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Short Communication: Development of selected PGPR consortium to control *Ralstonia syzygii* subsp. *indonesiensis* and promote the growth of tomato

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Abstract. *Yanti Y, Warnita, Reflin. 2018. Short Communication: Development of selected PGPR consortium to control Ralstonia syzygii subsp. indonesiensis and promote the growth of tomato. Biodiversitas 19: xxxx.* A microbial consortium is a group of different species of microorganisms and acts as a community. Combinations of biocontrol strains are expected to have a better result to suppress multiple plant diseases. Our previous research had selected four plant growth promoting rhizobacteria (PGPR) strains from chili (*B. pseudomycoides* strain NBRC 101232*, B. cereus* strain CCM 2010, *Bacillus toyonensis* strain BCT-7112, *Serratia nematodiphila* strain DZ0503SBS1) and three strains from tomato (*Bacillus pseudomycoides* strain NBRC 101232, *Bacillus toyonensis* strain BCT-7112, *Bacillus thuringiensis* strain ATCC 10792) which had ability to promote growth and control *Ralstonia syzygii* subsp. *indonesiensis* indigenously. The strains were used in the development of PGPR consortiums to increase their ability for biocontrol agent of *Ralstonia syzigii* subsp. *indonesiensis* and promoting the growth of tomato. Results showed that not all strains had good compatibility to grow together. Ten consortiums were developed based on their compatibilities. All consortiums exhibited the capability to reduce bacterial wilt disease development and also promote the growth of tomato. The consortium consisted of *Serratia nematodiphila* strain DZ0503SBS1, *B. cereus* strain CCM 2010, *Bacillus aryabhattai* strain B8W22 and *Bacillus cereus* strain IAM 12605 resulted in the best ability to reduce disease development and promote growth and yield of tomato.

Keywords: *Bacillus*; interaction compatibility; PGPR consortium; *Serratia*

INTRODUCTION

Bacterial wilt disease caused by *Ralstonia syzigii* subsp. *indonesiensis* (former name *R. solanacearum* (Safni et al. 2014)) causes a considerable amount of damage to tomato and many other crops in tropical, subtropical and warm temperate regions of the world (Ji et al. 2005). The pathogen is widespread and one of the economically important bacterial plant pathogens (Horita and Tsuchiya 2001). Management of bacterial wilt of tomato was difficult due to the soil-borne nature of the pathogen. Several pesticides had been recommended to be used against this pathogen. However, they were not considered to be long-term solutions due to the concerns of expense, exposure risks, residues, toxicity to non-target organisms and other health and environmental hazards. Nowadays, the idea of controlling soil-borne plant pathogens with chemical pesticides had been shifted to biological control options that might play an important role in agriculture.

Recent studies had been focused on developing eco-friendly, safe, and effective agents for plant pathogens and disease control options (Sundaramoorthy et al. 2012), such as Plant Growth Promoting Rhizobacteria (PGPR). The used of biofertilizers containing PGPR strains might serve as a better alternative and more environmentally friendly practice to improve plant growth through the supply of plant nutrients and increase of soil productivities (Rajasekar and Elango 2011). PGPR had been well known as the crops biofertilizer (Karlidag et al. 2007), due to the fact that the PGPR strains may promote growth by fixation of atmospheric nitrogen, minerals solubilization (Karthikeyan et al. 2008), and plant growth regulators production (Jaleel et al. 2007). Most of the biocontrol agents used single biocontrol agent against a pathogen in controlling plant disease. This approach had partially reported for its inconsistent performance due to the use of single biocontrol agent is not likely to be active in all ecosystems it was applied or against all the host pathogens. Nowadays, more attention is being concerned with the use of mixed strains of PGPR (Raupach and Kloepper 1998; Nandakumar et al. 2001b; Canaday 2003). Direct interactions that occur among different microbial types often result in the promotion of key processes benefiting plant growth and health. Syntrophic relationships among different microorganisms have been demonstrated in several microbial ecosystems. Therefore, the combination of microorganism inoculants that interacts synergistically was currently being devised, which yield better and quick results (Bashan 1998).

