

Efficacy of Rhizobacteria to Promote the Growth and Increase the Yield of Several Rice Cultivars

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Abstract

Rice is still play important role as staple food for most of Indonesian population; consequently Indonesia is greatly relying on rice production to maintain food security. This plant is commonly cultivated with intensive use of synthetic agrochemicals such as synthetic chemical fertilizers and pesticides. However, the improper use of agrochemicals may result in adverse effects to the environment including biotic and abiotic components. This study was conducted to evaluate the efficacy of rhizobacteria formulation to promote the growth and increase the yield of four rice cultivars that commonly grown in Indonesia *i.e.* Ciherang, Cigeulis, Inpari 33, and IR64. Experiment was done in a green house with three concentrations of rhizobacteria formulation *i.e.* 0%, 1%, and 2% (v/v). Several parameters were observed in this study such as seedling height, leaf chlorophyll content of seedling, plant height at 50 days after transplanting (DAT), leaf chlorophyll content at 50 DAT, number of tillers per plant at 50 DAT, number of productive tillers per plant, and yield per plant. Results showed that significant interactions were observed between rice cultivar and concentration of rhizobacteria formulation in all parameters observed. Rice cultivar IR64 consistently showed superior growth and yield among four rice cultivars tested. Except for cultivar IR64, rhizobacteria concentration at 2% showed superior results compared to 1% and 0% for all parameters observed. This study proved that rhizobacteria formulation containing *Stenotrophomonas maltophilia* Sg3 and *Enterobacter cloacae* A17K1a effectively promoted the growth and increase the yield of four rice cultivars that commonly cultivated in Indonesia, suggested that this rhizobacteria formulation is promising bio-agent to be used for rice production in Indonesia.

Keywords: Rhizobacteria, plant growth promotion, rice yield

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1. Introduction

Plant production is relying on the use of synthetic chemical fertilizers to provide enough macro nutrients such as N, P, and K. These macro nutrients are also very important in rice production in Indonesia. The use of macro nutrients in particular N is not entirely absorbed by the rice plant due to the poor root system. Consequently, part of N will remain in the soil; some lost because of evaporation, leaching through water runoff and potentially pollute water bodies in rivers as well as lakes. In addition, excessive use of N results in the plant growth become weak and susceptible to several plant pathogens such as blast disease caused by *Magnaporthe grisea* Barr (anamorf *Pyricularia grisea* Sacc., synonym *Pyricularia oryzae* Cav.) (Kato, 2001). Based on this reason, it is necessary to find bio-agents in particular from plant rhizospheres that posse's ability to enhance the formation of root hairs and improve the root system. Hence will increase the efficiency of absorption of nutrients.

Rice productivity in Indonesia was about 5.14 ton/ha (BPS, 2012) which is relatively lower than other countries such Australia 8.22 ton/ha, Japan 5.85/ton/ha and China 6.06 ton/ha (USDA, 2004). They gave of rice productivity in Indonesia with other rice growing countries suggested that there is a chance to increase the rice productivity through the use of rhizobacteria from rhizospheres of plants.

Several rhizobacteria have been known to have plant growth promotion potential such as *Pseudomonas*, *Enterobacter*, *Azospirillum*, *Azotobacter*, *Burkholderia*, *Bacillus* and *Serratia* (Alam *et al.*, 2001; Vidyasekaran *et al.*, 1997; Khalimi *et al.*, 2012). Seed treatment with *Pseudomonas fluorescens* strain Pfl obviously increased the rice yield (Vidhyasekaran *et al.*, 1997). Rice plant inoculated with *Rhizobium* sp. IRBG74 increased the rice yield by 11,6% (Biswas *et al.*, 2000), while Alam *et al.* (2001) found that treatment by soaking the root of rice seedling for 24 h with the suspensions of *Azotobacter nigricans*, *A.armeniacus*, *Bacillus sphaericus*, *B. megaterium*, *Enterobacter* and *Xhantobacter* increased the rice yield by 15,03%. Other study done by Khalimi and Suprpta (2012) showed that the treatment with *Pantoea agglomerans* isolates PaJ and BS2a isolated from the rhizosphere of groundnut increased the yield of rice cultivar Cicih Medang Selem (a local rice variety of Bali) by 154.17% when compared to control.

