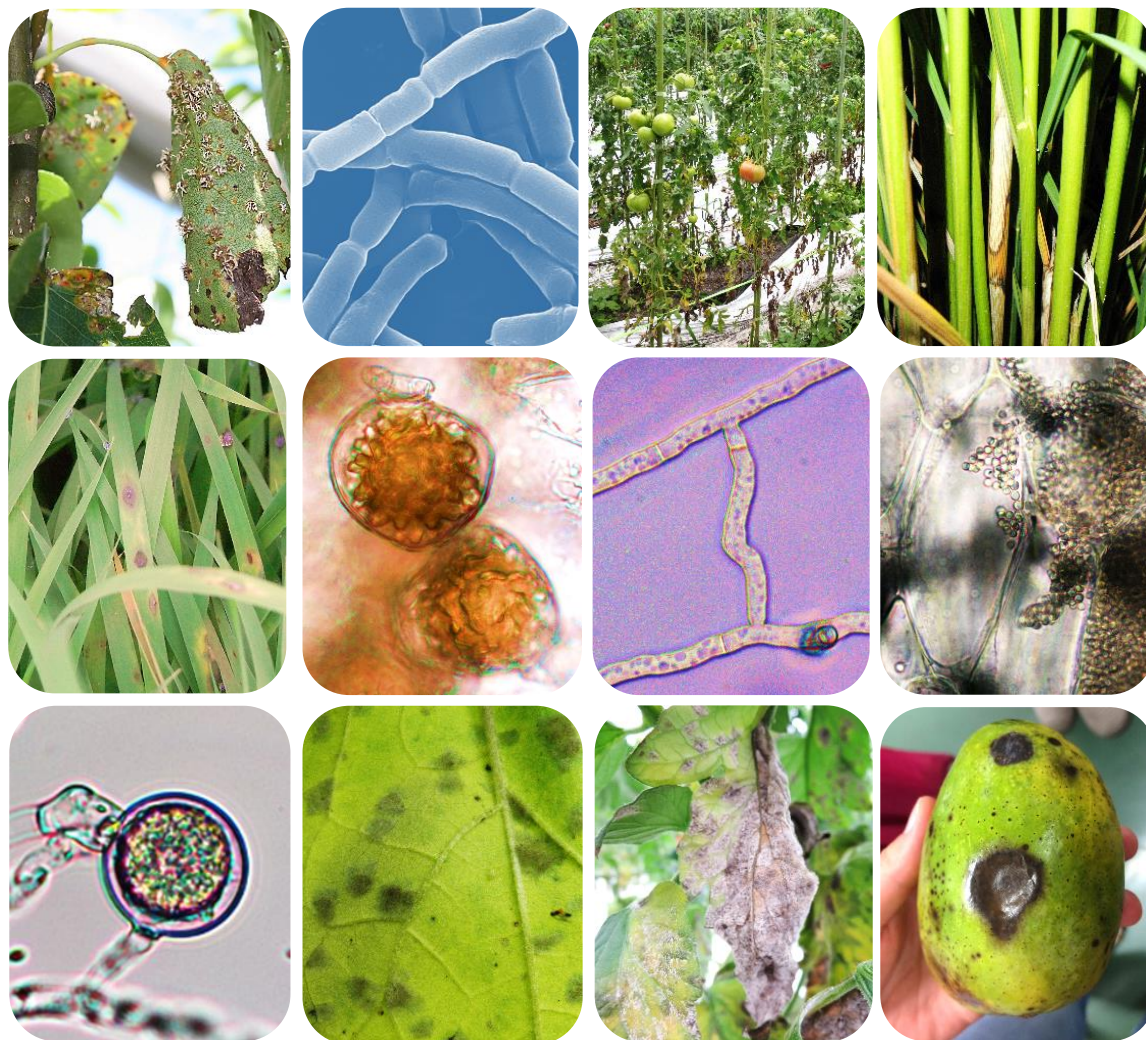


Proceedings of International Symposium on Innovative Crop Protection for Sustainable Agriculture 2018

