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Effectiveness of Rhizobacteria to Reduce Rice Blast Disease Intensity

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Abstract

Rice blast disease caused by *Magnaporthe grisea* Barr (anamorf *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) is one of important diseases for rice cultivation in Indonesia. Five isolates of rhizobacteria isolated from the rhizospheres of rice in Bali were tested for their effectiveness to reduce the rice blast disease intensity on rice cultivar Ciherang under green house condition. All isolates were formulated in liquid formulas which were respectively contained bacterial suspension of *Enterobacter agglomerans* Ch2D, *Xanthomonas luminescens* Ch3D, *Enterobacter agglomerans* Ch4B, *E. agglomerans* Gg14D and *Serratia liquefaciens* Gh13D. Results of this study showed that all five formula of rhizobacteria effectively reduced the rice blast intensity on rice cultivar Ciherang. The rice blast intensity on plants treated with rhizobacteria were varied from 13.96 to 19.44%, while the disease intensity on control was 33.56%. Formula containing bacterial suspension of *E. Agglomerans* Gg14D resulted in the lowest blast disease intensity and the highest yield per hill. The yield increment resulted from this treatment was 40% when compared to control. This result suggested that *E. agglomerans* Gg14D is one of promising candidate for bio-control agent to manage rice blast disease. However, the field trial is needed in order to evaluate the stability and effectiveness of the rhizobacteria formula under field condition at several localities.
Keywords: rhizobacteria, rice blast disease, disease intensity

1. Introduction

Rice blast disease caused by *Magnaporthe grisea* Barr (anamorf *Pyricularia grisea* Sacc. synonym *Pyricularia oryzae* Cav.) is one of important diseases for rice cultivation in Indonesia and other rice growing areas (BPS, 2010, Kato, 2001, Chin, 1975). The yield losses caused by the disease vary by areas, between 1-100% in Japan (Kato, 2001), 70% in China, and 21-37% in Bali Indonesia (Suprapta and Khalimi, 2012), in South America and Southeast Asia between 30-50% (Baker *et al.*, 1997; Scardaci *et al.*, 1997).

Three strategies has been known to control the rice blast disease such as cultural practice, grow the resistant cultivar and the use of synthethic fungicides (TeBeest, 2007; Ghazanfar *et al*., 2009; BPTP, 2009; IRRI, 2010). The use of resistant cultivas has been known to be the most effective strategy, however, *Pyricularia oryzae* has been known to develop the new race rapidly that may breakdown the rice resistance. Thus the use of resistant cultivar is limited to certain place and time (BPTP, 2009). Based on this reason, the use of resistant cultivar should be combined with other control strategy which is more effective and friendly to the environment. One of the strategy is the use of antagonists as bio-control agent to suppress the development of rice blast disease. Soil microorganisms those associated with the rhizospheres of plants have been known to contribute in many processes in the soil which in turn may influence the plant growth (Tilak *et al*., 2005). The interaction of microorganism and plant in the rhizospheres could be neutral, beneficial or harmful to the plant as they may cause plant diseases (Husen, 2003).

Several important rhizobacteria have been reported as plant growth promoting agent and antagonists against plant pathogens namely Azospirillum, *Alcaligenes, Arthrobacter, Acinetobacter, Acetobacter, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Proteus, Rhizobium* and *Serratia* (Kloepper *et al*., 1989; Glick, 1995; Tilak *et al*., 2005; Naureen *et al*., 2005; Egamberdiyeva, 2005; Bhawsar, 2011). Khan *et al.* (1997) reported that antagonistic rhizobacteria *Actinoplanes* spp. could control the fungal disease caused by *Pythium ultimum* on sugar cane. Huang and Wong (1998) found that Burkholderia *cepacia* A3R could control *Fusarium graminiearum* on wheat. *Burkholderia cepacia* PHQM and *Pseudomonas fluorescens* VO61 was reported to be effectively control *Fusarium* sp. and *Pythium* sp. on corn (Hebber *et al*., 1998). Lewis and Larkin (1998) reported *Cladorrhinum foecundissinum* could be used as bio-control agent to control pathogenic fungi *Phythium ultimum* and *Rhizoctonia solani* on egg plant and chili pepper. *Bacillus* spp. could control the pathogenic fungi *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *tritici* on wheat (Ryder *et al.*, 1999). Other workers reported that Bacillus subtilis BACT-D could control *Pythium aphandermatum* on tomato (Utkhede *et al.*, 1999) while *Pseudomonas fluorescens* VO61 could control Rhizoctonia solani on rice (Vidhyasekaran and Muthamilan, 1999). This study was done in order to test the effectiveness of *Enterobacter agglomerans,* a rhizobacterium isolated from rhizosphere of rice in Bali to control the rice blast disease caused by *Pyricularia oryzae*.

