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Efficacy of Consortium Bacteria for Control Rice Diseases under System of Rice Intensification (SRI) in West Java-Indonesia

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Abstract

The objective of this work was aimed to determine the efficacy of consortium bacteria containing a mixture of bacterial antagonist for controlling major rice diseases under System of Rice Intensification practices. The experiment consist of three consortium bacteria *viz.* C 1 (**Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia* sp E 76, **B. cereus* C 29d, **B. licheniformis* CPKPP 35, **Bacillus* sp H, **Bacillus* sp IR), C 2 (**Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia* sp E 76, * *B. cereus* C 29d, * *B. licheniformis* CPKPP 35, **Azospirillum* sp Aj. 5252) and C 3 (**Bacillus* sp H, **Bacillus* sp IR). All consortiums were treated three times by spraying rice canopy leaves with consortium bacteria suspensions at 14 days, 28 days and 42 days after transplanting. The candidate's C 1 could reduced the bacterial blight and red stripe diseases severity when compared with control treatment (untreated plots), with the efficacy control less than chemical control, although not effective against sheath blight disease. The yield increase obtained by C 2, C 3 and C 4 applications were ranging from 8.7% to 12.2%.

Key words: efficacy, disease management, bacterial antagonist, rice

1. Introduction

Since 2000, System of Rice Intensification (SRI) has been practiced to increase yield in several countries, including Indonesia [1, 2]. One of the issues on SRI practices was microbial activity which influenced by its persistency as well as its effectivity under intermittent irrigated system. Rice productivity has been frequently limited in terms of quantity and quality due to rice pest and diseases complex attack such as bacterial blight (*Xanthomonas oryzae* pv. *oryzae*), sheath blight (*Rhizoctonia solani*), and red stripe (*Mycobacterium* spp) were considered as the three potential diseases throughout the planting seasons [3, 4].

To date the control of the diseases has been depended upon the applications of cultural practices, varietals resistance, agrochemicals as well as biological control. Bacterial agents have been an alternative use to control rice plant pathogens, However, the use of bacterial antagonist often fail to control diseases in the field and may not effective due to lower persistent performance in reduction of plant pathogens. This phenomenon may attribute to inappropriate use of bacterial strains virulence, mode of actions as well as type of applications [5].

Generally, a single biocontrol agents widely used as biocontrol of plant disease caused by a single pathogen, however; the effectiveness sometimes showed inconsistent results. In contrast, mixture of biocontrol agents differing in colonization pattern and taxonomically different organism may potential for biocontrol through different disease suppression mechanisms [3, 6].

In previous the experiment, we developed a selection procedure to screen several soil and endophytic bacteria against major rice diseases and further developed consortium bacteria containing potential bacterial antagonist. By this method we selected bacterial antagonistic strains that can suppresses complex rice diseases such as bacterial blight and sheath blight (ShB); hence the purpose of the study is to evaluate the efficacy of potentially antagonistic consortium bacteria against major rice diseases under field condition performing SRI practices.

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2. Material and Methods

Preparation of bacterial consortium inoculums

The soil and endophytes antagonistic strains of bacteria used in this study were collected from various agro ecological zones (Table 1). All bacterial isolates used in consortium development were maintained on Nutrient broth (NB) and King's B (KB) media. The inoculums preparation was prepared by culturing each isolates on agar media for 48 h at room temperature. Ten mL of bacterial suspension contained about 10^8 CFU/mL were transferred onto 1000 mL flask containing NB media and adjusted with constant shaking (125 rpm) using rotary shaker (Stuart Scientific SI 50) for 48 h at room temperature.

Table-1. List of bacterial isolates used in the study

Bacterial isolates	Host/Source origin	Date of collection
Bacillus sp E 64***	Rice, Sukabumi W. Java	2004
B. firmus E 65***	Rice, Sukabumi W. Java	2004
Bacillus sp E 76***	Rice, Sukabumi W. Java	2004
Bacillus cereus C 29D***	Soil, Kenjeran Surabaya, E. Java	2007
Pseudomonas aeruginosa CPKKP 35***	Soil, Pantai kukup, Yogyakarta	2007
Bacilus sp IR**	Rice, Sukamandi, W. Java	2008
Bacillus sp H**	Rice, Sukamandi, W. Java	2008
Azospirillum sp, Aj 5252***	Rice, Bandung W. Java	2009
Paenibacillus sp*	Rice, Jatisari, W. Java	2007

Notes: * Isolates was supplied by Pest and Disease Surveillance Agency, Directorate Plant Protection Jatisari (BW); ** Isolates provided from ICRR (TSK); *** Isolates from ICABIOGRAD culture collection (DNS).