Date: March 7-8, 2018

Venue: 6th Floor, UG\$AS 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 7 th (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 8 th (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)	

Plenary lectures (Main seminar room, 6th floor of UGSAS-GU Building)

PL-1 (March 7th, 11:40–12:10)

Chair: Dr. Haruhisa Suga (Gifu University)

Dr. Sotaro Chiba (Nagoya University)

“Farmer Field Schools leading to sustainable management of insect pests in Cambodian rice fields”

PL-2 (March 7th, 13:30–14:00)

Chair: Dr. Koji Kageyama (Gifu University)

Dr. Masafumi Shimizu (Gifu University)

“Endophytic *Streptomyces*. attractive biocontrol agents”

PL-3 (March 8th, 10:00–10:30)

Chair: Dr. Koji Kageyama (Gifu University)

Dr. Haruhisa Suga (Gifu University)

“Molecular characterization of *Fusarium fujikuroi* in Japan”

PL-4 (March 8th, 12:45–13:15)

Chair: Dr. Masafumi Shimizu (Gifu University)

Dr. Shigenobu Yoshida (National Agriculture and Food Research Organization)

“Perspective on the development of biopesticides applicable to both agricultural insect pests and disease”

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** **Dr. Achmadi Priyatmojo** (Gadjah Mada University)
(14:00–14:25) “Current status and management of *Rhizoctonia solani*, the causal pathogen of sheath blight disease on rice and maize in Indonesia”
- OS I-2** **Ms. Hanifah Ihsaniyati** (Sebelas Maret University)
(14:25–14:50) “Indonesian farmers problems in implementing integrated pest management (IPM)”
- OS I-3** **Ms. Dwiwiyati Nurul Septariani** (Sebelas Maret University)
(14:50–15:15) “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** **Dr. Tri Joko** (Gadjah Mada University)
(15:30–15:55) “Bacterial endophytes isolated from orchids and their influence on plant health”
- OS II-2** **Dr. Radix Suharjo** (Lampung University)
(15:55–16:20) “Potential of endophytic bacteria as plant growth promoter and antagonist against pineapple-fungal plant pathogen in Indonesia”
- OS II-3** **Dr. Hadiwiyono** (Sebelas Maret University)
(16:20–16:45) “Endophytic Bacillus as biological control agent of banana wilt”
- OS II-4** **Dr. Md. Rashidul Islam** (Bangladesh Agricultural University)
(16:45–17:10) “Molecular based identification and formulation of cyanogenic *Pseudomonas* spp. controlling *Phytophthora infestans*”
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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** **Dr. Siti Subandiyah** (Gadjah Mada University)
(10:30–10:55) “Utilization of chitosan and glukomanan for fruit coating of chili againts antrachnose disease”
- OS III-2** **Dr. Pongphen Jitreerat** (King Mongkut’s University of Technology Thonburi)
(10:55–11:20) “Antifungal effects of ethanolic shellac - Modified coconut oil (ES-MCO) combined with physical treatments against postharvest diseases of mango and mangosteen”
- OS III-3** **Dr. Kanlaya Sripong** (King Mongkut’s University of Technology Thonburi)
(11:20–11:45) “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** **Dr. Moslama Aktar Maya** (British American Tobacco Bangladesh Limited)
(13:15–13:40) “Management of fusarium wilt in cyclamen plants using multiple soil microbes (AMF and *Piriformospora indica*)”
- OS IV-2** **Dr. Maria Viva Rini** (Lampung University)
(13:40–14:05) “Mycorrhizal oil palms seedlings response to different sources of *Ganoderma boninense* as the causal agent of basal stem rot disease”
- OS IV-3** **Dr. Purnomo** (Lampung University)
(14:05–14:30) “Potency of watery extract compost plus *Beauveria* sp. after storage for controlling planthopper and rice bug”
- OS IV-4** **Dr. Yuyun Fitriana** (Lampung University)
(14:30–14:55) “Low pH-tolerant mutant of *Trichoderma* spp. induced by EMS, gamma rays and UV irradiation”
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Oral sessions –Day 2– (Main seminar room, 6th floor of UGSAS-GU Building)

