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Effectiveness of plant growth promoting bacteria isolated from phyllosphere and rhizosphere microbial consortium of rice growth

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Abstract

A group of microbes living together and interacting both with each other and with the host plant is known as a microbial consortium. Previous studies have tested the combination of microbial consortium phyllosphere Fm48 with rhizosphere R15, effectively improved the growth and production of rice. The role of microbial consortium is supported by the role of microbes that constitute the consortium. Therefore, the effectiveness of the member of microbial consortium on plant growth need to be evaluated when applied in the form of a single culture. The microbial consortium of phyllosphere Fm48 has four members, namely the isolate of Fm48(1)(95.5% 16S rRNA homology with *Serratia* sp. Strain SE-3), Fm4(2)(96.4% 16S rRNA homology with *Enterobacter* sp. Strain KDP6), Fm48(3)(96.2% 16S rRNA homology with *Enterobacter* sp. Strain MS5) and Fm48(4)(96.6% 16S rRNA homology with *Klebsiella oxytoca*. Strain LRC162). The microbial consortium of rhizosphere R15 has four members, namely the isolate of R15(1)(96.1% 16S rRNA homology with *Stenotrophomonas* sp. Strain U1370-101126-SW193), R15(2)(92.3% 16S rRNA homology with *Stenotrophomonas acidaminiphila*. Strain SZH19), R15(3)(86.0% 16S rRNA homology with *Bacillus* sp. Strain SC59) and R15(4)(95.9% 16S rRNA homology with *Stenotrophomonas* sp. Strain BCc6). The isolate of microbial consortium of phyllosphere Fm48 and rhizosphere R15 having roles to fix N_2, to dissolve inorganic P and as a consortium produces plant growth hormones. The consortium of phyllosphere Fm48 improved rice growth significantly compared with their single culture, in contrast, there is not any different between rhizosphere R15 and their single culture on its positive impacts on rice growth.

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Introduction

A microbial population is a mixture of various kinds of microbes, inhabit both the phyllosphere and the rhizosphere. A group of microbes living together and interacting both with each other and with the host plant is known as a microbial consortium (Lindquist, 1975). Mutual interaction is referred to as an associative relationship; and detrimental interaction is referred to as an antagonistic relationship. The living habit of microbes is a complex of populations. Sutedjo *et al.* (1996) states that under abundant number of certain type of microbes in a complex of microbial populations, the speed of their development and physiological activities are largely influenced by the presence of other microbial development that are also abundant in number.

The phyllosphere and rhizosphere microbes have a role to fix N_2, dissolve inorganic and organic P, and produce plant growth hormones. Inoculation of N_2 fixing rhizobacteria contributes around 15 kg N/ha/year (Hindersah and Tualar, 2004). Pati (1992) showed that spraying bacterial isolates capable of fixing the N_2 on the leaves of rice plants increased yields up to 20-34 percent. The phyllosphere bacteria can also produce, the phytohormone auxin (Akbari *et al.*, 2007), increase up take N, P, K by rice plant (Gusmaini, 2005).

This study aimed to evaluate the effect of microbial consortium and its bacterial members of phyllosphere Fm48 from the young leaves of *Emmerrilia ovalis* Miq Dandy plants and rhizosphere R15 from the rhizosphere of *Physalis angulata* L. plants on rice growth.

Materials and methods

The field experiment was carried out in the green house of ICBB (Indonesian Center for Biodiversity and Biotechnology), Bogor, Indonesia in March 2014. The experimental design was a randomized block design (RBD) one factor with three replications. The study consisted of two experiments: 1. Treatment isolate microbial consortium members of phyllosphere Fm48 consists of 5 treatments, namely : Consortium Fm48 (control), Fm48(1) isolate, Fm48(2) isolate, Fm48(3) isolate and Fm48(4) isolate. 2. The treatment of isolate microbial consortium members of rhizosphere R15 consists of 5 treatments, namely : Consortium R15 (control), R15(1) isolate, R15(2) isolate, R15(3) isolate and R15(4) isolate.

Data analysis

Data collected were analyzed using analysis of variance, and if a significant difference exists, the analysis continued with Duncan Multiple Range Test at 5%. Data were analyzed using SAS (Statistical Analysis System) for Windows version 9.1 (Matjik and Sumertajaya, 2006).