2. Materials and Methods

2.1. Isolates of Rhizobacteria

Five isolates of rhizobacteria that are used in this study have been identified and maintained in the Laboratory of Biopesticide, Faculty of Agriculture, Udayana University, namely *Enterobacter agglomerans* Ch2D, *Xanthomonas luminescens* Ch3D, *Enterobacter agglomerans* Ch4B, *E.agglomerans* Gg14D and *Serratia liquefaciens* Gh13D. All of these isolates have been tested and showed strong antagonistic activities against *Pyricularia oryzae* on potato dextrose agar (PDA) medium. Their inhibitory activities against the colonial growth of *P. oryzae* ranged from 39.46 to 46.66%.

2.2. Formula of Rhizobacteria

The formula of rhizobacteria was prepared as follows. The formula was developed in liquid type containing: rhizobacterial suspension (5 ml, 10^6 CFU/ml), yeast extract (1 g/l), Tween-80 (5 ml/l), molasses (50 ml/l) and potato broth to make one liter. This formula was incubated under room temperature in the dark for 14 days before they are used. Five isolates of rhizobacteria were used in this study viz., *Enterobacter agglomerans* Ch2D, *Xanthomonas luminescens* Ch3D, *Enterobacter agglomerans* Ch4B, *E.agglomerans* Gg14D and *Serratia liquefaciens* Gh13D. The formula developed from these isolates, were designated as formula A, B, C, D and E, respectively.

2.3. Green House Experiment

Formulas of rhizobacteria were tested for their activity to suppress the blast disease on rice under green house condition. The rice cultivar for this test was Ciherang, the most common rice cultivar cultivated by farmers in Indonesia. The rice seed was soaked in distilled water for 24 h, and then was drain up and put in a tray with wetted tissue paper and incubated for 24 h to allow the seed to germinate. The germinated seeds were then soaked in formula at 2% concentration for 30 minutes. The germinated seed for control was soaked in distilled water. For seedling preparation, the seed was then sown on a tray that has been filled with sterile fertile soil. Treated seed and control were sown on separate trays. The 12-day old seedlings (12 days after sowing) are used for transplanting.

Plastic pots (surface diameter: 30 cm and height 35 cm) were filled with cultural medium. Cultural medium consisting of fertile soil (the top soil of paddy field at 20 cm depth) and compost (3:1). Fertilizers TSP and KCl were added into the pot at the dose of 1 g/pot and 0.75 g/pot respectively. A 5 ml each of respective formula was incorporated into the soil, after which two 15-day old rice seedlings of the cultivar Ciherang were transplanted. A completely randomized block design (CRBD) was implemented in this experiment consisting of 6 treatments, namely treatment with formula A, B, C, D and E and Control. Each treatment was replicated four times, and each experimental unit consisting of 10 pots. Treatment with rhizobacterial formula was also done at 19 days after transplanting (a day before inoculation with *P. oryzae*) by spraying a 25-ml /plant of respective formula onto the whole rice plant.

Pyricularia oryzae that has been maintained on PDA slant agar was used for inoculation. Inoculation was done on rice plant at 20 days after transplanting (a day after treatment with rhizobacterial formula). Mycelial plugs of the fungus was grown on rice floor agar medium (consisting of 20 g/l rice floor, 2.5 g /l yeast extract, 1.5% (w/v) Bacto agar and distilled water) and incubated at 28 $\pm$ 2^{o}C for two weeks. Spores of the fungus were harvested using 7 ml sterile distilled water containing 0.5% gelatin. The suspension was filtered through 4 layers cheesecloth, and soon kept on a flask on ice to prevent the germination of the spore. The spore's density was adjusted to 10^5 spores/ml. Inoculation was done in the evening by spraying spore's suspension (25 ml/plant) onto the whole plant. After inoculation, the plant was covered with black plastic for 12 h to stimulate the infection. The third treatment with rhizobacterial formula was done at two days after inoculation by spraying a 25-ml/plant of formula onto the whole plant.

