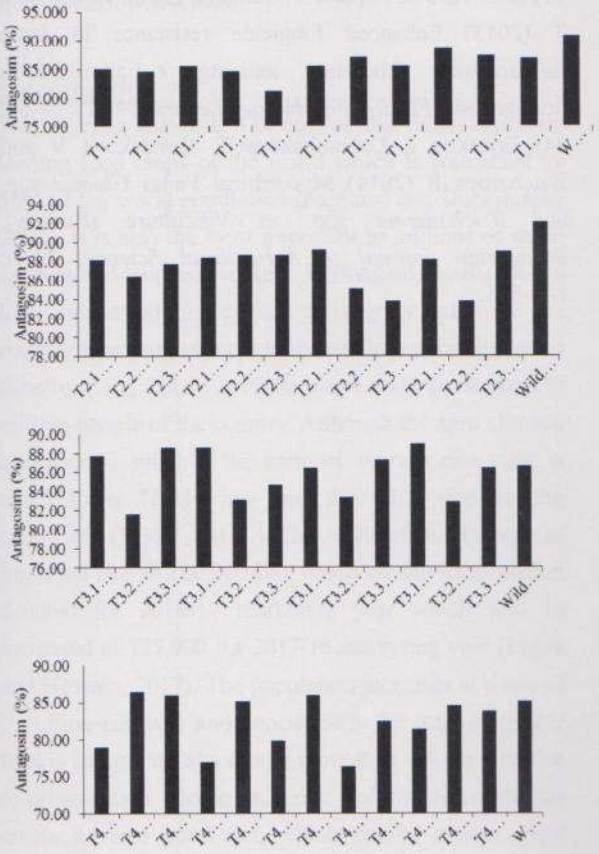

Fig.8 Antagonism to *G. boninense* of Low pH mutants from EMS solution

In this research, we used 3 mutation agents (mutagen) namely gamma ray irradiation, ultraviolet irradiation and Ethyl Methane Sulfonate (EMS). These three mutagens have generally been widely used to generate mutants (Darwis, 2006; Kava et al., 1995). Treatment with these three mutagens will lead to changes in various chemical and molecular bonds of reproductive cells from microorganisms (Darwis, 2006).

Piri et al. (2011) mentioned that gamma ray irradiation has been widely used to produce mutants from plants and microorganisms. Gamma rays irradiation have also been successfully used to produce resistance fungicide of *Isaria fumosorosea* and thermotolerant mutant of *Metarhizium anisopliae* s.l (Shinohara et al., 2013; Fitriana et al., 2014). UV irradiation and EMS have also been reported successfully used to create mutants from microorganisms (Pelczar and Chan, 1986). Radha et al. (2012) reported that the use of EMS can produce *Aspergillus niger* mutants capable of producing proteases 1.5 times higher than wild-type. UV Irradiation was also reported to be able to make mutant of *Aspergillus niger* capable of producing proteases 2 times higher.

From this research, in total, we obtained 109 mutants isolates from three mutagens (UV irradiation, gamma ray irradiation and EMS) that were low pH resistant. All of the mutant isolates have different performance with wild-type. After its treated with mutagen, almost all mutant isolates have better performance than wild-type when it were grown on low pH medium. The results showed that some mutant isolates had different colonies colors compared with wild-type. However, there are also isolates that do not change colonies color either on PDB media or after being transferred back using PDA media.

The ability of mutant isolates to grow, sporulation, spore viability and antagonism to *Ganoderma* sp. were also various. There were some mutant isolates that had performance lower than wild-type, some of they are similar to wild-type and some else had better performance than wild-type.

Najafi and Pezehki (2013) reported that mutations in microorganisms can affect or not affect the nature of these living things. The other research reported that thermotolerant resistant of *Metarhizium anisopliae* obtained from gamma-ray irradiation had lower virulence with wild-type, but others had higher virulence than wild-type (Fitriana, 2015).

Conclusion

The mutant isolates were able to grow and produce spores in the pH 2-Potato Dextrose medium but not for the wild type. Only 1 out of 109 potentially low pH-tolerant mutant of Trichoderma consistently showed better colony growth, sporulation, viability and antagonist to *G. boninense* than the wild type. These results showed that EMS, gamma rays and UV irradiation can be used to improve low-pH tolerant of *Trichoderma* spp.

Acknowledgement

This work was made possible through various facilitations. We thank to BPDP 2015 and Financial Department. We also thank our former students (Fransiska Dina and Fransiskus Ellyando Sinaga) for valuable assistance during research.

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