AGRIVITA VOLUME 37 No. 3 *OCTOBER - 2015* *ISSN : 0126 - 0537*

POPULATION DYNAMICS OF RHIZOBACTERIA AND ITS POTENCY AS A BIOLOGICAL CONTROL AGENT TO CONTROL FUSARIUM DISEASE IN THE NURSERY OF AGARWOOD (*Aquailaria malaccensis* Lamrk*)*

Tri Wahyuni [*)], Mulawarman and Nurhayati Damiri

University of Sriwijaya
Jl. Padang Selasa No. 549, Bukit Besar, Palembang 30139, South Sumatera, Indonesia
[*)] Corresponding author Email: ami_hanif@yahoo.com

Received: October 20, 2014/ Accepted: September 1, 2015

ABSTRACT

Agarwood is a resin product produced by particular trees and has a certain high comercial value. In Central Bangka Regency, agarwood is the main commodity of forest. The research was aimed to determine the dynamic population of rhizobacteria and its potential as a biological control agent to control Fusarium disease in the nursery of agarwood (*Aquailaria malaccensis* Lamrk*)***.** The research was carried out by using exploration and identification methods. Sixty nine bacterial isolates were obtained from 20 samples. The samples taken were from Pangkalan Baru and Koba districts. After selection process, 49 bacterial isolates were tested for the capacity of inhibition. Results showed that 37.50 % of the bacterial isolates indicated a strong inhibition capacity, meanwhile 58.33% indicated a moderate and only 4.70% possessed a weak inhibition. *Pseudomonas fluorescens, P. aeroginosa, P. malthophilia* and *Klebsiella pnemoniae* were identified from the selected isolates. These bacteria were potentially able to protect plants against *Fusarium* disease and to promote plant growth. This research needed to be continued at the field level in order to know the real effects on plant.

Keywords: agarwood; biocontrol agents; endophytic bacteria; *Fusarium* disease; rhizobacteria

INTRODUCTION

Agarwood is a forest product which has a high economical value compared to other forest products, therefore it has potential to develop. In the wild nature, the production of agarwood is less than 5%. If agarwood had been formed, the amount of it was usually less than 10% of the biomass of the infected tree. Because of the valuable price, exploitation of natural agarwood was conducted without proper consideration of its sustainability. As a result, population of agarwood species declined rapidly, so that this species was included in *Appendix* II CITES. As a consequence, in formal trade, agarwood should be produced from cultivation, not from nature. The cultivation of agarwood had problem such as pests and diseases especially in the seedling. Murdan (2008) reported that there are seven plant pathogenic funguses associated with a rotten root of agarwood and one isolate was identified as *Fusarium* sp.. Leaves of agarwood fell continuously. It caused a bare crown, rotten root, and finally caused plant die. Those were the symptoms of Fusarium disease. The disease attacked agarwood from the beginning of seedbed to the two years old of plant (with diameter of stem > 2 cm).

Fusarium sp. was one of the pathogens found in most of the plants and was causing a lot of economic loss. Murdan (2008) reported that Agarwood Development Center in Senaru village, West Lombok covering an area of 225.7 hectars, had beenbeen attacked by Fusarium disease and the loss reached 9.75% (or equivalent to Rp 274,015,599). Nowadays, there have been some ways to control pest and disease biologically. One of biological agents used are endophytic bacteria and rhizobacteria inside and around the plant itself. Natural bioactive compounds are produced by endophytic microbes potential for application in the fields of health, agriculture and industry (Joseph and Priya, 2011). Endophytic bacteria species diversity reflects many possible ways to reduce plant pathogens by produced antibiotic compounds (Bacon and Hinton, 2007). The way of endophytic bacteria work as biological control are to

Accredited SK No.: 81/DIKTI/Kep/2011
http://dx.doi.org/10.17503/Agrivita-2015-37-3-p276-283

produce a mixture of anti-microbial materials, to compete a space and nutrients, to compete a micro nutrients such as iron and siderophores production, and to cause the host plant becomes resistant.