Molecular microbial identification

The method of DNA isolation refers to Atashpaz *et al.* (2010) which has been modified. The 16S rRNA gene was amplified using the *Polymerase Chain Reaction* (PCR, dice mini TAKARA) machine. The primers were 16F27 5'AGA GTT TGA TCM TGG CTC AG-3' and R1492 5'-TAC GGY TAC CTT GTT ACG ACTT-3' (Santosa, 2001). PCR *master mix* (*Thermo scientific dreamtaq green PCR Master mix(2x)*) is prepared in a final volume of 50 µl consisting of 25µl PCR *master mix*, 5 µM primer forward, 5 µM primer reverse, 1 µl DNA extract, and nuclease-free water until the final volume is reached. Amplification process is carried out as follows : initial denaturation of 3 minutes at 95 ^{o}C, denaturation at 95 ^{o}C for 30 seconds, annealing at 48 ^{o}C for 30 seconds, and extension at 72 ^{o}C for 1.5 minutes. After the 30 cycles are over, final extension at 72 ^{o}C for 10 minutes was done. The PCR product was run on agarose gel 1.5% added with ethidium bromide stain using TAE 1x buffer. The PCR product was delivered to the PT. Genetika Science Indonesia, Jakarta for sequencing. DNA sequence was compared to sequence at the European Bioinformatic Institute (EBI) database using FASTA 3 software at http://www.ebi.ac.uk.

Testing the ability of isolates to fix N_2

The test for the ability of isolates to fix N_2 qualitatively was done by growing the isolates on NFb (*nitrogen free bromthymol*) medium with composition per liter of malic acid 5.0 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 0.1 g, $CaCl_2.2H_2O$ 0.02 g, minor element stock solution of 2 ml ($CuSO_4.5H_2O$ 0.4 g, $ZnSO_4.7H_2O$ 0.12 g, H_3BO_3 1.13 g, Na_2MoO_4 1.0 g, $MnSO_4.H_2O$ 1.5 g per liter), bromthymol blue solution 0.5% in 2 N KOH 2 ml, FeEDTA 1.64% ml, vitamin solution 1 ml (Biotin 10 mg and Pyriodoxol-HCl 20 mg and made into 100 ml) and agar 1.75 g. pH of the solution was adjusted to 6.8 with KOH. The sample was suspended 10 ml of pure culture into 90 ml physiologically sterile saline solution (NaCl 0.85% in distilled water), and diluted until 10^{-7}. 1 ml of the dispension added to NFb medium and incubated for 1 week. The procedure was repeated 3 times. After one week, the color of the medium change from green to blue and the white pellicle was formed beneath the surface. N_2 fixation by microbes will cause increase the pH of the media due to the formation of $NH4^+$. After a few days, the pH normally increases by more than 1.0 units (Saraswati *et al.,* 2007). Nitrogenase activity of the diazotroph was also defected indirectly by acetylene reduction assay using Chromatoghraphy Gas (GC). Isolate was grown on N-free media. Then 0.5 to 1 ml of the sample was introduced into the incubation tube with a capacity of 30 ml. Acetylene was added and incubated for 1-2 hours. 1 ml of gas was sucked and injected in to Gas Chromatoghraphy. The amount of produced ethylene was used to calculate N_2 fixation of the isolates (Hidayati, 2014).

Testing the ability of isolates to dissolve P

The dissolve P test was done on Pikovskaya medium contains 5 g glucose, 5 g $Ca_3(PO_4)_2$, 0,5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.001 g $MnSO_4$, 0.001 $FeSO_4$, 0.5 g yeast extract, 15 g gelatin in 1000 ml aquades. 1 ml of diluted 10^{-4}, 10^{-5}, and 10^{-7} solution was spread into petri dish. Then, poured with Pikovskaya medium and incubated at room temperature (37 ^oC) for 1 – 3 days. The building of a crystal clear zone around the bacterial isolates indicated the ability of the isolate to dissolve phosphate (Saraswati *et al.,* 2007).