As rhizobacteria may have specificity in their host plant, in this study a formulation of rhizobacteria containing *Stenotrophomonas maltophilia* Sg3 and *Enterobacter cloacae* A17K1a was tested for its potential to promote the growth and increased the yield of 4 (four) rice cultivars that commonly cultivated in Indonesia. The aim of this study is to know variation of responses among 4 (four) rice cultivars against rhizobacteria treatment.

2. Materials and Method

Isolates of Rhizobacteria

Two isolates of rhizobacteria used in this study are *Stenotrophomonas maltophilia* Sg3 isolated from rhizosphere of groundnut (*Arachis hypogea*) and *Enterobacter cloacae* Al7K1a was isolated from rhizosphere of *Imperata cylindrica*. Both of these isolates are maintained in the Laboratory of Biopesticide Faculty of Agriculture Udayana University.

Rice Cultivars

Four rice cultivars that commonly cultivated by the farmers in Indonesia were tested in this study namely: Ciherang, Cigeulis, Inpari 33, and IR64. The seeds were obtained from agricultural shop in Denpasar Bali.

Formulation Preparation

Formulation of rhizobacteria was developed with the following ingredients:

Na₂HPO₄·12 H₂O (0.3 g), KH₂PO₄ (0.1 g), potato broth (200 ml), bacto peptone (1 g), yeast extract (1 g), dextrose (10 g), NH₂CONH₂ (0.1 g), P₂O₅ (0.5 g), K₂O (0.5 g), MgSO₄ (0.1 g), leaf extract of aloe vera (100 ml), Tween-80 (20 ml), suspension of *Stenotrophomonas maltophilia* Sg3 (10 ml, 10⁷ CFU/ml), and *Enterobacter cloacae* Al7K1a (10 ml, 10⁷ CFU/ml) and water to make one liter. All ingredients except bacterial suspension were sterilized in an autoclave for 20 minutes at 121°C and keep under room temperature for about 6 h and inoculated with bacterial suspension in a laminar air flow cabinet. This formulation is ready for use.

Treatment with Rizobacteria Formulation

Seeds of five rice cultivars were soaked in sterile distilled water for 24 h, and drained up and kept for 24 h in a plastic tray under room temperature in the dark for germination. Germinated seeds were then soaked in a formulation at concentration of 2% (v/v) for 30 minutes while for control were soaked in sterile distilled water. The treated seeds were then sown on plastic trays filled with sterile fertile soil and maintained in a green house. The rice seedlings were transplanted 15 days after sowing.

Transplanting

A factorial experiment with randomized block design were applied in this study consisting of two factors, i.e. factor rice cultivars consisting of 4 levels (Ciherang, Cigeulis, Inpari 33, and IR64) and factor concentrations of rhizobacterial formulation consisting of 3 levels (0%, 1%, and 2%). From these two factors, there are 12 combined treatments were tested in this study. Each treatment was replicated three times, thus there are 36 experiment units were prepared. Each experiment unit consisting of 10 pots of rice plants. Plastic pots with diameter 30 cm filled with fertile soil were used to grow the rice plant. The rice seedlings were pull out and then the roots were dipped for several seconds in a formulation at concentration 0%, 1%, and 2% after which two seedlings each were transplanted into plastic pots filled with fertile soil. All plants were grown in a green house until ready for harvest.

Parameters Observed

Plant growth parameters such as plant height, number of tillers, leaf chlorophyll content were measured every two weeks after transplanting, while yield components such as number of productive tillers per hill, length of panicle, number of grain per panicle, and weight of grain per hill were determined after harvest.

Data Analysis

All data were subjected to the analysis of variance (ANOVA) and continued by Duncan's multiple range test (DMRT) at 5% level.