2.4. Determination of disease intensity

Observation to determine the blast disease intensity was done weekly, started from three days after inoculation. The disease intensity was determined followed the formula developed by IRRI (1996). Three plants were used as samples for rice blast disease intensity. The formula used is as follows:

$$DI = \frac{\Sigma (ni \times v)}{(N \times V)} \times 100\%$$

DI : disease intensity
ni : number of leaves with i score
N : number of total leaves observed
V : the highest scale of disease severity
v : scales of disease severity (0-9)

Scale description :
0 = no lesions
1 = small brown, specks of pinhead size.

2 = larger brown specks.
3 = small, roundish to slightly elongated, necrotic gray spots about 1-2 mm in diameter.
4 = typical blast lesions, elliptical, 1-2 cm long usually confined to the area of the two main veins infecting < 2% of the total leaf area.
5 = typical blast lesions infecting < 10% of the leaf area
6 = typical blast lesions infecting 10-25% of the leaf area
7 = typical blast lesions infecting 26-50% of the leaf area
8 = typical blast lesions infecting 51-75% of the leaf area
9 = all leaves dead

2.5. Determination of Peorxidase activity

Determination of peroxidase activity was done followed the method developed by Simons and Ross (1970) with modification. Sample (leaf of rice) was collected 7 days after inoculation with *P. oryzae*. The leaf was grounded in a mortar and added with 0.01 M phosphate buffer pH 6.0. The filtrate was centrifuged at 5,000 rpm for 10 min at 4^{o}C. Supernatant was collected and used to determine peroxidase activity. The peroxidase activity was expressed as unit per minutes per mg of protein.

2.6. Data analysis

All data were subjected to the analysis of variance (ANOVA) and followed by the least significance different test (LSD) at 5% level.

3. Results and Discussion

3.1. Effect of rhizobacteria to rice blast disease intensity

Treatment with formula of rhizobacteria significantly (P<0.05) suppressed the disease intensity of rice blast on rice cultivar Ciherang grown on pots in a green house. Inoculation of the spore's suspension of *P. oryzae* successfully produced typical leaf blast disease on rice indicated that the fungus successfully infected the plant. Observation of the leaf tissues of the disease plants under microscope showed that the spore formation has occurred on the diseased-leaf. In general, there has been an increase in the disease intensity from 10 days after inoculation (DAI) up to 30 DAI. All artificially inoculated rice plants showed typical rice blast symptom, however the disease intensity among treatment were varied.

The disease intensity of the rice plant of control was 33.56% which is significantly different (P<0.05) from the rice plants treated with rhizobacteria formula. No significant differences (P>0.05) were found among rice plants treated with rhizobacteria formula. The disease intensity for treated plants varied from 13.96 to 19.44% (Table 1). However, formula D (containing *E. agglomerans* isolate Gg14D) showed the lowest disease intensity among other formula tested.

Treatment with rhizobacteria formula significantly increased the activity of peroxidase enzyme in the rice leaf and reduced the disease intensity. There is a correlation between peroxidase activity and the disease intensity. Average peroxidase activity on control plants was 0.364 unit/min/mg protein with the disease intensity by 33.56%, while on plants treated with rhizobacteria formula the peroxidase activity varied from 0.402 to 0.474 unit/min/mg protein with disease intensity ranged from 13.96 to 19.44%.

Table 1. Effects of rhizobacteria treatment to the peroxidise activity and disease intensity

Treatment	Peroxidase activity (unit/min/mg protein)	Disease intensity (%) 30 days after inoculation
Control	0.364 a*	33.56 b
A	0.425 bc	16.76 a
B	0.411 b	17.65 a
C	0.402 b	19.44 a
D	0.474 c	13.96 a
E	0.448 c	15.66 a
LSD 0.05	0.0361	7.4058

*Means followed by the same letters are not significantly different (P >0.05) according to LSD 0.05.

Whipps (2001) reported that antibiotic compound produced by antagonist contribute to the growth suppression against plant pathogen through direct contact. While peroxidase enzyme is one of enzymes that produced by rice plant when they are infected by the pathogen (Vidhyasekaran *et al*., 2001). Several enzymes have been reported to increase after treatment with bio-control agent such as peroxidase, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase (Chen *et al*., 2000).