Testing the ability of isolates to produce plant growth hormones

Phytohormones produced by microbes consist of three types, namely auxin, gibberellins, and sitokonin and are analyzed using HPLC (High Performance Liquid Chromatography). The testing of the ability of the consortium of microbes to produce IAA (Indole Acetic Acid) and GA3 (gibberellin) quantitatively was conducted using modified method of Unyayar *et al.* (1996). The microbial consortium was inoculated on media NB + 20 ml methanol media and incubated at room temperature for 24 hours at 200 rpm. The supernatant was evaporated by evaporator machine at 35 °C. The extract was adjusted to pH 2.5 with 2 M HCl. Further was done three times with ethyl acetate at the same volume. Furthermore, the extract was filtered, and then injected into the HPLC. Mobile phase of 12 % acetonitrile at a wavelength of 265 nm. The ability of microbial consortium to produce cytokines was also measured by using HPLC. The extract was diluted with 0.1 M KH_2PO_4 at pH 2.4, centrifuge at 8000 rpm at 4 °C for 15 minutes. Supernatant was collected in a bottle containing 1 g polyvenil polypirolidone, shaken and filtered with a Sep-Pak C18 (water + 10 ml of the sample and 80% MeOH 10 ml), then rinsed with water. The solution was injected into HPLC mobile phase of 80 % methanol.

Results and discussion

Molecular characterization of the consortium

The members of microbial consortium from phyllosphere and rhizosphere were isolated and identified by using molecular methods based on sequence of 16S rRNA gene of phyllosphere Fm48 and rhizosphere R15. The phyllosphere consortium FM48, was identified to consist of 4 members from 3 genus. The rhizosphere consortium R15, was identified to consist of 4 members from 3 genus, are presented in Table 1.

Table 1. Results of identification microbial consortium of phyllosphere Fm48 and rhizosphere R15.

Isolate code	equivalent species	Strain	Homology (%)	Colony morphology
Phyllosphere Fm48(1)	*Seratia* sp.	SE-3	95.5	Red, round, convex, flat edge, wet texture *(smooth)*
Fm48(2)	*Enterobacter* sp.	KDP6	96.4	Milk beige, round, convex, flat edge, texture wet
Fm48(3)	*Enterobacter* sp.	MS5	96.2	Clear, beige, not too convex surface, flat edge, wet texture
Fm48(4)	*Klebsiella oxytoca*	LRC162	96.6	Beige, round, flat edge, convex surface, texture wet
Rhizosphere R15(1)	*Stenotrophomonas* sp.	U1370-101126-SW193	96.1	beige, round, wet texture, flat edge, dense texture
R15(2)	*Stenotrophomona acidaminiphila*	SZH19	92.3	beige, round shape there is a box in it, clear, flat edge, dense texture
R15(3)	*Bacillus* sp.	SC59	86.0	Clear, beige, round, wide growth, wavy edges, moist texture
R15(4)	*Stenotrophomonas* sp.	BCc6	95.9	beige, round, flat edge, convex surface, texture wet

Description: Fm48 = the microbial consortium of phyllosphere Fm48 from young leaves of *Emmerrilia ovalis* Miq Dandy, R15 = the consortium microbial of rhizosphere from rhizosphere of *Physalis angulata* L.

Ability to fix N_2 and dissolve P

All of the isolates showed the capacity to fix N_2 and most of them, except *Klebsiella oxytoca* {Fm48(4)} and *Stenotrophomonas* sp. {R15(1)}, could dissolve P inorganic (Table 2.).

Table 2. Bacterial members of the consortium isolates and the ability to fix N_2 and dissolve P.

Species	Ability to fix N_2			Dissolve P
	NFb medium	A pellicle formed	*ARA* (ppm)	
Seratia sp.	+	+	7.336	+
Enterobacter sp. Strain KDP6	+	+	7.976	+
Enterobacter sp. Strain MS5	+	+	8.826	+
Klebsiella oxytoca	+	+	9.526	-
Stenotrophomonas sp. Strain SW193	+	+	7.100	-
Stenotrophomonas. acidaminiphila	+	+	5.004	+
Bacillus sp.	+	+	7.976	+
Stenotrophomonas sp. Strain BCc6	+	-	3.668	+

Description: (+) capable, (-) are not able to, Fm48 = the microbial consortium of phyllosphere Fm48 from young leaves of *Emmerrilia ovalis* Miq Dandy, R15 = the microbial consortium of rhizosphere from rhizosphere *Physalis angulata* L.