OS V : Resistant cultivars

March 8th, 15:10–16:50

Chair: Dr. Pongphen Jitareerat (KMUTT)

OS V-1 **Dr. Abu Shamim Mohammad Nahiyah** (Advanced Seed Research & Biotech Centre)
(15:10–15:35) “Management of rice and wheat blast pathogen in Bangladesh”

OS V-2 **Dr. Triwidodo Arwiyanto** (Gadjah Mada University)
(15:35–16:00) “Control of eggplant and tomato bacterial wilt by grafting in Indonesia”

OS V-3 **Dr. Md. Motaher Hossain** (Bangabandhu Sheikh Mujibur Rahman Agricultural University)
(16:00–16:25) “Detection and characterization of Asia soybean rust in Bangladesh”

OS V-4 **Dr. Nandariyah** (Sebelas Maret University)
(16:25–16:50) “In vitro selection of sugarcane (*Saccharum officinarum* L) for Fusarium-pokah bung (Pb) resistance”

Poster session (Seminar room, 6th floor of UGSAS-GU Building)

- P-1 **Fumonisin production recovery in a *Fusarium fujikuroi* strain by complementation of *FUM21*, *FUM6* and *FUM7* genes**
Sharmin Sultana, Hironori Kobayashi, Ryuou Yamaguchi, Masafumi Shimizu, Koji Kageyama, Haruhisa Suga
- P-2 **Genetic mapping of chromosome No.1 region associated with pathogenicity in *Fusarium* head blight pathogen**
Rina Okumura, Maho Ikawa, Yuki Hirata, Masafumi Shimizu, Koji Kageyama, Haruhisa Suga
- P-3 **Isolation of plant probiotic *Bacillus* spp. from tea rhizosphere**
Nusrat Ahsan, Tomoki Nishioka, Haruhisa Suga, Hiroyuki Koyama, Masafumi Shimizu
- P-4 **Microbial basis of *Fusarium* wilt suppression by *Allium*-cultivated soils**
Tomoki Nishioka, Malek Marian, Haruhisa Suga, Masafumi Shimizu
- P-5 **Isolation of novel deoxynivalenol-degrading microorganisms from Poaceae planted soils**
Hiroyuki Morimura, Sotaro Chiba, Daigo Takemoto, Kazuhito Kawakita, Ikuo Sato
- P-6 **Plant growth-promoting traits of rhizospheric *Flavobacterium* and *Chryseobacterium***
Fumiya Mizutani, Tomoki Nishioka, Haruhisa Suga, Koji Kageyama, Masafumi Shimizu
- P-7 **Establishment of global *Phytophthora* database for quarantine control**
Ayaka Hieno, Mingzhu Li, Kayoko Otsubo, Haruhisa Suga, Koji Kageyama
- P-8 **Morphological and molecular identification of causal agent of cocoa pod rot disease in Indonesia**
Masanto, Ayaka Hieno, Arif Wibowo, Siti Subandiyah, Masafumi Shimizu, Haruhisa Suga, Koji Kageyama
- P-9 **Biocontrol of tomato bacterial wilt using *Ralstonia* and *Mitsuaria* species**
Malek Marian, Tomoki Nishioka, Hiroyuki Koyama, Haruhisa Suga, Masafumi Shimizu
- P-10 **Comprehensive evaluation of the resistance of root-stock-used *Cucumis melo* stock to *Meloidogyne incongnita***
Wanxue BAO
- P-11 **Population genetics analysis of *Phytophthium helicoides* in Japan**
Auliana Afandi, Emi Murayama, Ayaka Hieno, Haruhisa Suga, Koji Kageyama
- P-12 **Study of a transcriptional regulator of plant pathogenic genes in a soft rot disease causing bacterium, *Dickeya dadantii***
Dina Istiqomah, Naoto Ogawa
-

Poster session (Seminar room, 6th floor of UGSAS-GU Building)