Microbial consortium for plant growth promotion was suggested as a strategy to increase the activity and viability of the PGPR. Each of the constituent strains of the consortium not only outcompetes with the others for rhizospheric establishments, but complement functionally

for plant growth promotion (Shenoy and Kalagudi 2003). Our previous research had selected four PGPR strains from tomato (*B. pseudomycoides* strain NBRC 101232*, B. cereus* strain CCM 2010, *Bacillus toyonensis* strain BCT-7112, *Serratia nematodiphila* strain DZ0503SBS1), 3 strains from potato (*Bacillus toyonensis* strain BCT-7112, *Bacillus aryabhattai* strain B8W22, *Bacillus cereus* strain IAM 12605) and 3 strains from chili pepper (*Bacillus pseudomycoides* strain NBRC 101232, *Bacillus toyonensis* strain BCT-7112, *Bacillus thuringiensis* strain ATCC 10792). The strains had the ability to control *Ralstonia syzigii* subsp. *indonesiensis* indigenously and promote the growth of chili (Yanti et al. 2017). Developing suitable consortiums are necessary to increase the strains' ability to suppress pathogen attack and also promote growth. This present study purposed to develop consortiums of biocontrol agents to control *Ralstonia syzigii* subsp. *indonesiensis* and promote the growth of tomato.

MATERIALS AND METHODS

Bacteria strain preparation

All bacteria strains used in this study (Table 1) were separately re-cultured in Nutrient Agar (NA) media in petri-dish by streaking each of the strains to the agar and incubated for 72 h. The pure colony was then used for further study.

Compatibility assay

All strains were tested for their antagonistic for microbial consortium development following the method of Kumar et al. (2011). The strains were inoculated in Nutrient Broth (NB) and incubated on 110 rpm shaker for 24 h in room temperature (± 27ºC). Two colonies of two different strains were re-suspended with sterile water (10^7 CFU/mL) then sprayed to a petri-dish containing NA and incubated for 24 h. The presence of the inhibition zone was observed for each strain. Each treatment was replicated thrice. The PGPR consortia were developed based on the compatibility of the strains (Table 2).

Development of PGPR consortia

One pure colony of each strain was inoculated into 25 mL of NB in culture bottle (50 mL) and incubated in rotary shaker 110 rpm for 24 hours. Four mL of the culture then was transferred to 400 mL of sterile coconut water in Erlenmeyer flask for the main culture and incubated for 2x24 hours (Yanti et al. 2017). Suspension of rhizobacteria strains in the coconut water culture was diluted with a comparison to McFarland scale 8 (Density estimated 10^8 CFU/mL). The consortiums (Table 2) were made by mixing the strains with the same volume (50 mL/strain) and homogenized.

PGPR consortium assay on tomato

Tomato seeds were grown in seed tray for 21 days. The seeds were previously dipped in the PGPR consortium for 15 minutes and then sown in the seed tray containing soil and organic manure (2: 1 v/v). The 21-days old tomato seedlings were then planted in the polybag containing soil and organic manure (2: 1 v/v) with previously introduced with the same consortium suspensions by dipping the root in the suspension for 15 minutes. All treatments were replicated three times, with five plants for each unit. No PGPR consortium treatment was applied for control plants.

Pathogens innoculation

The *Ralstonia syzygii subsp. indonesiensis* was isolated from infected plants by dipping the stem in sterilized water, and the suspension then streaked in triphenyl tetrazolium (TZC) agar medium. The isolates were then assayed on two weeks old tomato plants to select the most virulence pathogens by injecting 1 mL of *Ralstonia* suspension (10^8 CFU/mL) to the tomato plant base stem. The most virulence bacteria (the fastest wilt disease development) was then re-cultured in TZC agar and used for plant disease infection. The pathogen was inoculated on the two weeks old plants by root wounding methods described by Yanti et al. (2018a). The roots were cut in 2 sides of the plants using scissors and poured with 10 mL (10^8 CFU/mL) of the *R. syzigii subsp. indonesiensis* suspensions.