Ability to produce plant growth hormones

Both consortium of phyllosphere Fm48 and rhizosphere R15 produced plant growth hormones, i.e Auxin, Sitokinin and Gibberellin. Quantity of IAA hormone produced by consortium phyllosphere Fm48 and rhizosphere R15 was moderate, otherwise the gibberellin production was high compared with other researches. Agustin *et al.* (2010) reported that, the content of IAA in maize rhizosphere was 67.30 ppm. Gusmaini *et al.* (2013) reported that endophytic bacterial isolates from Sambiloto plant produced IAA 205.4 to 585.7 ppm and Gibberellins 39-60 ppm (Table 3).

Table 3. The results of hormones content of phyllosphere and rhizosphere microbial consortium.

Phytohormones	Microbial concortium	
	Phyllosphere Fm48 (ppm)	Rhizosphere R15 (ppm)
Auxin		
- IAA	185.382	178.513
Sitokinin		
- Zeatin	121.126	118.775
- Kinetin	135.047	137.424
Gibberellin	211.776	193.855

Description: Fm48 = the microbial consortium of phyllosphere Fm48 from young leaves of *Emmerrilia ovalis* Miq Dandy, R15 = the microbial consortium of rhizosphere from rhizosphere of *Physalis angulata* L.

The result showed that treatment of the microbial consortium of phyllosphere Fm48 significantly increased plant height, number of leaves, fresh eight, and dry weight better than the treatment using its single culture isolated form the consortium (Table 4).

Table 4. The average plant height, number of leaves, fresh weight, and dry weight on 60 days after planting at microbial concortium of phyllosphere treatment.

Treatment	Plant height (cm)	∑ leaves	Fresh weight (g)	Dry weight (g)
Concortia of Fm48 (control)	61.683a	20.500a	92.974a	23.525a
Fm48(1) = *Seratia* sp.	50.167b	6.333b	13.161b	2.594b
Fm48(2) = *Enterobacter* sp. KDP6	49.083b	8.000b	15.824b	2.996b
Fm48(3) = *Enterobacter* sp. MS5.	51.350b	8.333b	20.956b	3.985b
Fm48(4) = *Klebsiella oxytoca*	62.017a	10.500b	28.230b	5.562b

Description: The average followed with the same alphabet at the same column shows no significant different based on DMRT test at 0.05, Fm48 = the microbial consortium of phyllosphere Fm48 from young leaves of *Emmerrilia ovalis* Miq Dandy.

Contrary, the results showed that there was no significant difference between the consortium of rhizosphere R15 and its bacterial member on their capacity to increase the growth of rice. Treatment of *S. acidaminiphila* {R15(2)} slightly increased plant height, number of leaves, and dry weight (Table 5).

Table 5. The average plant height, number of leaves, fresh weight, and dry weight on 60 days after planting at microbial concortium of phyllosphere treatment.

Treatment	Plant height (cm)	∑ leaves	Fresh weight (g)	Dry weight (g)
Concortia of R15 (control)	51.517a	8.000a	24.804a	3.9805a
R15(1) = *Stenotrophomonas* sp R15(2) = *S. acidaminiphila*	48.583a	7.833a	21.701a	3.2302a
	54.933a	9.333a	23.750a	4.5721a
R15(3) = *Bacillus* sp.	44.583a	6.500a	13.711a	2.4073a
R15(4) = *Stenotrophomonas* sp.	50.517a	8.000a	20.167a	3.4106a

Description: The average followed with the same alphabet at the same column shows no significant different based on DMRT test at 0.05, R15 = the microbial consortium of rhizosphere from rhizosphere *Physalis angulata* L.