Peroxidase is an enzyme which acts as catalisator on the final stage of lignin and hydrogen peroxidase

biosynthesis. The fuction of peroxidase is to increase the resistance of the cell wall against degrading enzymes produced by the pathogen (Vance *et al.*, 1980). Hydrogen peroxidase may suppress the pathogen directly or through the production of free radicals that posse's antimicrobial effect (Silva *et al*., 2004).

Galston and Davies (1970) reported that in addition peroxidase enzyme, there are several enzymes have been known to contribute to the plant resistance against plant pathogens viz. phenylalanine ammonia lyase, tyrosine ammonia lyase, monophenolase, diphenolase, diphenol oxidase and polyphenol oxidase. According to Huang (2001), peroxidase is one of pathogenesis-related protein (PR-protein) which is a group of plant protein with diverse structures. This protein sometime toxic to pathogen, and has been found in the healthy plant tissue, but its concentration remarkably increased when the plant infected with the pathogen. Michel *et al*. (2002) reported that *Bacillus polymyxa* and *Pseudomonas fluorescens* can increase the plant health through the increase of peroxidase activity in the plant.

Result of this study showed that there is a close correlation between peroxidase activity in the leaf of rice plant with the rice blast disease intensity with the regression equation y = 88.42-163.8 x with the coefficient of determination R^2 = 0.767 (Fig.1). This result is in agreement with previous results of studies in which the peroxidase axtivity affected the disease intensity in plant (Michel *et al*., 2002; Huang, 2001; Chen *et al*., 2000; Vance *et al*., 1980).

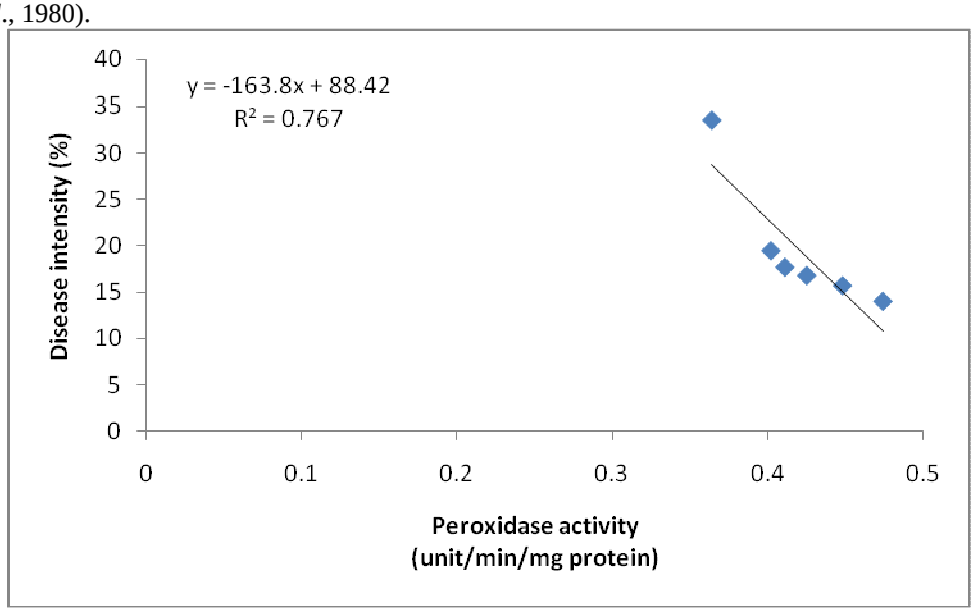

Figure 1. Correlation between peroxidase activity and blast disease intensity (30 days after inoculation)

3.2. Effect of rhizobacteria to the plant growth

Treatment with rhizobacteria formula did not significantly (P>0.05) affect the plant height and number of tiller. This result suggested that all isolates tested in this experiment did not act as plant growth promoting rhizobacteria. This is probably due to the absence of plant growth hormone such as indole acetic acid (IAA) produced by the isolates. Indole acetic acid has been known as one of plant growth hormones that stimulate the plant growth. According to Rondriguez and Wetzstein (1994) that the excessive concentration of auxin for a certain plant may inhibit the growth rate, while in the low concentration of auxin may promote the plant growth. Husen (2009) stated that the plant growth can be promoted directly by rhizobacteria due to their ability to produce plant growth hormones such as auxin, ethylene, cytokinin and gibberellin.