Table 1. The strain used for consortium development

Strain	Code	Source of isolates *)
B. pseudomycoides strain NBRC 101232	C1	Chili plants
B. cereus strain CCM 2010	C2	
Bacillus toyonensis strain BCT-7112	C3	
Serratia nematodiphila strain DZ0503SBS1	C4	
Bacillus toyonensis strain BCT-7112	T1	Tomato plants
Bacillus aryabhattai strain B8W22	T2	
Bacillus cereus strain IAM 12605	T3	

Note: *) Yanti et al. (2017)

Table 2. PGPR consortia

Code	Strain
C1	*S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605
C2	*S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112
C3	*B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605
C4	*B. Pseudomycoides* strain NBRC 101232*, B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112, *B. toyonensis* strain BCT-7112
C5	*B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22
C6	*B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112
C7	*B. Pseudomycoides* strain NBRC 101232*, B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112,
C8	*B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22, *S. nematodiphila* strain DZ0503SBS1
C9	*B. toyonensis* strain BCT-7112, *S. nematodiphila* strain DZ0503SBS1
C10	*B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112

Data collection

Parameter observed in this research were disease development such as incubation time (observed when the first symptoms of bacterial wilt disease appear) and disease incidence (total of plant diseased until last observation days), and plant growth such as plant height, number of leaves, first flowering time and yields at the first harvest.

Data were analyzed by analysis of variance (ANOVA) at 0.05 probability level. The difference between two means was analyzed using Least Significance Difference (LSD) at 0.05 probability level. All analysis was performed using Statistics 8 Software. Effectivity of all treatments towards control also calculated using the formula of Sivan and Chet (1989).

RESULTS AND DISCUSSION

Compatibility among bacterial strains

All PGPR strains were tested for their compatibility. Some of the strains were incompatible showed by the inhibition zone indicating that the strains compete for each other (Table 3). The absence of inhibition zone suggesting that the biocontrol agents were compatible (Fig. 1). All the consortiums were further designed based on the compatibility of the strains.

Growth promoting activity of tomato introduced with PGPR consortiums

The tomato plant introduced with consortiums had better growth than the control plant, showed from the increase of plant height and leaves number (Table 4). All consortiums could promote tomato heights ranging from 26.95 to 105.19% compared to control (Fig. 2). Consortium C1 consisted of *S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605 was the best strains combination in increasing plant height and leave number.

Besides promoting the vegetative phase of the tomato plants, the consortia also promoted the generative stage of the plants indicated by the strain's ability in promoting flowering time and yields (Table 4). All consortia increased the yields differently. C1 (*S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605) was the best consortium to accelerate the flowering time and increase yields with an effectivity of 161.9% compared to control.

Table 3. Compatibility of PGPR strains

Strain	C1	C2	C3	C4	T1	T2	T3
C1							
C2	√						
C3	√	√					
C4	-	-	√				
T1	√	√	√	-			
T2	-	√	√	√	√		
T3	√	-	√	√	-	√	

*Note: √= compatible (no inhibition zone appear); - = incompatible (inhibition zone appear)

Table 4. Plant height and number of leaves of tomato introduced with PGPR consortia

Consortium	Height		Number of leaves	
	(cm)	Effectivity (%)	(leaves)	Effectivity (%)
C1	126.4a	105.19	54.4a	177.55
C2	115b	86.69	44.8b	128.57
C3	111.4b	80.84	450b	129.59
C4	99.8c	62.01	37.6c	91.84
C5	100c	62.34	37.2c	89.8
C6	86d	39.61	28.8d	46.94
C7	81.6de	32.47	24.8de	26.53
C8	82.2de	33.44	24.8de	26.53
C9	81.6de	32.47	22.8ef	16.33
C10	78.2e	26.95	22.8ef	16.33
Control	61.6f		19.6f	