Some endophyticbacterial could be used as a biocontrol agent. It could protect plants from microbial pathogens attack such as *Xanthomonas campestris, Pseudomonas solanacearum, Colletotricum gloeosporioides* and *Fusarium oxysporum*, control of the disease in Siam citrusplants, banana plants (Melliawati *et al*., 2006; Hadiba, 2009; Munif and Hipi, 2011; Ningsih *et al*., 2012). Wibowo and Munif (2013) isolated endophytic bacteria from forest plants successfully such as from mahogany (*Swietenia mahogany* Jacq), tamarind (*Albizia saman* (Jacq.) Merr.), agarwood (*Aquilaria malaccensis*) and meranti (*Shorea* sp.). Endophytic bacterial also could improve the plant growth of potato and corn (Hallmann *et al*., 1997; Munif dan Hipi, 2011).

Based on the explanation above this research was significant to be conducted, since the information on science and technology of agarwood pest and disease and also the controlling was still limited. The objective of this research was to assess the population dynamics of rhizosphere bacteria of agarwood (*Aquailaria malaccensis* Lamrk*)* and its potency as a Bioagents against pathogenic (*Fusarium* sp.) in the nursery.

MATERIALS AND METHODS

The research was conducted in the District of Pangkalan Baru and Koba in Central Bangka regency, Laboratory of the Ministry of Health Center Palembang, and Phytopathology Laboratory of the Department of Plant Pests and Diseases Faculty of Agriculture, Sriwijaya University Inderalaya South Sumatra, from February to August 2014. Exploration and identification methods were used to conduct this research.

Isolation of Endophytic and Rhizobacteria

Isolation of endophytic rhizobacteria was carried out by collecting of soil rhizosphere and root of agarwood from 20 sites soil rhizosphere and root of agarwood in the Garden Growers and Nursery of Horticulture and Forestry Department, Central Bangka regency. The procedure followed the method of isolation of endophytic bacteria which had been modified. The roots of agarwood was cut and washed with water, then dried. 1 g of roots were taken and the surface sterilized successively with 70% alcohol (30 seconds), then dipped in a solution of 2% NaOCl (for 2 minutes), and then dipped in sterile water. Before grinding, root of agarwood onis was applied on petridish containing TSA media (as a control). If bacteria grew on the control group, it was necessary to identify the bacteria that would grow as a marker of bacterial endophytic bacteria using the controls group. Roots were transferred into a mortal and crushed and added to 9 ml of sterile aquadesh. 1 ml of root extract was inserted into a test tube containing 9 ml of sterile aquadesh, then shaken until homogeneous, the next 1 ml of the extract was taken and diluted in series to 10-4 dilution. Next 0.1 ml of extract from the series of dilution 10-3 and 10-4 was put in sterile petridish that had been filled by media TSA.

Rhizobacteria was isolated by means of soil around the roots of plants that had been dried, taken as 1 g put in 9 ml of sterile distilled water, then mixed until homogeneous. 1 ml of the extract was put into a test tube containing 9 ml of sterile distilled water, then shaked until homogeneous and 1 ml was transferred to the next tube, and did it continually until there was dilution series 10^1-10^4. 0.1 ml of extract dilution series 10^3 and 10^4 inserted into the filled sterile petridish TSA medium and then spread evenly in petridish. The extract that had been deployed in petridish, incubated for 3 days, then counted the number of colonies of bacteria growing.

Isolation of *Fusarium* sp.

Pathogens were obtained from the isolation of the pathogen on the roots and stems of the infected agarwood from planting directly method. The pieces of tissue which had a size 5 mm x 5 mm soaked in 1% NaOCl for 1 minute, then rinsed with sterile water and dried on sterile filter paper. The tissue pieces were then placed on plates containing *potato dextrose agar* medium (PDA) and incubated at room temperature for 48 hours. Colonies of fungus that grows were identified. The results showed that the isolated fungus were identified as *Fusarium* sp., then they were purified and used in further tests.

Antagonist Test

A total of 49 isolated endophytic bacteria and antagonists tested rhizobacteria against *Fusarium* sp. were obtained. Antagonists testing by in vitro was conducted usinga *dual–culture technique*.