- P-13 Identification of freshness marker of stored soybean sprouts**
Syukri, D., Thammawong, M., Kuroki, S., Tsuta, M., Yoshida, M., Nakano, K.
- P-14 Studies on acetaldehyde tolerance system in the budding yeast using *myo*-inositol**
Annisiya Zarina Putri, Mizuho Inagaki, Masaya Shimada, Takashi Hayakawa, Tomoyuki Nakagawa
- P-15 Identification of bioaerosols from environmental samples in the AIST, Tsukuba, Japan**
Panyapon Pumkaeo, Wenhao Lu, Youki Endou, Tomohiro Mizuno, Junko Takahashi, Hitoshi Iwahashi
- P-16 The effect of persimmon (*Diospyros kaki*) on the prevention of sarcopenia**
Nayla Majeda Alfarafisa, Tomio Yabe
- P-17 Transcriptional biomarkers for managing pulse crop production in acid soil region**
Raj kishan Agrahari, Hiroyuki Koyama
- P-18 The accumulation of carotenoid in mango during fruit maturation**
W. Yungyuen, T.T. Vo, G. Ma, L.C. Zhang, P. Jitareerat, A. Uthairatanakij, M. Kato
- P-19 Augmented nuclease resistance and gene silencing with 3'-end modified small interfering RNAs and dendrimer based drug delivery**
Akash Chandela, Yoshihito Ueno
- P-20 Protein-based functional analysis of renin and (pro)renin receptor genes in hypertensive and diabetic Bangladeshi population: Pursuing the environment-induced molecular traits**
Jobaida Akther, A. H. M. Nurun Nabi, Tsutomu Nakagawa, Fumiaki Suzuki, Akio Ebihara
- P-21 Proposals for countermeasures to reduce risk of hydraulic fracturing adjacent to culvert – A case study**
Duy Quan Tran, Shinichi Nishimura, Masateru Senge, Tatsuro Nishiyama, Fumitoshi Imaizumi
- P-22 Droughts hotspot distribution by long term assessment the Standardized Precipitation Index (SPI) in Indonesia**
Yudhi Pramudya, Takeo Onishi
- P-23 The role of floral volatiles for attracting pollinators and reproductive isolation in *Mimulus* species**
Muhammad Arifin, Tomoko Okamoto
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**Potential of endophytic bacteria as plant growth promoter and antagonist against
pineapple-fungal plant pathogen in Indonesia**

Radix SUHARJO¹, Titik Nur AENY¹, Udin HASANUDIN¹, TE SUKMARATRI², Ruslan KRISNO², Thoriq KHOIRONI¹, Diyan Adinda SAFITRI¹

(¹Faculty of Agriculture, Lampung University, Indonesia; ²Great Giant Food Company, Lampung, Indonesia)

SUMMARY

This study was aimed to investigate potential of 15 endophytic bacteria (3C, AK, CH, GKSKK, GBSH, AM, B1, GKSKC, GBSK3, GKSKW, GKSKP, A31, GKSKKn, NS and AP) that were isolated from healthy leaves of pineapple as plant growth promoter and antagonist of pineapple-fungal plant pathogens. The isolates were investigated on their hypovirulence, ability as plant growth promoter and ability to inhibit three pineapple-fungal pathogens, namely *Phytophthora* sp., *Curvularia* sp. and *Thielaviopsis* sp. The result showed that 10 out of 15 isolates were hypovirulent. Among 10 hypovirulence isolates, 7 isolates had potential as plant growth promoter (3C, AK, GKSKK, AM, B1, GKSKC and GBSK3). In the case of their antagonistic capability, the isolates which were produced the highest percentage of inhibition against *Phytophthora* sp. and *Curvularia* sp. were GKSKK at 72,48% and 66,08% of inhibition, respectively. Meanwhile, the highest percentage inhibition against *Thielaviopsis* sp. was obtained by CH at 64,82% of inhibition. In this study, we found that some of the endophytic bacteria can be plant growth promoter or antagonist or both as plant growth promoter and antagonist.