Note: Values followed by the same letters in the same column differed insignificantly at 5% probability level according to LSD test

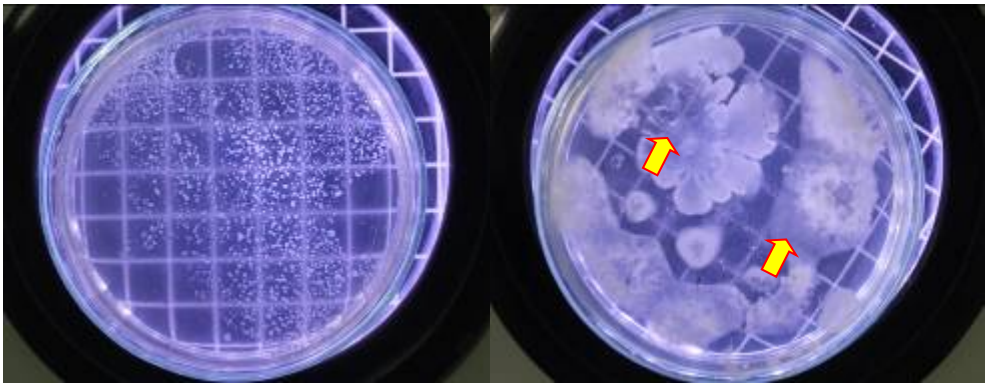

Figure 1. Rhizobacteria compatibility assay, compatible strains (no inhibition zone appear) of *B. pseudomycoides* strain NBRC 101232 and *B. cereus* strain CCM 2010 (*left*) and incompatible strains (inhibition zone appear) of *B. pseudomycoides* strain NBRC 101232 and *B. aryabhattai* strain B8W22 (*right*)

A B C D

Figure 2. Growth comparison of tomato plants introduced by PGPR consortia. A. C1, B. C2, C. C3, and D. control

Table 4. First flowering time and yields of tomato introduced with PGPR consortiums

Consortium	First flowering time (days after planting)	Effectivity (%)	Yields (g)	Effectivity (%)
C1	21.6f	50.46	260a	161.9
C2	25.2e	42.2	264a	157.94
C3	27.2de	37.61	239.6ab	137.7
C4	27.6de	36.7	215.6bc	113.89
C5	280d	35.78	198.0cd	96.43
C6	31.6c	27.52	186.0d	84.52
C7	41.6ab	4.59	152.2e	50.99
C8	41.2ab	5.5	152.2e	50.99
C9	40.4b	7.34	151.4e	50.2
C10	42.4ab	2.75	155.2e	53.97
Control	43.6a		100.8f	

Note: Values followed by the same letters in the same column differed unsignificantly at 5% probability level according to LSD test

Table 5. Disease development of *R. syzygii* subsp. *indonesiensis* on tomato introduced with PGPR consortium

Consortium	Incubation time (days after inoculation)	Disease incidence (%)	Effectivity (%)
C1	42.00*	0.00	100.00
C3	42.00*	0.00	100.00
C4	42.00*	0.00	100.00
C2	42.00*	0.00	100.00
C6	42.00*	0.00	100.00
C5	42.00*	0.00	100.00
C7	41.00	40.00	50.00
C10	41.20	40.00	50.00
C8	39.60	40.00	50.00
C9	37.60	60.00	25.00
Control	32.80	80.00	0.00

Note: *= no symptoms appear until the last day of observations

Figure 3. *Ralstonia syzigii* subsp. *indonesiensis* disease development on tomato treated with C1 consortium (*right*) and control (*left*)

Biocontrol activity against *R. syzigii* subsp. *indonesiensis* on tomato introduced with PGPR consortiums

Besides of having good abilities in promoting growth rate, the consortia also had good ability to suppress the infection of *R. syzygii* subsp. *indonesiensis* on tomato (Fig 3). Six out of ten consortia had the best ability in controlling the bacterial wilt disease, in which no symptoms developed on the plants until the last day of observations (Table 5). Those consortia were C1, C2, C4, C5, and C6.