Identification of Bacteria

Bacteria with potential biological agent were identified based on the strong inhibition zone (after the dual-culture technique), and they were identified by biochemical tests: motility test, acid and gas production test of some carbohydrates tests, gelatin hydrolysis test, casein hydrolysis test, starch hydrolysistest, nitrate and nitrite reduction tests, indole and H_2S gas production tests, oxidase test, catalase test, methylred (MR) test and vogespraskauer (VP) test (Cowan, 1985).

Parameters observed in this study were a population of bacteria, inhibitory power against *Fusarium* sp, the biochemical bacteria reaction obtained for identification.

RESULTS AND DISCUSSION

Population of Endophyitic Bacteria and Rhizobacteria

The population densities of indigenous endophytic bacteria and rhizobacteria in agarwood roots and soil between the plants were varied. The isolated agarwood from 20 samples from the district of Pangkalan Baru and Koba were obtained 69 bacteria. Most of bacterial population presented in the roots, and they were about 209 colonies from Pangkalan Baru districts compared to the root from Mesu.

The number of bacteria colonies obtained from the root were more than bacteria obtained from the soil, even in some petri dishes there were no bacteria grown. The number of bacteria colonies obtained from Pangkalan Baru district were more than bacteria obtained from Koba district (Table 1 and Table 2). It had been predicted, since the demographic circumstances and the way of farming in Pangkalan Baru district was different with Koba. Population density of bacteria from the soil was less than the density of the bacteria population derived from the root, and it was the same as that reported McInroy and Kloepper (1995) who stated that the population of endophytic bacteria was commonly found in the roots and ramifications (Misaghi and Donndelinger, 1990; Bell *et al*., 1995). Endophytic bacteria population density was very dependent on the type of selected plant tissue and roots that seem highest and lowest of the trunk and acropetal decline. Root was considered as a preferrable place for bacteria to enter the plant, and in particular it explained the high number of bacteria in roots in the early stages of growth. The root system seemed to be more supportive of this habitatas if it was related to water availability and temperature changes (Sessitsch *et al*., 2004; He *et al*., 2009; Sarr *et al*., 2010). But factors such as crop rotation, organic matter, temperature, rainfall, soil physical properties and chemical constituents might have an effect on bacteria populations (Mahaffee and Kloepper, 1997; Sessitsch *et al*., 2004) and some of these factors might have an influence in these research.

Table 1. Population density of endophytic bacteria and rhizobacteria from agarwood from Koba District and Pangkalan Baru District of Central Bangka Regency

Sample	From	Number of colony (10^5) (cfu)/g soil; g fresh root	
		Koba	Pangkalan Baru
T1	Soil	32	129
T2	Soil	14	173
T3	Soil	10	30
T4	Soil	10	137
T5	Soil	10	0
T6	Soil	51	33
T7	Soil	50	6
T8	Soil	47	14
T9	Root	110	68
T10	Root	119	116
T11	Root	69	16
T12	Root	114	30
T13	Root	28	209
T14	Root	41	95
T15	Root	23	36
T16	Root	94	9
T17	Root	83	41
T18	Root	153	21

Tabel 2. In vitro antagonism of endophytic and rhizobacteria isolated from agarwood Koba District toward *Fusarium* sp. showing various degrees inhibition

Isolates	Inhibition		
	Weak (<1 cm)	Moderate (1-2 cm)	Strong (>2 cm)
TT1	-	-	0.60
TT2	-	1.50	-
TT3	-	1.20	-
AT1	-	1.30	-
TT4	-	1.05	-
AT2	-	1.10	-
AT4	-	1.10	-
AT3	-	1.80	-
TT5	-	-	0.50
TT6	-	1.15	-
TT7	-	1.35	-
TT8	-	1.50	-
AT5	-	1.25	-
TT9	-	1.05	-
AT6	-	1.70	-
TT10	-	1.75	-
TT11	-	-	0.75
AT7	2.50	-	-
AT8	-	-	0.95
TT12	2.40	-	-
AT9	-	-	0.60
AT10	-	1.30	-
TT13	-	1.10	-
TT14	-	1.05	-
TT15	-	-	0.90
AT11	-	-	0.45
AT12	-	1.70	-
TT16	-	-	0.80
AT13	-	1.35	-
AT14	-	-	0.35
AT15	-	-	0.50
	2.00	19.00	10.00

Remarks: AT: from root; TT: from soil

In vitro antagonism of Endophytic Bacteria and Rhizobacteria Towards Fungal Pathogens (*Fusarium* sp.)