Treatment using the isolates of microbial consortium of phyllosphere Fm48 produced better effect than treatment using single culture affect of the consortium. This suggests that the consortium members cannot effectively influence the growth of rice when applied as a single culture, although each single culture has their own role in plant growth, such as fix N_2, dissolve P, and produce plant growth hormones. They require the presence of other microbes member of consortium to effectively influence the growth of rice. Phyllosphere microbial consortium is composed of various microbes that interact in the population to produce a chemical response. Sunatmo (2009) stated that the presence of other microbes determines the physiological response of the microbial population. Microbial cells can respond to chemical signals from the environment including products of other microbes.

Sutedjo *et al.* (1996) affirm that microbial populations form a mutual relationship and antagonistic relationship, the first is beneficial, the latter is harmful for other microbes. Mutual relationship creates good conditions and provides nutrients for their members. In contrast, antagonistic relationship often takes place in the presence of microbial populations that affect or harm other microbial activity. A type of reciprocal relationships among microbes, among others, is the understanding of the specific ecological nature and metabolites products produced in a variety of activities. Effective microbes refer to a mixture of cultures of several kinds of beneficial microbes that can be used as inoculants to increase the microbial diversity (Rao, 1995).

In some of the phyla of bacteria dominating phyllosphere plants, plant factors involved in shaping phyllosphere communities adaptation and shows a special relationship and, either with host plants and among members of the community (Vorholt, 2012). Very complex interactions among microbes and between microbes and host plants are expected to occur. Microbes that inhabit the leaves have an important effect on plant growth (Redford *et al.,* 2010). Reisberg *et al.* (2013) report that environmental factors such as sunlight irradiation, season, geographical location and sampling sites are recognized as an important factor in shaping phyllosphere micro-biotic. In addition, it has been reported that phyllosphere microbial community is a species- specific. Leaf properties, such as size, color, mineral content, the presence of vascular tissue, stomata, and trachoma, and complementary surfaces including hydathodes, affect microbial community composition.

The effectiveness of rhizosphere microbial is greatly influenced by rhizosphere environment (Sharma *et al.,* 2003). Microbe is a major component of the rhizosphere and the composition of which is very often different from the surrounding soil due to changes in plant species and as a result of the diversity of interactions between plant and microb. Some interactions involve mutual exchanges, such as increasing N_2 fixation or producing hormones. Environment around the root system of plants have a major influence on the productivity of plants. However, the complex system in which a series of interactions occurred is under the influence of biotic and abiotic factors.

Healthy rhizosphere colonized by microbial communities both the microflora and microfauna will interact to form a stable and dynamic rhizosphere microbial community structure. Rhizosphere microbial community structure is strongly influenced by the type and age of plant, as well as physical and chemical properties of the soil. In line with the growth of plants, changes happen in the rhizosphere microbes due to changes of microbial structure associated with differences in root exudates and organic matter produced by plant roots during growth. Interactions of microbes in the rhizosphere can accelerate the process of decomposition of organic matter. Egamberdieva (2008) confirms that microbes in the soil play an important role in the process of decomposition, humification, and mineralization. However, Rao (1995) states that the

antagonism between microbes is a common symptom in the soil, caused by antibiotics produced by these microbes. Mwajita *et al.* (2013) report that many phyllosphere and rhizosphere microbes can dissolve phosphorus making it available, increasing the uptake of nitrogen, and synthesize growth hormones.

Conclusion

The isolate of microbial consortium of phyllosphere Fm48 as well as rhizosphere R15 having roles to fix N_2, to dissolve inorganic P and as a consortium produces plant growth hormones. The consortium of phyllosphere Fm48 improved rice growth significantly compared with their single culture, in contrast, there is no significant difference between rhizosphere R15 and their single culture on its positive impacts on rice growth. Therefore, phyllosphere microbes should be applied as a consortium, while rhizosphere microbes could be applied as a single culture.

Reference

Agustin Nuriyani Maira L, Emalinda O. 2010. Rhizobakteria penghasil fitohormon IAA pada rhizosfir tumbuhan semak karamunting, titonia dan tanaman pangan. Jurnal Solum **7(1),** 49-60.

Akbari Gh A, Arab SM, Alikhani HA, Allahdadi I, Arzanesh MH. 2007. Isolation and selection of indigenous Azospirillium spp. And IAA of superior strain on wheat roots. World journal of Agricultural Science **3(4),** 523-529.