3. Results and Discussion

Growth of Rice Seedlings

The growth of rice seedlings of 5 rice cultivars at 18 days after sowing significantly ($p < 0.05$) promoted by treatment with rhizobacteria formulation indicated by the plant height and leaf chlorophyll content as presented in Tables 1 and 2. Treatment at concentration of 1% and 2% resulted in higher plant height as well as chlorophyll content for all 5 rice cultivar tested. Interaction between rice cultivars and concentration of rhizobacteria concentration was also significant ($p < 0.05$) suggested that both rice cultivars and concentration of rhizobacteria affected the growth of rice seedling. The highest plant height was obtained from rice cultivar Cigeulis treated with 2% rhizobacteria concentration (Table 1), while for the highest leaf chlorophyll content was obtained from rice cultivar IR64 (Table 2).

Table 1. Plant height (in cm) of seedlings on four rice cultivars treated with rhizobacteria formulation at 18 d after sowing

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	19.22 d*	22.67 c	23.57 c
2	Cigeulis	22.86 c	29.24 a	29.28 a
3	Inpari 33	19.85 d	23.38 c	23.86 c
4	IR64	19.48 d	27.27 b	28.54 ab

*Values followed by the same letters are not significantly different according to Duncan's Multiple range tests at 5% level.

Table 2. Leaf chlorophyll content (SPAD unit) of seedlings on four rice cultivars treated with rhizobacteria formulation at 18 d after sowing

No	Rice cultivar	Concentration of rhizobacteria formulation		
		0	1	2
1	Ciherang	13.62 f	24.67 d	24.71 d
2	Cigeulis	13.76 f	25.79 cd	26.34 cd
3	Inpari 33	14.29 f	27.45 bc	28.31 b
4	IR64	17.99 e	29.18 ab	30.72 a

*Values followed by the same letters are not significantly different according to Duncan's Multiple range tests at 5% level.

Plant Height, Chlorophyll Content and Number of Tillers

The growth of 5 rice cultivars significantly ($p < 0.05$) affected by the treatment with rhizobacteria formulation indicated by the plant height, leaf chlorophyll content, and number of tiller measured at 50 days after transplanting (DAT) as presented in Tables 3, 4, and 5. Interaction between rice cultivars and concentration of rhizobacteria formulation was also significant ($p < 0.05$) for all three parameters observed suggested that both rice cultivars and concentration of rhizobacteria formulation affected plant height, leaf chlorophyll content, and number of tiller per hill measured at 50 DAT. In term of plant height, rice cultivar IR64 treated with rhizobacteria concentration at 2% showed the highest value (90.57 cm), while rice cultivar Ciherang treated with 0% rhizobacteria concentration showed the lowest value (70.12 cm) as presented in Table 3. Similar results were also observed on parameters leaf chlorophyll content and number of tiller per hill 50 DAT as shown in Tables 4 and 5.

Table 3. Plant height (in cm) of four rice cultivars treated with rhizobacteria formulation at 50 Days after transplanting

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	70.12 h	75.96 g	78.98 f
2	Cigeulis	85.54 cd	87.59 bc	89.34 ab
3	Inpari 33	81.23 ef	85.41 cd	87.27 bc
4	IR64	83.58 de	89.32 ab	90.57 a

*Values followed by the same letters are not significantly different according to Duncan's Multiple range test at 5% level.

Table 4. Leaf chlorophyll content (SPAD unit) on four rice cultivars treated with rhizobacteria formulation at 50 days after transplanting

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	37.78 e	42.13 cd	44.74 a
2	Cigeulis	41.23 d	43.05 abcd	43.49 abc
3	Inpari 33	41.58 cd	42.47 bcd	44.17 ab
4	IR64	41.56 cd	44.22 a	44.49 ab

*Values followed by the same letters are not significantly different according to Duncan's Multiple range tests at 5% level.

Table 5. Number of tillers per plant of four rice cultivars treated with rhizobacteria formulation At 50 days after transplanting

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	40.07 e	44.56 d	45.62 d
2	Cigeulis	48.19 c	60.03 b	62.18 ab
3	Inpari 33	45.24 d	47.40 c	50.15 c
4	IR64	60.57 b	64.26 a	64.46 a

*Values followed by the same letters are not significantly different according to Duncan's Multiple range tests at 5% level.