A total of 49 isolated bacteria were tested with antagonist fungus *Fusarium* sp. Level antagonist indicated by the variation of zone inhibition such as weak (<1 cm), moderate (1 to 2 cm) and strong (>2 cm) (Rocha *et al*., 2009) (Table 3). and each of bacterial isolated power had a different inhibitors (Figure 1). After selection, 49 isolates were tested for inhibitation power. Results showed that 37.50% of the bacterial isolates indicated a strong inhibition capacity, 58.33% indicated a moderate and only 4.70% possessed a weak inhibition. In this study the antagonistic test used *Nutrient Agar* (NA) because it had been predicted that by using this medium the fungus and bacteria would grow optimally, furthermore the selection of the growing medium could provide a high impact on the inhibition shown (Yang *et al*., 2011). This results might be affected by medium used for antibiosis in vitro assay. Antagonism test of bacteria towards pathogenic fungi by using invitro method provided a fast way to select initial candidate biological control based on antibiosis.

Tabel 3. In vitro antagonism of endophytic and rhizobacteria isolated from agarwood Pangkalan Baru District toward *Fusarium* sp. showing various degrees inhibition

Isolates	Inhibition		
	Weak (<1 cm)	Moderate (1-2 cm)	Strong (>2 cm)
AM3	-	1.35	-
AM4	-	1.25	-
TM3	-	1.10	-
AM5	-	-	0.80
AM6	-	1.50	-
TM4	-	-	0.90
TM5	-	-	0.75
AM7	-	1.80	-
AM8	-	1.45	-
TM6	-	1.30	-
TM10	-	-	0.40
AM9	-	1.05	-
TM7	-	-	0.80
TM8	-	-	0.80
AM10	-	-	0.40
TM9	-	-	0.35
AM11	-	1.00	-
AM12	-	-	0.45
Total		9.00	9.00

Remarks: AT: from root; TT: from soil

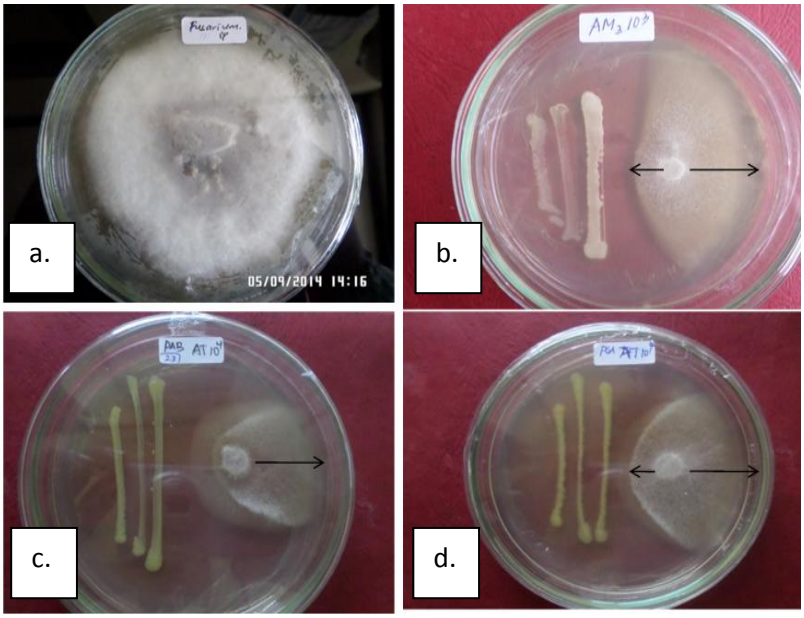

Figure 1. In vitro antagonism of endophytic and rhizobacteria isolated from agarwood toward *Fusarium* sp. and in the presence of endophytic bacteria showing various degrees of inhibition: a. *Fusarium* sp., b. weak inhibition, c. strong inhibition and d. moderate inhibition.