3.3. Effects of Rhizobacteria Treatment to the Yield of Rice

Treatment with rhizobacteria did not significantly (P>0.05) affect the weight of 1000 grains, but significantly (P<0.05) increased the weight of filled grains per hill (Table 3). Treatment D (*E. agglomerans* Gg14D) resulted in the highest (62.33 g/hill) filled grains followed by treatments E (*Serratia liquefaciens* Gh13D), B (*Xanthomonas luminescens* Ch3D), A (*E. agglomerans* Ch2D), C (*E. agglomerans* Ch4D), and Control which were respectively resulted in 59.29 g/hill, 55.27 g/hill, 49.27 g/hill, 47.02 g/hill and 44.58 g/hill. When the potential yield per hectare was calculated based on the weight of filled grains per hill multiplied by the number of plants per hectare (111,111 plants) showed that the estimated yield (ton/ha) were 4.95, 5.47, 6.15, 5.22, 6.93 and 6.59 which were given by Control, treatments A, B, C, D and E. These results suggested that treatment with rhizobacteria potentially increased the rice yield by 5.45 to 40.00% when compared to control. The highest

yield increment (40.00%) was obtained by treatment with formula D (*E. agglomerans* Gg14D). This is probably due to the highest ability of this treatment to suppress the rice blast disease as shown in Table 1.

Study done by Singh *et al*. (2012) showed that seed treatment with *Trichoderma harzianum* increased the seed germination, reduced the days of flowering and maturity and reduced the rice blast disease intensity. The disease intensities of rice plants derived from seeds treated with *Trichoderma* were 23.30 to 30.55% while on control the disease intensities varied from 40.50 to 48.09%. Other study done by Sireesha (2013) showed that treatment with the suspension of *Pseudomonas fluorescens* as spray fluid of 4 ml culture filtrate (containing 10^9 cgu/ml) was the most effective in controlling the leaf blast, and the percentage of the leaf blast incidence was 13.1% compared to 56.5% disease incidence on control. The rice yield obtained by the treatment with *Pseudomonas fluorescens* was 3,296 kg/ha, which is 127.94% higher than that of control (1,446 kg/ha). Our present study showed that treatment with *Enterobacter agglomerans* Gg14D obviously could reduce the blast disease incidence from 33.56% to 13.96% and increased the yield by 40% when compared to control. Results of these studies clearly indicated that none of bio-control agent tested completely suppressed the rice blast disease, suggested that it is necessary to integrate the use of bio-control agent with other control measures to give a better suppression to rice blast disease. In addition, our experiment was done under green house condition, thus it is necessary to conduct the field trial at several localities to prove the effectiveness of this agent under field condition.

Table 2. Weight of 1000 grains, weight of filled grains per hill, estimated yield per hectare and percentage of yield increment against control

Treatment	Weight of 1000 grains (g)	Weight of filled grain/hill (g)	Estimated yield per ha (ton)**	Percentage of increment against control (%)
Control	23.88 a*	44.58 a	4.95	-
A	23.91 a	49.27 ab	5.47	10.50
B	23.81 a	55.32 abc	6.15	24.24
C	23.90 a	47.02 ab	5.22	5.45
D	23.62 a	62.33 c	6.93	40.00
E	24.23 a	59.29 bc	6.59	33.13
LSD 0.05	0.62	12.87		

* Means followed by the same letters are not significantly different (P >0.05) according to LSD 0.05.
**Calculation was done based on the weight of filled grain per hill multiplied by the number of Plants per hectare (111,111 plants).

4. Conclusion

Five formula of rhizobacteria isolated from rhizospheres of rice which were respectively contained bacterial suspension of *Enterobacter agglomerans* Ch2D, *Xanthomonas lumininescens* Ch3D, *Enterobacter agglomerans* isolat Ch4B, *Enterobacter agglomerans* Gg14D, and *Serratia liquefaciens* Gh13D effectively reduced the rice blast intensity on rice cultivar Ciherang. Formula containing bacterial cells of *E. Agglomerans* Gg14D resulted in the lowest blast disease intensity and the highest yield per hill. The yield increment resulted from this treatment was 40% when compared to control. Field trial is needed in order to evaluate the stability and effectiveness of the rhizobacteria formula under field condition at several localities.

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