Number of Productive Tillers per Plant

Analysis of variance showed that number of productive tillers per plant of four rice cultivars treated with rhizobacteria formulation at different concentration significantly different ($p < 0.05$) among treatments. Interaction between rice cultivars and concentration of rhizobacteria formulation showed significant ($p < 0.05$) effect to the number of productive tillers per hill. The highest number of tillers per hill (59.78) shown by rice cultivar IR64 treated with rhizobacteria formulation at concentration 2%, while the lowest one was shown by rice cultivar Ciherang treated with 0% rhizobacteria formulation (33.43) as presented in Table 6.

Table 6. Number of productive tillers per plant of four rice cultivars treated with rhizobacteria formulation 98 days after transplanting

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	33.43 g	37.60 f	38.13 f
2	Cigeulis	50.69 c	52.69 bc	54.74 b
3	Inpari 33	42.14 e	47.20 d	50.32 c
4	IR64	46.64 d	57.50 a	59.78 a

*Values followed by the same letters are not significantly different according to Duncan's Multiple range test at 5% level.

Yield per Plant

Based on the analysis of variance, a significant interaction ($p < 0.05$) was observed between rice cultivars and concentration of rhizobacteria formulation regarding their effect to the yield of rice per plant. The highest yield per plant was obtained from cultivar IR64 treated with rhizobacteria formulation at concentration of 2%. Weight of grains for this treatment was 139.55 g/plant or equal to 15.5 ton/ha. The lowest yield was observed on cultivar Ciherang with only 55.38 g/plant or equal to 6.1 ton/ha. Interesting result obtained in this study that treatment with rhizobacteria formulation at concentration 1% was not significantly different ($p > 0.05$) with that of concentration 2% in all parameters observed for rice cultivar IR64, while for other rice cultivars treatment with concentration 1% was significantly different ($p < 0.05$) with concentration 2% for most of parameters observed. This result suggested that for rice cultivar IR64 treatment with rhizobacteria formulation at concentration 1% is enough to promote the growth and increase the yield per plant, while for other cultivars *i.e.* Ciherang, Cigeulis, and Inpari 33, concentration 2% is necessary to obtain a maximum both growth promotion and yield per plant.

Treatment with rhizobacteria formulation increased the yield of four rice cultivars between 13,48% (obtained from rice cultivar Cigeulis treated with 1% rhizobacteria formulation) to 43.00% (obtained from rice cultivar Ciherang treated with 2% rhizobacteria formulation) when compared to those treated with 0% rhizobacteria formulation. These results proved that rhizobacteria formulation effectively increased the yield of four rice cultivars tested in this study.

Table 7. Effects of rhizobacteria formulation to the weight of grain per hill (gram) measured at harvest (98 days after transplanting) on four rice cultivars

No	Rice cultivar	Concentration of rhizobacteria formulation (%)		
		0	1	2
1	Ciherang	55.38 h	72.67 g	79.27 f
2	Cigeulis	101.58 e	115.27 c	121.85 b
3	Inpari 33	80.15 f	105,07 d	112.62 c
4	IR64	103.81 de	137.46 a	139.55 a

*Values followed by the same letters are not significantly different according to Duncan's Multiple range tests at 5% level.

Present study proved that formulation containing rhizobacteria significantly ($p < 0.05$) promoted the growth of four rice cultivars and increased the yield. The plant height, leaf chlorophyll content, number of tillers per

plant, number of productive tiller per plant and yield per plant consistently higher on four rice cultivars treated with rhizobacteria formulation (1% or 2%) when compared to treatment with 0% formulation. Several studies indicated similar results with our study. Study done by Murphy *et al.* (2000) showed that treatment of tomato plant with rhizobacteria obviously promoted the plant growth. The growth of plant was increased because rhizobacteria produced growth hormones such as auxin, ethylene, cytokinin and gibberellins which are necessary for plant growth (Husen, 2009).