2.1 Treatment

The field experiment was carried out in rice disease endemic area at Pusakanagara Expt. Station-West Java during 2009/2010 dry season (DS) using a completely randomized block design (RCBD) consist of consortium bacteria treatments with different practices, and controls (Table 2). The treatment was replicated four times. Rice cv. "Sintanur" was germinated in the rice seedbeds, then the 10 d-old seedling plant were transplanted into 10 x 22 m^2 rice plots with 30 cm x 30 cm plant spacing. All treatments were applied in System of Rice Intensification (SRI) practices following application of different biological agents (consortium bacteria); Integrated crop management (ICM) practices; Semi-SRI (treated using local microorganisms obtained from plant debris), chemical check, and untreated plot based on SRI farmer practices (without consortium).

Table-2. Treatment of bacterial isolates selected in forming antagonistic consortium bacteria to control rice diseases

Treatment	remarks
C 1-SRI	**Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia* sp E 76, **B.cereus* C 29d, **B. licheniformis* CPKPP 35, **Bacillus* H, **Bacillus* IR
C 2-SRI	* *Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia sp* E 76, **B. cereus* C 29d, * *Azospirillum* sp Aj 5252
C 3-SRI	**Bacillus* H, **Bacillus* IR
C 4-SRI	**Paenibacillus* sp
Semi-SRI	treated using local microorganisms obtained from plant debris (without consortium bacteria)
ICM practices-SRI	use of inorganic fertilizer and chemical when necessary
Chemical control-SRI	Streptomycin sulphate (3 cc/L)
Untreated control-SRI	control without consortium bacteria (farmer check)

The inoculums suspensions (10^8 ^{CFU}/mL) were sprayed using knapsack sprayer in the field. Streptomycin sulphate was used as standard chemical control. Two weeks-old seedlings of the rice field plots were sprayed (3 cc/L) according to each treatment following candidate of consortium bacteria. Further spraying using final concentration of consortium bacteria adjusted to 10^{7-8} CFU/mL was carried out at 28 and 42 days after planting (DAP), respectively.

Observation and statistical data analysis

Disease assessment was based on natural infection. Plants were recorded by scoring disease severity on the scale basis using standard evaluation system (SES) for rice [7]. Plants were observed at 2 weeks before harvesting time by recording disease severity, and yield per plot. Diseases severities were calculated using the formula:

DS = 100% x ∑ (n x v)/ N x V, where DS = disease severity, n = number of leaves infected, v = value score of each category infection, N = number of leaves observed, V = value of the highest score of infection.

All data were statistically analyses by analysis of variance (ANOVA), and the means significant differences were calculated based on Duncan Multiple Range test (DMRT) at P=0.05.

3. Results and Discussion

The rice cultivation under SRI practices in the field condition at Pusakanagara Expt. Station-W. Java, used excess of cow-manuring as organic soil amendment which may induced chemical and physical changes of soil and affect soil microflora. Nevertheless, visual performance of plant responses against field spray applications by consortium bacteria in SRI practices were not much differed as compared with semi-SRI practices using local microrganisms (Figure 1).

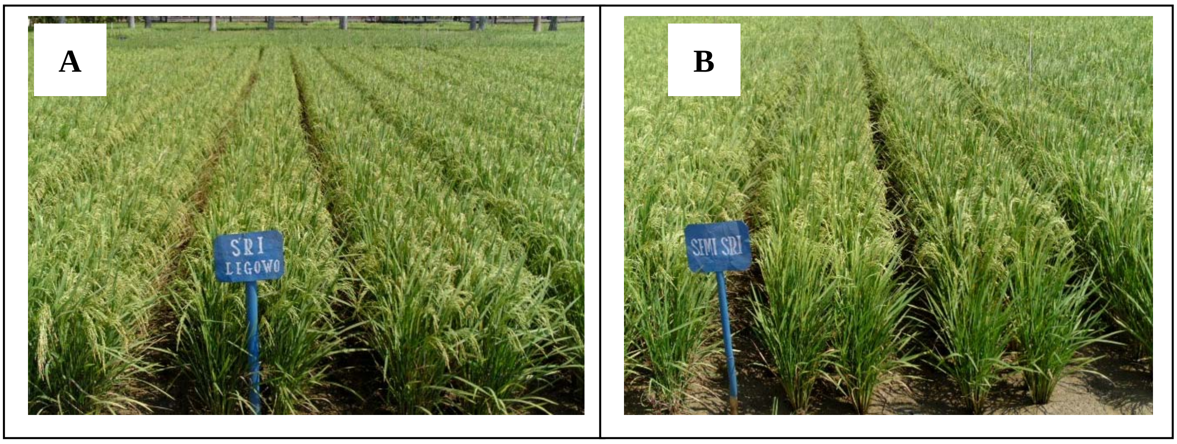

Figure 1. Performance of plant response against field spray application of bacterial consortium on rice cv. Sintanur under SRI practices. A= SRI treated using formulated bacterial consortium; B= semi –SRI practice treated using local microorganisms**.**

Table 3. Efficacy of consortium bacteria against major rice diseases.

