

PLANT SCIENCE

Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium

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Abstract

Rice blast caused by *Pyricularia oryzae* is a major disease affecting rice production grown in upland and wet-land rice. Application of beneficial bacteria as seedling root dip and spraying method to protect against the disease may be an alternative strategy to chemical control. This research was aimed to explore the bacterial consortium that may control blast disease on rice plants. In this study, the following bacterial cultures and their consortiums were used: *Bacillus firmus* E65, *Serratia marcescens* E31, *Pseudomonas aeruginosa* C32b, *Bacillus cereus* II.14, and its combination for their suppression ability against *P. oryzae* under in-vitro conditions. The results showed that A2 (*Bacillus firmus* E65) and A6 consortium (*Bacillus firmus* E65, *Bacillus cereus* II.14, and *Pseudomonas aeruginosa* C32b) significantly reduced the mycelial growth of *P. oryzae* with the percentage inhibition of 73-85% and 66-83%, respectively. Further greenhouse testing conducted with use of formulative preparation of the two selected best treatments using talc, bentonite, palm oil, and suspension-based carriers showed that spraying with suspension formulation had good effect in suppressing blast disease compared with that of other carriers evaluated.

Key words: *Bacillus firmus*, Blast disease, Consortium of bacteria, *Pseudomonas aeruginosa*, *Pyricularia oryzae*, Rice, *Serratia marcescens*

Introduction

Blast disease caused by *Pyricularia oryzae* is a major disease affecting rice cultivation that affects about 12% of the total area of rice fields in Indonesia (BPS, 2008). Rice blast was reported to infect rice causing a lowered yield of about 30-50% in Southeast Asia and South America (Shimamoto, 2001). Disease severity has increased recently due to the use of intensive agronomic practices that favor disease development. Blast disease severity was triggered by the excess of N fertilization (Faria et al., 1982; Huber, 1990) as well as rainfall and high humidity conditions.

The current control strategy of the disease is through fungicides application, however; their adverse effects on environment and beneficial soil

micro-organisms are quite evident. Most studies have shown that various types of microbial are a potential substitute for inorganic chemical compounds (fertilizer and pesticides) that can be applied in the field on a wide scale.

A number of microbial agents have been reported to be effective as biological control of plant diseases i.e. *Bacillus*, *Bdellovibrio*, *Dactylella*, *Gliocladium*, *Penicillium*, *Pseudomonas*, and *Trichoderma* (Fravel, 1988). Biocontrol approach for managing blast disease is considered to be a practical and economical alternative. Production of secondary metabolites such as antibiotics, Fe-chelating siderophores, and cyanide are most often associated with fungal suppression by fluorescent *Pseudomonads* in the rhizosphere of several crops (Howell and Stipanovic, 1980). Most antagonistic studies of *Bacillus* were conducted on *B. subtilis* and *B. cereus*, and occasionally on *B. firmus*.

The success of biocontrol may depend on suitable formulations as well as survival of the microbial agents. Bacteria as biological control agents have advantages over fungal biological control agent i.e. the bacterial cells mass can be

Received 28 May 2012; Revised 28 August 2012; Accepted 30 August 2012; Published Online 01 March 2013

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produced more easily and faster than the fungus. In addition, they are generally effective when applied as a preventive application to suppress the disease. As in line with this study, we have previously evaluated several strains of bacteria isolated from rice plant for their antagonistic ability against *M. grisea* and *R. solani* (Suryadi et al., 2011).

This study was aimed to test the candidate microbial consortium (*B. cereus* II.14, *B. firmus* E65, *P. aeruginosa* C32b, and *Serratia marcescens* E31) using various formulations as biological agents to control blast disease caused by *P. oryzae*.

Materials and Methods

Isolation and in-vitro screening of bacterial consortium against rice blast

The indigenous Indonesian bacterial isolates viz. *B. firmus* (E 65), *P. aeruginosa* (C 32b), *S. marcescens* (E 31), and *B. cereus* (II.14) were obtained from the microbial gene banks culture collection of Microbiology Laboratory (BiogenCC), and Department of Biology, Faculty of Mathematics and Natural Sciences (IPB). Stock cultures of bacteria were prepared using Nutrient Broth (NB) medium. Colonies were selected, purified and used for further studies. The list of bacterial isolates and their origin is presented in Table 1.