Several species of rhizobacteria have been reported for their growth promotion to rice plant, such as *Pantoea agglomerans* (Khalimi *et al.*, 2012); *Bradyrhizobium* sp. IRBG271 (Biswas *et al.*, 2000); *Burkholderia caryophylli* ACC7 (Shaharoon *et al.*, 2007), *Rhizobium leguminosarum* 128C56C (Han and Lee, 2005); *Azospirillum brasilense* (Kannan and Ponmurugan, 2010). Souza (2012) proved that inoculation of strains *Agrobacterium*, *Burkholderia*, *Enterobacter*, and *Pseudomonas* could promote the growth of rice. Application of *Serratia marcescens* NBRI1213 significantly increased the plant height, the dry weight of shoot, root length and dry weight of root of *Piper betle* L. by 81%, 68%, 152% and 290% respectively (Lavania, 2006). Treatment with *Pantoea agglomerans* could increase the number of maximum tiller of rice cultivar Cicih Medang Selem by 93.27% to 120.19% when compared to control (Khalimi *et al.*, 2012). Other study done by Hussain *et al.* (2009) showed that treatment of the root of rice with *Mesorhizobium ciceri* strain CRI-32 increased the number of maximum tillers by 25,34%.

Other study showed that the number of panicles per plant increased by 92.13% to 110.95% by the treatment of *Pantoea agglomerans* (Khalimi *et al.*, 2012). A study done by Hussain *et al.* (2009) showed that the rice plants treated with *Rhizobium leguminosarum* strain LSI-30 could increase the number of panicles per plant by 27.33%. Similar result was also reported by Khorshidi *et al.* (2011) in which the treatment of rice seedling with suspension of *Azospirillum lipoferum* for 12 h increased the number of grains per panicle by 5.28%.

Treatment with *P. agglomerans* on rice local variety, Cicih Medang Selem, significantly increased the yield per hill, in which the weight of grains per plant on rice treated with isolates Pj, Bs and PB were 114.03%, 134.33% and 154.17% higher than that of control (Khalimi *et al.*, 2012). Khorshidi *et al.* (2011) reported that treatment of rice seedling with the suspension of *Pseudomonas fluorescens* for 12 h increased the yield by 33.3%. Treatment with *Pseudomonas fluorescens* BRM-32111 and *Burkholderia pyrrocinia* BRM-32113 was proven to promote the growth of rice. The rice plant inoculated with *P. fluorescens* BRM-32111 or *B. pyrrocinia* BRM-32113 resulted in the increase of biomass by 88%, the length of leaf increased by 40%, and chlorophyll content increased by 21% (Rego *et al.*, 2018). Most (89.7%) of isolates of rhizobacteria isolated from rhizospheres of rice plant in Afghanistan produced IAA. Isolates AF134 and AF137 including *Enterobacter ludwigii* and *Pseudomonas putida* produced IAA by 92.3 $\mu\text{g mL}^{-1}$. More than 70% isolates significantly increased dry weight of root and shoot (Habibi *et al.*, 2019). Treatment with *Azospirillum lipoferum* and *Azotobacter chroococcum* as seed treatment increased the yield of rice. Number of panicles per m^2 and number of grains per panicle increased by 9% and 18% respectively (Aminpanah and Firouzi, 2019).

Results of our study enriched the data about the potential and use of rhizobacteria as bio-agent to promote the growth and increase the yield of rice. Hence, the rhizobacteria formulation developed in this study may be used for field testing and further used as an alternative bio-stimulant for rice production.

4. Conclusion

Rhizobacteria formulation containing suspension of *Stenotrophomonas maltophilia* Sg3, and *Enterobacter cloacae* A17Kla effectively promoted and increased the yield of four rice cultivars that commonly grown in Indonesia. Responses of four rice cultivars to the treatment with rhizobacteria formulation in term of plant growth parameters and yield to some extent varied among cultivars. Treatment with rhizobacteria formulation increased the yield of four rice cultivars between 13.48% to 43.00% when compared to untreated one, suggested that this rhizobacteria formulation potentially may be used to increase rice production in particular in Bali Island, Indonesia. The field test is needed to evaluate the stability and effectiveness of the formulation under real field condition in different localities and season.

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