Treatment	Means of disease severity (%)					
	Bacterial blight	DR	*Sheath blight*	DR	*Red stripe*	DR
C 1-SRI	9.33 ^{a}	33.16	7.67 ^{a}	-	2.33 ^{e}	93.16
C 2-SRI	10.67 ^{a}	23.78	5.33 ^{ab}	30.51	4.00 ^{de}	83.10
C 3-SRI	9.00 ^{a}	35.71	5.00 ^{ab}	34.81	8.00 ^{cde}	66.20
C 4-SRI	8.33 ^{a}	40.50	4.33 ^{ab}	43.54	8.33 ^{cde}	64.81
Semi SRI	11.33 ^{ab}	19.07	6.18 ^{ab}	19.42	20.67 ^{ab}	12.67
ICM practices-SRI	9.33 ^{a}	33.36	3.0 ^{b}	60.89	15.33 ^{abc}	35.23
Streptomycin sulphate (chemical check)-SRI	9.33 ^{a}	33.36	5.67 ^{ab}	26.07	13.33 ^{bcd}	43.68
Untreated control (farmer check)-SRI	14.00 ^{b}	-	7.67 ^{a}	-	23.67 ^{a}	-

Note: Means of four replications. Means followed by the same letter are not significantly different by DMRT (P=0.05). DR (disease reduction) = (untreated control – treated)/untreated control x 100%. SRI= System of Rice Intensification, ICM= Integrated Crop Management.

In the previous study it was reported that cultural practices using intermittent condition using 7 days irrigated interval at vegetative until generative stage of rice could reduce infection of fungal soil-borne pathogens such as ShB and stem rot diseases; however limited data on its effect on foliar pathogens still unavailable since no other combined control treatment applied to the rice plant. Based on final observation at generative stage, several major rice disease occurred in the rice field *viz.* bacterial blight, ShB and red stripe with the average disease severity ranging from 8.33% - 14.00%, 3.0% - 7.67% and 2.33% - 23.67%, respectively (Table 3).

Biological control was considered as an alternative method to control plant diseases and pests [8]. Generally single use of consortium bacteria with 4 to 6 times foliar spraying showed lower control efficacy than that of chemical sprays [9]. In this trial, the suspension-type formulation of consortium bacteria was developed using three times foliar spraying applications (14, 28 and 42 DAP) that exhibited antibacterial or antifungal activity against rice diseases.

It was suggested that the use of consortium bacteria containing isolates of *Bacillus* sp, *B. firmus, B. licheniformis, Azospirillum* sp and *Paenibacillus* sp in this study could suppress rice diseases. Inline with the present study, it was reported that under field spray condition the use of *B. subtilis* could reduce incidence of bean rust (*Uromyces appendiculatus*) up to 74% [5]. In addition, used of *B. subtilis* could also inhibit the growth of *Sclerotium rolfsii* fungus up to 13.05% [10]. The mechanism reduction of consortium containing *Bacillus* isolates may influenced by its ability to inhibit mycelia growth as well as production of sclerotia of the sheath blight pathogen.

The potentially antagonistic consortia used in this study viz. C 1 (containing bacterial isolates **Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia* sp E 76, **B. cereus* C 29d, **B. licheniformis* CPKPP 35, **Bacillus* sp H, **Bacillus* sp IR) was isolated from various source of environment. Efficacy of three times application using consortia C 1 showed good effect in reducing bacterial blight and red stripe disease severity which produced the lowest severity of 8.33% and 2.33%, respectively; however; this treatment had lower efficacy on ShB disease severity. Red stripe of rice was first reported in Indonesia in 1988. The widespread of the disease was also reported in Vietnam, Philippines and Malaysia. The symptom was initiated with water soaked then the lesion turned yellow to orange [4].

Two candidate consortium bacteria C 3 and C 4 containing *Bacillus* sp and *Paenibacillus* sp produced less sheath blight disease severity. Streptomycin sulphate as standard chemical control used in this study was less effective in suppressing rice foliar diseases severity. Disease reduction (DR) or control efficacy of chemical control was ranging from 26.07% to 43.68%, whilst protective application of consortium bacteria was ranging from 23.78% to 93.16% (Table 3). This indicated that the consortium bacteria more likely in a protective mode. Semi SRI treatment showed fewer efficacies than that of ICM practices.