Introduction

Pineapple is one of the most important fruit commodities in Indonesia. Recently, although not very significant, pineapple production in Indonesia continues to decline. This is due to the decreased of soil fertility and infection of some plant pathogens. Three important plant pathogens that have been reported causing severe economic losses are *Phytophthora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. Application extra chemicals fertilizer and fungicides to solve the problems can cause more severe harms to the environment and future cultivation and efforts to improve pineapple production. Thus, it need to find alternative methods that is safe to be used to solve the complications, and one of which are reducing the chemicals and using bio agents.

Endophytic bacteria is one of the promising bio agents that can be used to improve production of cultivated plants, including pineapple. Endophytic bacteria is bacteria that live internally inside the plant tissue, can be isolated from the plant after surface disinfection and does not cause negative effects on plant growth (Wilson, 1995; Gaiero et al., 2013). It has been reported that endophytic bacteria has capability as plant growth promoter (Gaiero et al., 2013; Santoyo et al., 2016), plant resistance inducer against plant diseases (Costa et al.,

2013; Lanna-Filho et al., 2013; Yi et al, 2013; Egamberdieva et al., 2017; Leiwakabessy et al., 2018) and antagonist of many kinds of plant pathogens (Duffy and Defago, 1999; Gaiero et al., 2013).

Fifteen endophytic bacteria were successfully isolated from healthy leaves of pineapple. However, study on their potential as agricultural bio agents has not been performed. This study was conducted in order to investigate virulence, ability as plant growth promoter and antagonist of the fifteen isolates of above mentioned endophytic bacteria against three pineapple fungal pathogens, namely *Phytophthora* sp., *Curvularia* sp., and *Thielaviopsis* sp..

Material and Method

Endophytic bacteria. As much as 15 isolates of endophytic bacteria used in this study. All the strains were isolated from healthy leaves of pineapple.

Hypovirulence test. Hypovirulence test was performed using method developed by Worosuryani (2005). Sprouts of cucumber were used as indicator. Inoculation each of endophytic bacteria was repeated 3 times. Observation was performed until 14 days after inoculation. Disease severity Index (DSI) was calculated using formula : $(\sum N/Z)$; N : total score of disease severity on each individu, Z : total individu used. Score of the disease

severity that was used can be explained as follows: 0 : healthy, there was no infection on hypocotyl; 1 : one or two brown spot observed with <0.25 cm of diameter; 2 : brown spot observed with < 0.5 cm of diameter with <10% of wetness area of hypocotyl; 3 : brown spot observed with > 1 cm of diameter with 10%<x<100% of wetness area of hypocotyl; 4 : black spot observed, wilt and sprouts death. The endophytic bacteria with DSI <2 was put in the group of hypovirulent bacteria.

Investigation on its capability as plant growth promoter. Cucumber plant was used as indicator plant. Investigation was conducted using methods developed by Worosuryani (2005). As much as 10 ml of bacterial suspension (~10⁸ CFU/ml) was pured into planting medium of plant indicator. Inoculation each of the bacteria was repeated 3 times. Observation of plant height was performed every two days. Greenish leaves level was conducted once at 16 days after inoculation using chlorophyll content meter CCM 200 plus (opsi science) at the 3 of leaves position i.e. top, midle and bottom. Weight of wet and dry of shoot and root was conducted at 21 days after inoculation. In the case of dry weight of shoot and root, the fresh shoot and root were put into envelope and it was incubated at 80°C for 3 days. After incubation, it was weight using digital balance EG 4200-2NM (Kern).