Table 1. List of isolates used in this study.

Bacterial codes	Host	Source
<i>Bacillus cereus</i> II.14	chili	IPBCC
<i>Bacillus firmus</i> E65	rice	BiogenCC
<i>Pseudomonas aeruginosa</i> C32b	soil	BiogenCC
<i>Serratia marcescens</i> E31	rice	BiogenCC

Table 2. Treatment code and bacterial isolates used in the study.

Treatment code	Isolates/mix cultures
A1	<i>B. cereus</i> II.14
A2	<i>B. firmus</i> E65
A3	<i>P. aeruginosa</i> C32b
A4	<i>S. marcescens</i> E31
A5	<i>B. firmus</i> E65 + <i>P. aeruginosa</i> C32b
A6	<i>B. firmus</i> E65 + <i>P. aeruginosa</i> C32b + <i>B. cereus</i> II.14
A7	<i>B. cereus</i> II.14 + <i>P. aeruginosa</i> C 32b + <i>S. marcescens</i> E31
A8	<i>B. firmus</i> E 65 + <i>P. aeruginosa</i> C32b + <i>B. cereus</i> II.14 + <i>S. marcescens</i> E31
A9	Copper sulphate 56% (chemical control)
A10	Untreated Control (water)

Dual culture test formulative bacteria against *Pyricularia oryzae*

Bacterial isolates were grown on NB medium in the Erlenmeyer flask until the population reaches 10^6 - 10^7 CFU/ ml; incubated at room temperature for 24-48 hours, then stored in a refrigerator at 4 °C. Mixing the bacterial suspension was then performed as indicated in Table 2.

P. oryzae fungus inoculums was isolated from blast-infected rice cv. Inpari 13 obtained in Kuningan district, West-Java. Rice seeds cv. Inpari 13 used in this study were obtained from Muara experiment station, Bogor, West Java. Pure cultures of *P. oryzae* were grown on petri dishes containing potato dextrose agar (PDA) medium and incubated for one week at room temperature. Production of *P. oryzae* spores was carried out by growing the fungus on oat meal agar (OMA) medium, incubated for 8-10 days at room temperature. Fungal colonies were added with 2 µg/50 ml streptomycin to eliminate aerial hyphae; Isolates were then irradiated with fluorescent lamps 300 volts to induce the growth of spores for 5-6 days; then washed with 1 liter of sterile distilled water containing 0.2% (v/v) Tween 20. Spores were harvested by rubbing using a brush to the surface of the fungus colony that has been sterilized by immersion in absolute alcohol. Spore suspension was then filtered and collected in sterile Erlenmeyer flasks, and the spore density was observed using Neubauer-haemocytometer.

The isolates and mix cultures were screened for their suppression ability against rice blast pathogen, *P. oryzae* by dual culture technique following the method of Rabindran and Vidyasekaran (1996). Bacterial isolate used as bacterial whole cell cultures (BWC) was streaked at one side of petri dish (3 cm away from the edge) containing PDA. Five mm mycelial plug from seven-day-old PDA cultures of *P. oryzae* were placed at the opposite side of petri dishes (diameter 9 cm) perpendicular to the bacterial streaks. Petri dishes inoculated with fungal discs alone served as control. The treatments were arranged in completely randomized design with three replications. The plates were incubated at 28°C until fungal mycelia completely covered the agar surface in control plate. Observations were done by measuring inhibition zone and mycelial growth of the pathogen, and percent inhibition of pathogen growth was calculated using formula $I\% = \frac{C-T}{C} \times 100$; where I = inhibition of mycelial growth, C= growth of pathogen in the control plate (cm) and T= growth of pathogen in dual cultures (cm).

Dual culture test using culture filtrates of the selected best formulation

Two effective consortiums resulting from the above dual culture studies were selected and tested for their suppressing ability using bacterial culture filtrate (BCF). An agar plug (9 mm diameter) taken from actively growing fungal culture was placed on the surface of the plate-enhancing medium. Bacteria initially were grown on NB medium and incubated for 24 hours. One ml of culture suspension was centrifuged at 8944g for 5 minutes. A total of 100 mL supernatant of BCF was transferred into a petri dish and then poured to the homogenized PDA medium. Plates inoculated with fungal agar plugs alone were used as control. A piece of *P. oryzae* isolate was placed by using a cork bore diameter of 0.5 cm into the PDA medium in petri dishes. Each treatment was performed using 8 replications. The entire petri dish containing treatment was incubated for one week at room temperature, and then the radial growth of *P. oryzae* was measured and percent inhibition of pathogen growth was calculated (Kumar et al., 2000).