Table 4. Biochemical characterization of bacteria and rhizobacteria isolated from Bangka Tengah Regency

Biochemical characterization	***Pseudomonas* spp.**			***Klebsiella sp.***
	(P. fluorescens)	***(P. aeruginosa)***	***(P. maltophilia)***	***(pneumoniae)***
Motility	(+)	(+)	(+)	(-)
Katalase	(+)	(+)	(+)	(+)
Oxidase	(+)	(+)	(+)	(-)
Production of acid:				
Glukosa	(+)	(+)	(+)	(+)
Lactosa	(-)	(-)	(-)	(+)
Manitol	(+)	(+)	(+)	(-)
Maltosa	(-)	(-)	(-)	(-)
Sucrosa	(-)	(-)	(-)	(-)
Hidrolisis:				
Gelatin	(+)	(-)	(+)	(-)
Pati	(-)	(+)	(+)	(+)
Kasein	(-)	(-)	(-)	(-)
Production of H_2S	(-)	(-)	(-)	(-)
Production of Indol	(-)	(-)	(-)	(-)
Nitrat Reduction	(+)	(+)	(+)	(+)
Urea	(+)	(+)	(+)	(+)
Metyl Red (MR)	(-)	(-)	(-)	(-)
VP	(-)	(-)	(-)	(+)

Remarks: (+) : positive test result, (-) : negative test result

Identification of Bacteria

Based on the results of the antagonism test there were 11 antagonistic isolated bacteria that had a strong inhibition against *Fusarium* sp. After identification by biochemical tests (Table 4), it was known that bacteria were *Pseudomonas fluorescens, P.aeroginosa, P.malthophilia and Klebsiella pnemoniae.* Pseudomonas group had been studied for biological control of *P. flourescens* primarily as an inducer of plant

resistance to pathogens (Van Loon *et al*., 1998; Van Loon, 2000; Junita, 2010) as antagonist agents and as a plant growth promoter (Kloepper, 1991; Kloepper *et al*., 1999). *P. fluorescens, P. putida* was able to suppress the growth of soil borne pathogens.

From these results known that *P. Fluorescens* was the most common of the 11 isolates that had the greatest percentage of inhibition, four isolates. The suppression of pathogenic fungi *Fusarium* sp by bacteria *P. flourescens* occured because bacteria were able to remove antibiotics such as pyoverdine, pyoluteorin, 2,4 diacetylphloroglucinol and monoacetylphloroglucinol that could inhibit the growth of pathogens (Blanco and Bakker, 2007). Besides, *P. flourescens* could also inhibite the development of the disease by nutrient competition of iron Fe (III) and carbon element, HCN production, stimulate phytoalexin accumulation so that the plant became resistant, colonized roots and stimulated plant growth (Defago *et al.*, 1990; Notz *et al*., 1990; Widodo *et al*., 1993).

P. maltophilia produced siderophores in a form of maltophilin, produced extra cellular protease enzyme that could control *Pythium ultimum* in sugar beet rhizosphere of plants and able to induce resistance of onion toward the bacterial leaf blight (Jakobi *et al*., 1996; Dunne *et al*., 1997; Ernita *et al*., 2010). The success of biological control of plant diseases was determined by the mechanism of inhibition of biological agents. The mechanism of inhibition was commonly found in biological agents such as siderophores, antibiosis, competition of mycoparasitism, PGPR, impactresistance, enzymesandtoxins (Soesanto, 2008).