Table 4. Efficacy of bacterial consortium on rice yield

Treatment	Grain weight/hills (g)	Yield (t/ha)
C 1-SRI	60.83 [a]	5.8 [a]
C 2-SRI	67.02 [b]	6.4 [b]
C 3-SRI	62.87 [a]	6.4 [b]
C 4-SRI	59.83 [a]	6.2 [a]
Semi SRI	60.50 [a]	6.0 [a]
ICM	60.80 [a]	5.9 [a]
Streptomycin sulphate	65.73 [b]	7.1 [c]
Untreated control (farmer check)	59.20 [a]	5.7 [a]

Note: Means of four replications. Means followed by the same letter are not significantly different by DMRT (P=0.05).

Based on data presented in Table 4, it was shown that consortium bacteria applications were significantly affected grain weight/hills and yield. Thakur *et al,* [11] explained that the main factors responsible for the yield enhancement in SRI management were better grain filling and a significant increase in grain weight.

The present study indicates that use of consortium bacteria tends to improve rice yield ranging from 8.7% to 12.2% compared with that of untreated plot (control treatment without consortium). The efficacy of consortium bacteria C 2, C 3 and C 4 increased yield of rice as compared with the untreated plot. This result may have been due to indirect effect of antagonism as well as competitions with pathogens for essential nutrients. To obtain more stable efficacy to suppress other major rice diseases, similar experiment need to be done at different rice agro ecological zone.

4. Conclusions

When compared with control treatment (untreated plot without consortium bacteria), the candidate C 1 (**Bacillus* sp E 64, * *B. firmus* E 65, * *Burkholderia* sp E 76, * *B. cereus* C 29d, **B. licheniformis* CPKPP 35, **Bacillus* H, **Bacillus* IR) and consortium C 2 (* *Bacillus* sp E 64, **B. firmus* E 65, **Burkholderia sp* E 76, **B. cereus* C 29d, * *Azospirillum* sp Aj 5252) could reduce the severity of red stripe disease under SRI practices. Rice plant treated with consortium bacteria C 3 (**Bacillus* H, **Bacillus* IR) and C 4 (**Paenibacillus* sp) showed less sheath blight disease compared with control treatment. Efficacy of the consortium was significantly increased rice yield ranging from 8.7% to 12.2%.

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6. References

1. Gani A, Kadir TS, Jatiharti A, Wardhana IP, Las I: **The System of Rice Intensification in Indonesia.** *Proceedings Assessments of the System of Rice Intensification*, 2002, 58-63.
2. Uphoff N: **Higher yields with fewer external inputs? The system of rice intensification and potential contributions to agricultural sustainability**. *International Journal of Agricultural Sustainability* 2003, **1:**38–50.
3. 3.Gnanamanickam SS: **Biological control of rice diseases***:* Springer, New York; 2009.
4. Mew TW, Castilla NP, Elazegui FA, Vera Cruz CM: **The etiology of red stripe of rice: current status and future directions.** *IRRN* April 2001, p: 4-10.
5. Cook RJ, Baker KF: **The nature and practice of biological control of plant pathogens**: APS. Press. St. Paul, Minnesota; 1983.
6. Wilson M, Backman PA: **Biocontrol of plant pathogens**. In: Handbook of pest management: Marcel-Dekker Inc; 1999: 309-335.
7. IRRI: **Standard evaluation system for rice**: IRRI, Manila, the Philippines; 1996.
8. 8.Souto GI, Correa OS, Montechia MS, Kerber NL, Pucheu NL, Bachur M, Garcia AF: **Genetic and functional characterization of *Bacillus* sp strain excreting surfactin and antifungal metabolites partially identified as iturin-like compounds**. *Journal Applied Microbiology* 2004, **97**:1247-1256.
9. Kim GH, Lim MT, Hur JS, Yun KJ, Koh YJ: **Biological control of tea anthracnose using an antagonistic bacterium *B. subtilis* isolated from tea leaves**. *Plant Pathology Journal* 2009, **25**(1):99-102.
10. Nalisha I, Muskhazli M, Nor Farizan T: **Production of bioactive compounds by *B. subtilis* against *S. rolfsii.*** *Malaysian Journal of Microbiology* 2006, **2**(2):19-23.
11. Thakur AK, Uphoff N, Anthony E: **An assessment of physiological effects of system of rice intensification (SRI) practices compared with recommended rice cultivation practices in India.** *Experimental. Agriculture* 2010, **46** (1): 77-98.