Discussion

Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake, and support the health of plants (Adesemoye et al. 2009). Our present study had shown that compatible strains developed

as consortiums could increase plant growth and have biocontrol activity.

The combinations of PGPR strains consistently reduced the bacterial wilt disease of tomato under field conditions. The disease incidence varied from 0 to 60% (Table 5). No disease symptom was observed in the six consortia (C1, C2, C3, C4, C5, and C6) until the last day of observations. This showed a better result compared to the previous study that used single strain (Yanti et al. 2017). The results provide evidence that the compatibility of PGPR strains in the consortia effectively suppress disease development of *R. syzygii* subsp. *indonesiensis.* Combination of biocontrol agents is a strategic approach to control plant disease and pest (Nandakumar et al. 2001a; Latha et al. 2009). Furthermore, interactions among the bacterial strains may have synergistic effects that could induce systemically. These results could happen due to consortium may also improve their efficacy, reliability, and consistency under various soil and environmental conditions (Stockwell et al. 2010).

Our previous study showed that all the strain could inhibit disease development by *R. syzigii* subsp. I*ndonesiensis* bythe increase of defense-related enzyme activity such as Phenylalanine ammonia lyase, Polyphenol oxidase, and peroxidase produced by the strains (Yanti et al. 2017). The inhibition of various plant pathogen and disease management by using several biocontrol agents through the induction of systemic resistance (ISR) in plants had reported in several bibliographies (van Loon and Bakker 2005; Saravanakumar et al. 2007). Increased activity of ISR induced by consortiums may be due to the increased number of strains involved in the treatment and moreover due to the cooperation among the strains.

In this study, the PGPR consortia increased plant height and leaf number of tomato (Table 3). The growth performances were better compared to the previous study using the single strain. This may occur due to the synergistic ability of the strains. All the strains used in this study can also produce indole acetic acid and some other PGPR traits such as solubilize phosphate (Yanti et al., 2017) that could lead to the plant growth promoting activity. Earlier reports had shown that combined inoculation of *Azospirillum*, *Azotobacter chrococcum*, *Pseudomonas fluorescens* and *B. megaterium* for sorghum was significantly increased grain yield. The stimulatory effects of this PGPR strains on the yield and growth of the crops were attributed to the N2 fixation ability, plant growth regulator production and phosphate solubilizing capacity (Cakmakci et al. 2007; Kevinvessey 2003; Karlidag et al. 2007; Salantur et al. 2006).

The consortiums could accelerate flowering and increase yields of tomato (Table 4). This effect may relate to the growth increase of the tomato. The yields also higher compared to the single strain inoculant. The beneficial effect of seed inoculation with bacterial consortia on the shoot, dry weight, and yield of maize was also reported by Shaharoona et al. (2006). Son et al. (2006) also found that combination treatment of rhizobacteria strains of *Bradyrhizobium japonicum* and *Pseudomonas* spp. could increase yield of soybean.

In conclusions, the application of PGPR consortiums found to be effective in controlling the bacterial wilt disease caused by *R. syzygii* subsp. *indonesiensis*. The present study clearly indicated that the combination of biocontrol agents showed the maximum effects on reduction of *R. syzygii* subsp. *indonesiensis* disease development, compared to individual agents used as the previous study suggesting the synergistic effect of consortiums against the pathogen. The PGPR combinations of C1 (*S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605), C2 (*S. nematodiphila* strain DZ0503SBS1, *B. toyonensis* strain BCT-7112), C3 (*B. aryabhattai* strain B8W22, *B. cereus* strain IAM 12605), C4 (*B. Pseudomycoides* strain NBRC 101232*, B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112, *B. toyonensis* strain BCT-7112), C5 (*B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112, *B. toyonensis* strain BCT-7112, *B. aryabhattai* strain B8W22) and C6 **(***B. cereus* strain CCM 2010, *B. toyonensis* strain BCT-7112) were the promising consortia for the management of wilt disease and enhance the growth of tomato plants.

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