Besides the group of *Pseudomonas*, there was another bacteria that had been identified. It was *Kleibsella pneumoniae*a diazotrof endophytic bacteria. Diazotrof endophytic bacteria generally did not cause a disease, proliferates in plant tissues, however it did not form an endosymbiont inside cells of living plant. Diazotrof endophytic bacteria normally lived in the intercellular spaces or xylem vessels of roots, stems, leaves, and seed surface (James *et al*., 2000). The colony of diazotrof endophytic bacteria inplant tissue could exploit the carbon substrate supplied by plants without competing with other microbes. Some diazotrof endophytic bacteria was not only able to tie up nitrogen but also secrete acid indole-3-acetic (Ladha *et al*., 1997). *Klebsiella* sp isolated from tomato plants could produce IAA and also siderophores, hydrogencyanide (HCN) and salicylic acid (Nandhini *et al*., 2012). *Azospirilium brasilense, Azotobacter chroococcum* and *K. pneumoniae* could inhibit *F.oxysporum f.sp lycopersicy*, *Rhizoctonia solani* and *Pythium* sp. that attacked cucumber plants in vitro (Hassouna *et al*., 1998).

CONCLUSION

Endophytic bacteria and rhizobacteria in Indonesia forest especially agarwood (*A. malaccensis*) had a potency as abiological control agents that was useful to protect from pathogenic *Fusarium* sp. A total of 69 isolated bacteria were obtained from Pangkalan Baru and Koba District in Central Bangka, and After selection, 49 isolates were tested for inhibitation power. Results showed that 37.50 % of the bacterial isolates indicated a strong inhibition capacity, 58.33% indicated a moderate and only 4.70% possessed a weak inhibition. *Pseudomonas fluorescens, P. aeroginosa, P. malthophilia* and *Klebsiella pnemoniae* were identified from the selested isolates. These bacteria were potentially able to protect plants against *Fusarium* disease and promote plant growth.

ACKNOWLEDGEMENT

The deepest gratitude is delivered to the Government of Central Bangka regency for supporting all funding and permission in doing parts of research in nursery garden of agarwood in Central Bangka Regency.

REFERENCES

Bacon, C.W. and Hinton, D.M. 2007. Potential for control of seedling blight of wheat caused by *Fusarium graminearum* and related species using the bacterial endophyte *Bacillus mojavensis*. Biocontrol Sci. Tech. 1 (1-2): 81-94. doi: 10. 1080/ 09583150600937006

Bell, C.R., G.A. Dickie, W.L.G. Harvey and J.W.Y.F. Chan. 1995. Endophytic bacteria in grapevine. Can. J. Microbiol. 41(1): 46-53. doi: 10.1139/m95-006

Blanco, J.M. and P.A.H.M. Bakker. 2007. Interactions between plants and beneficial *Pseudomonas* spp.: exploiting bacterial traits for crop protection. Springer. Netherlands. A. van Leeuw. J. Microb.

92(4): 367-389. doi: 10.1007/s10482-007-9167-1

Cowan, S.T. 1985. Cowan and steel's manual for identification of medical bacteria. 2nd edition. Cambridge University Press. Cambridge, London.

Defago, G., C.H. Berling, U. Burger, D. Hass, G. Kahr, C. Keel, C. Voisard, P. Wurthner and B. Wuthrich. 1990. Suppresion of black root rot of tobacco and other root diseases by strains of *Pseudomonas flourescens*: Potential applications and mechanisms. In: Biological control of soil-borne plant pathogens. D. Hornby, R.J. Cook, Y. Henis, W.H. Ko, A.D. Rovira, B. Schippers and P.R. Scott (eds.). CAB International. Oxon, United Kingdom. p. 93-108.

Dunne, C., J.J. Crowley, Y.M. Loccoz, D.N. Dowling, F.J. de Bruijn and F. O'Gara. 1997. Biological control of *Phytium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. Microbiology. 143: 3921-3931. doi: 10.1099/00221287-143-12-3921

Ernita, M., Nasrun and N. Suharti. 2010. Characterization and physiological response of onion-induced rhizobacteria indigenus (in Indonesian). J. Embrio 30 (2): 110-116.

Hadiba, N.H. 2009. Selection and characterization of endophytic bacteria for disease control and blood on the banana *Fusarium* Wilt (in Indonesian). Thesis Post Graduate Program. Gadjah Mada University. Yogyakarta.