Antagonistic capability agains pineapple fungal-plant pathogens. Three pineapple fungal plant pathogens used in this study i.e. *Phytophthora* sp., *Curvularia* sp., and *Thielaviopsis* sp.. Antagonistic test was performed by scraping the bacteria using inoculating loop with a distance of 2 cm from the edge of petridish (diam 9 cm) contains Potato Sucrose Agar (PSA) medium (Potato extract 1000 ml, Sucrose 20 g, Agar 20 g) in both side. One culture of 7 old days of each of the fungal pathogens (diam 0.5 cm) was placed in the middle of petridish. As control, one culture of each of plant pathogens was put in the middle of petridish contains PSA medium without any endophytic bacteria. All the petridish were incubated at room temperature. Observation was conducted at 1, 3, 5 and 7 days after inoculation on the wide of fungal colony that was measured in milimeter. Percentage of inhibition was calculated using formula : $[L1-L2/L1] \times 100\%$. L1: wide of fungal colony without endophytic bacteria, L2 : wide of fungal colony with endophytic bacteria.

Result and Discussions

In this study, 15 endophytic bacteria was investigated on

their hypovirulence, capability as plant growth promoter and antagonist against 3 pineapple plant pathogens, namely *Phytophthora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. The result showed that 10 out of 15 isolates showed hypovirulent (Table 1). Among those 10 hipovirulent isolates, 7 isolates showed potential as plant growth promoter. Application of the bacterial isolates resulting better growth on indicator plant compared to the untreated plants. Application of endophytic bacteria consistently improve plant height, greenish leaves, wet and dry weight of shoot and root and root length (Fig 1). Gaiero et al (2013) and Santoyo et al. (2016) stating that endophytic bacteria also could promote growth of their host plant. The bacteria release phytohormones (Bloemberg & Lugtenberg, 2001) that can improve plant growth such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Gaiero et al., 2013; Santoyo et al., 2016), jasmonate, indole acetic acid, and abscisic acid (Patten and Glick, 2002; Forchetti et al., 2007). Beside their ability as growth promoter, endophytic bacteria was also reported as plant resistance inducer (Romeiro et al., 2005; Lanna-Filho et al., 2013). Application of endophytic bacteria has been reported can improve plant resistance against plant diseases such as bacterial leaf spot of pepper (Yi et al, 2013), bacterial leaf spot of

Table 1 Disease severity index resulted by inoculation of the bacterial isolates on cucumber sprouts and its role as plant growth promoter

Isolates	Disease Severity	
	Index	Role as plant growth promoter
AP	2.75	Not tested
GKSKKn	2.58	Not tested
NS	2.50	Not tested
A31	2.50	Not tested
GKSKP	2.42	Not tested
3C	2.00	Yes
AK	1.92	Yes
CH	1.83	No
GKSKK	1.67	Yes
GBSH	1.67	No
AM	1.33	Yes
B1	1.17	Yes
GKSKC	1.00	Yes
GBSK3	0.75	Yes
GKSKW	0.33	No
Kontrol	0.00	-

tomato (Lanna-Filho et al., 2013), bacterial leaf blight of rice (Leiwakabessy et al., 2018), damping off on

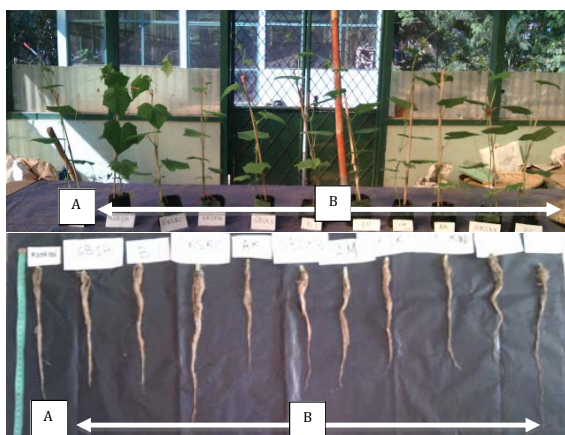


Fig.1 Indicator plant after application of bacterial isolates. A. control, B. treated plants

cucumber (Costa et al., 2013) and root rot of chickpea (Egamberdieva et al., 2017). Some of endophytic bacteria have also been reported produce anti microbial compounds, such as siderophore and antibiotics, that can inhibit growth of plant pathogens (Duffy and Defago, 1999; Gaiero et al., 2013) such as *Enterobacter*, *Pseudomonas* sp., *Bacillus* sp.. (Muzzamal et al., 2012), *Fusarium oxysporum* f.sp. *lycopersici* (Shahzad et al., 2017), *Phytophthora capsici*, *Alternaria panax* and *Botrytis cinerea* (Paul et al., 2013).