Atashpaz S, Khani S, Barzegari A, Barar J, Vahed SZ, Azarbaijani R, Omidi Y. 2010. A robust universal method for extraction of genomic DNA from bacterial species. Microbiology **79(4),** 538-542. http://dx.doi.org/10.1134/S0026261710040168.

Egamberdieva D. 2008. Plant growth promoting properties of rhizobacteria isolated from wheat and pea grown in loamy sand soil. Turkish Journal Biology **32(1),** 9-15.

Gusmaini. 2005. Pemanfaatan konsorsium mikrob daun berasal dari tumbuhan ekosistem air hitam untuk memacu pertumbuhan vegetatif dan generatif padi. PhD thesis, Bogor Agricultural University, Bogor, 44-55.

Gusmaini Azis SA, Munif A, Sopandie D, Bermawie N. 2013. Potency of endophytic bacteria to increase the growth, biomass and andrographolide yields of the bitter king. Jurnal Penelitian Tanaman Industri **19(4),** 167-177.

Hidayati U. 2014. Potensi bakteri endofit asal tanaman karet sebagai pemacu pertumbuhan bibit batang bawah tanaman karet (*Hevea brasiliensis* Mull.Arg.). PhD thesis, Bogor Agricultural University, Bogor 12-13.

Lindquist JA. 1975. Bacteriological and ecological observation on the northem pitcher plant, *Sarracenia pupurea* L. University of Wisconsin-Madison Press, 36-40.

Matjik AA, Sumertajaya IM. 2006. Perancangan percobaan dengan aplikasi SAS dan MINITAB. Bogor : IPB Press, 275p.

Pati BR. 1992. Effect of spraying nitrogen fixing phyllospheric bacterial isolates on rice plants. Zentralblatt fur Mikrobiologie **147(7),** 441-446. http://dx.doi.org/10.1016/S0232-4393(11)80312-4.

Rao NSS. 1995. Soil microorganism and plant growth. 3rd. Edition. Science Publishers, Inc., New Hampshire, 100-121.

Redford AJ, Bowers RM, Knight R, Linhart Y, Fierer N. 2010. The ecology of the phyllosphere: geographic and phylogenetic variability in the distribution of bacteria on tree leave. Environmental Microbiology **12(11),** 2885-2893, http://dx.doi.org/10.1111/j.1462-2920.2010.02258.x.

Reisberg EE, Hildebrandt U, Riederer M,

Hentschel U. 2013. Distinct phyllosphere bacterial communities on arabidopsis wax mutant leaves. PLoS ONE **8(11),** 1-12. http://dx.doi.org/10.1371/journal.pone.0078613.

Santosa DA. 2001**.** Rapid extraction and purification of environmental DNA for moleculer cloning application and moleculer diversity studies. Molecular Biotechnologi **17(1),** 59-64. http://dx.doi.org/10.1385/MB:17:1:59.

Saraswati R, Husen E, Simanungkalit RDM. 2007. Metode analisis biologi tanah. balai besar litbang sumberdaya pertanian. Badan penelitian dan pengembangan pertanian. Jakarta : Departemen Pertanian, 23-55.

Sharma A, Sahgal M, Johri BN. 2003. Microbial communication in the rhizosphere: Operation of quorum sensing. Current Science **85(8),** 1164-1172.

Sunatmo TI. 2009**.** Mikrobiologi Esensial 1. Jakarta : Ardy Agency, 30-61.

Sutedjo MM, Kartasapoetra AG, Sastroatmodjo RDS. 1996. Mikrobiologi tanah. Edisi kedua Jakarta: Rineka Cipta, 1-68.

Unyayar S, Topcuoglu SF, Unyayar A. 1996. A modified method for extraction and identification of indole-3-acetic acid (IAA), gibberellic acid (GA3), abscicid acid (ABA), and zeatin produced by *Phanerochaete* and *Chrysosporium* ME446. Bulgarian Journal of Plant Physiology **22(3-4),** 105-110.

Vorholt JA. 2012**.** Microbial life in the phyllosphere. Nature Reviews Microbiology **10(12)**, 828-840. http://dx.doi.org/10.1038/nrmicro2910.