Hallmann, J., A. Quadt-Hallmann, W.F. Mahaffee and J.W. Kloepper. 1997. Bacterial endophytes in agricultural crops. Can. J. Microbiol. 43 (10): 895-914. doi: 10.1139/m97-131

Hassouna, M.G., M.A.M. El-Saedy and H.M.A. Saleh. 1998. Biocontrol of soil-borne plant pathogens attacking cucumber (*Cucumis sativus*) by Rhizobacteria in a semiarid environment. Arid Soil Res. Rehab. 12 (4): 345-357.doi:10.1080/ 15324989809381523

He, R.L., G.P. Wang, X.H. Liu, C.L. Zhang and F.C. Lin. 2009. Antagonistic bioactivity of an endophytic bacterium isolated from *Epimedium brevicornu* Maxim. Afr. J. Biotechnol. 8 (2): 191-195.

Jakobi, M., G. Winkelmann, D. Kaiser, C. Kempter, G. Jung, G. Berg and H. Bahl. 1996. Maltophilin: A new antifiingal compound produced by *Stenotrophomonas maltophilia* R3089. J. Antibiot. 49 (11): 1101-1104. doi: http://doi.org/10.7164/ antibiotics. 49.1101

James, E.K., P. Gyaneshwar, W.L. Barraquio, N. Mathan and J.K. Ladha. 2000. Endophytic diazotrophs associated with rice. In: The quest for nitrogen fixation in rice. J.K. Ladha and P.M. Reddy (eds.). International Rice Research Institute. Los Banos, Philippines. p. 119-140.

Joseph, B. and R.M. Priya. 2011. Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am. J. Biochem. Mol. Biol. 1 (3): 291-309. doi: 10.3923/ajbmb.2011.291. 309

Junita, A. 2010. The effectiveness of the control of white root fungus using biological agents rhizobacteria *Pseudomonas fluorescens* in rubber (in Indonesian). Thesis. Department of Plant Science. Agricultural Faculty. Sriwijaya University. Palembang.

Kloepper, J.W., R. Rodriguez-Ubana, G.W. Zehnder, J.F. Murphy, E. Sikora and C. Fernandez. 1999. Plant root-bacterial interactions in biological control of soilborne diseases and potential extension to systemic and foliar diseases. Australas. Plant Path. 28 (1): 21-26.

Kloepper, J.W., R.M. Zablotowicz, E.M. Tipping and R. Lifshitz. 1991. Plant growth promotion mediated by bacteria rhizosphere colonizers. In: The rhizosphere and plant growth. D.L. Keister and P.B. Cregan (eds). Beltsville Symposia in Agricultural Research. Beltsville, Maryland. Kluwer Academic Publishers. 14: 315-326. doi: 10.1007/ 978-94-011-3336-4_70

Ladha, J.K., F.J. de Bruijn and K.A. Malik. 1997. Introduction: assessing opportunities for nitrogen fixation in rice – a frontier project. Kluwer Academic Publishers. Netherlands. Plant Soil 194(1-2): 1-10. doi: 10.1023/A: 1004264423436

Mahaffee, W.F. and J.W. Kloepper. 1997. Temporal changes in the bacterial communities of soil, rhizosphere, and endorhiza associated with field-grown cucumber (*Cucumis sativus* L.). Springer - Verlag. New York. Microb. Ecol. 34 (3): 210-223. doi: 10.1007/s002489900050

McInroy, J.A. and J.W. Kloepper. 1995. Survey of indigenous bacterial endophytes from cotton and sweet corn. Kluwer Academic Publishers. Netherlands. Plant Soil 173 (2): 337-342. doi: 10.1007/BF00011472

Melliawati, R., N.W. Dian, C.D. Apridah and S. Harmastini. 2006. Study of endophytic bacteria producing bioactive compound for plant protection (in Indonesian). J. Biodiversitas 7(3): 221-224.

Misaghi, I.J. and C.R. Donndelinger. 1990. Endophytic bacteria in symptom-free cotton plants. Phytophatology. 80: 808-811.