In this study, we found that some of endophytic bacteria used had capability to inhibit *Phytophthora* sp., *Curvularia* sp. and *Thielaviopsis* sp.. (Fig. 2). Inhibition was in the range of 5.13 to 72.48% (*Phytophthora* sp.), 2.33 to 66.08% (*Curvularia* sp.) and 1.33 to 64.82% (*Thielaviopsis* sp.). The best capability to inhibit *Phytophthora* sp., and *Curvularia* sp. was produced by GKSKC. Meanwhile, the highest inhibition of *Thielaviopsis* sp. was produced by CH (Table 2). It was shown that one endophytic bacteria can inhibit more than one pathogens.

Ability of endophytic bacteria to inhibit more than one kinds of pathogens have also been reported. Endophytic bacteria isolated from potato (Berg et al., 2005) and chilli pepper (Paul et al., 2013) have been proven to be antagonist of more than one kinds of pathogens.

Study performed by Berg et al. (2005) revealed that endophytic bacterium isolated from potato could inhibit *Verticillium dahliae* or *Rhizoctonia solani*. Paul et al. (2013) stated that edophytic bacteria isolated from chilli pepper can inhibit *Fusarium oxysporum* or *Alternaria panax* or *Colletotrichum acutatum* or *Phytophthora capsici* or *Botrytis cinerea*.

Conclusion

In conclusion, not all endophytic bacteria used in this study were plant growth promoter and antagonist. There

was endophytic bacteria that play a role as plant growth promoter or antagonist or both plant growth promoter and antagonist. The best inhibition to *Phytophthora* sp. and *Curvularia* sp. were produced by GKSKC, meanwhile, the highest inhibition against *Thielaviopsis* sp. was obtained by CH.

Acknowledgement

We thank to Great Giant Food Company for providing

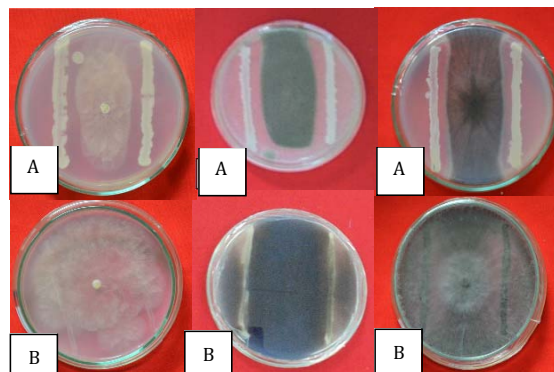


Fig. 2 Antagonist test of endophytic bacteria against 3 pineapple fungal pathogens 7 days after inoculation. From left to right : *Phytophthora* sp., *Curvularia* sp., *Thielaviopsis* sp.. A. Endophytic bacteria that had antagonistic capability, B. Endophytic bacteria that did not has antagonistic capability.

Table 2 Percentage of inhibition of endophytic bacteria against the pineapple fungal pathogens

Isolates	Percentage of inhibition (%)		
	Phytophthora	Curvularia	Thielaviopsis
AP*	14.76	30.18	5.58
GKSKKn*	0.00	27.77	1.53
NS*	0.00	16.73	5.44
A31*	61.85	13.35	46.15
GKSKP*	62.74	6.37	61.85
3C*	0.00	15.40	63.71
AK	64.30	29.94	4.94
CH	67.15	2.40	64.82
GKSKK	0.00	30.53	25.87
GBSH	20.00	14.48	29.68
AM	0.00	5.67	1.50
B1	0.00	9.40	8.72
GKSKC	72.48	66.08	4.88
GBSK3	5.13	2.33	0.00
GKSKW	64.11	24.88	1.33
Kontrol	0.00	0.00	0.00

* Isolates which were virulent (DSI>2) on the result of hypoverulence test

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