Munif, A. and A. Hipi. 2011. The potency of endophytic bacteria and rhizosphere to increase the growth of corn (in Indonesian). Proceedings of a National Workshop on Cerealia. 3-4 October 2011. Maros. Indonesia. p. 1-8.

Murdan. 2008. Inventarisation and description of fungus that associated with agarwood root infected root rot in senaru agarwood development centre (in Indonesian). Faculty of Agriculture. University of Mataram. p. 1-5.

Nandhini, S., V. Sendhilvel and S. Babu. 2012. Endophytic bacteria from tomato and their efficacy againts *Fusarium oxysporum* f.sp. *lycopersici*, the wilt pathogen. J. Biopest. 5 (2): 178-185.

Ningsih, R., Mukarlina and R. Linda. 2012. Isolation and identification of fungi of damaged parts at Citrus Siam (*Citrus nobilis* var. *microcarpa*) (in Indonesia). Jurnal Protobiont 1 (1): 1-7.

Notz, R., M. Maurhofer, U. Schnider-Keel, B. Duffy, D. Hass and G. Defago. 2001. Biotic factors affecting expression of the 2,4-Diacetylphloroglucinol biosynthesis gene phlA in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. Phytopathology 91(9): 873-881. doi: 10.1094/PHYTO.2001.91. 9.873

Rocha, R., D.E da Luz, C. Engels, S.A.V. Pileggi, D.S.J. Filho, R.R. Matiello and M. Pileggi. 2009. Selection of endophytic fungi from comfrey (*Symphytum officinal*e L.) for in vitro biological control of the phytopatogen *Sclerotia sclerotium* (Lib.). Braz. J. Microbiol. 40(1): 73-78. doi: 10.1590/ S1517-83822009000100011

Sarr, P.S., T. Yamakawa, S. Asatsuma, S. Fujimoto and M. Sakai. 2010. Investigation of endophytic and symbiotic features of *Ralstonia sp*. TSC1 isolated from cowpea nodules. Afr. J. Microbiol. Res. 4(19): 1959-1963.

Sessitsch, A., B. Reiter and G. Berg. 2004. Endophytic bacterial communities of field-grown potato plants and their plant-growth-promoting and antagonistic abilities. Can. J. Microbiol. 50(4): 239-249. doi: 10.1139/ w03-118

Soesanto, L. 2008. Introduction to Biological Control of Plant Diseases (in Indonesian). Raja Grafindo Persada. Jakarta.

Van Loon, L.C. 2000. Systemic induced resistance. In: Mechanisms of resistance to plant diseases. A.J. Slusarenko, R.S.S. Fraser and L.C. Van Loon (eds.). Kluwer Academic Publishers. Netherlands. p. 521-574.

Van Loon, L.C., P.A.H.M. Bakker and C.M.J. Pieterse. 1998. Systemic resistance induced by rhizosphere bacteria. Annu. Rev. Phytopathol. 36: 453-483.

Wibowo, A.R. and A. Munif. 2013. Isolation of endophytic bacteria from plants forest and their potential to control *Meloidogyne* spp. in tomato (in Indonesian). Mini-Thesis. Department of Plant Protection. Bogor Agricultural University. Bogor. p. 1-37. http://repository.ipb.ac.id/handle/ 123456789/64977. Accessed on February 22, 2013.

Widodo, M.S. Sinaga, I. Anas and M. Mahfud. 1993. The use of *Pseudomonas* spp. (*P. fluorescens*) to control the roots cudgel (*Plasmodiophora brassicae* Wor.) on caisin (*Brassica campestris* L. var. *chinensis* Rupr. Olson) (in Indonesian). Bull. Plant Pest-Disease 6 (2): 94-105.

Yang, C.J., X.G. Zhang, G.Y. Shi, H.Y. Zao, L. Chen, K. Tao and T.P. Hou. 2011. Isolation and Identification of endophytic bacterium W4 againts tomato *Botrystis cineria* and antagonistic activity stability. Afr. J. Mcrobiol. Res. 5 (2): 131-136. doi: 10.5897/AJMR10.815