Efficacy of biocontrol agents against *P. oryzae* (green house test)

The carrier materials used in this study were talc, bentonite, oil, and suspension with the composition and mixing process of the materials as follows: (a) talc: 300 ml suspension of bacterial isolates, 1 kg of talc, 10 g of carboxymethyl cellulose (CMC), 15 g of CaCO_3 , (b) bentonite: 300 ml of suspension bacterial isolates, 1 kg of bentonite, 10 g of CMC, 15 g of CaCO_3 , (c) oil: 300 ml suspension of bacterial isolates, 6 ml of palm oil, 0.15 ml of Triton X-100, and (d) suspension: 300 ml of bacterial suspension (10^9 CFU/ml).

Rice seeds cv Inpari 13 was sown for 18 days in a plastic bag ($15 \times 30 \text{ cm}^3$) containing field soil. Prior to transplanting the seedlings were immersed in the following treatment regimens: immersion in talc and bentonite for one night; whilst immersion in oil and suspension was given for 3 hours; respectively.

Fungal inoculation was done by spraying using *P. oryzae* spores with a density of 5.5×10^7 spores/ml. Inoculated plants were placed under humid conditions with 90% relative humidity. Rice plants treated without *P. oryzae* infection and formulations served as healthy control; whilst plants inoculated with *P. oryzae* alone without formulation served as untreated control. 100 ml of bacterial formulations was applied using three

times spraying at 3 days, 7 days, and 9 days after inoculation (d.a.i). Data was taken from eight plants (4-5 leaves per plant) for each treatment. Observations was done at 14 d.a.i based on blast disease assessment given by the standard evaluation system of IRRI (1996), using 9 scale basis i.e.; 0= no lesions; 1= small, brown, specks of pinhead size; 3= small, roundish to slightly elongated, necrotic, gray spots about 1-2mm in diameter; 5= typical blast lesions infecting < 10% of the leaf area; 7= typical blast lesions infecting 26-50% of the leaf area; 9= typical blast lesions infecting >51% leaf area and many dead leaves. Then the severity of blast is calculated using the formula:

$$DS = \frac{\sum n \times v}{N \times V} \times 100\%$$

DS = disease severity

n = number of leaves infected by blast

v = value score of each category attack

N = number of leaves observed

V = value the highest score

Results

Isolates of bacteria can grow well after 48 hours and fresh pure culture was obtained on NA media, while the *P. oryzae* fungus require around 7 days to grow up on PDA media. Hyphae in the early growth of *P. oryzae* were white in color and became dark after one week. Observation of spores using light microscopy showed the spores morphology was an avocado like shape with hyaline brownish color (Figure 1).

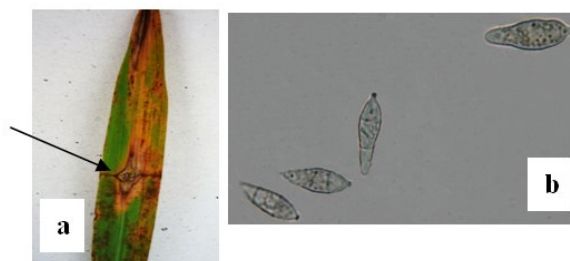


Figure. 1. Typical blast symptom on rice leaves 14 days after inoculation (a) an arrow indicate leaf spot; *P. oryzae* spores at magnification 400x (b).

Dual culture test of formulative bacteria against *Pyricularia oryzae*

Dual culture studies of these bacteria against the pathogen revealed that the inhibition of blast *P. oryzae* ranged from 10-53%. Among the isolates, E65 was found to be highly effective in controlling the pathogen with inhibition of 53.32%. The other effective strains were E31, and C32b that showed

inhibition of test pathogen in the range of 26.2 to 33.65% (Table 3).

Results from dual culture tests indicated the presence of inhibitory zones. This is evident of the formation of inhibitory activity against *P. oryzae* fungi by bacteria. The result revealed that isolates E65 (A2), C32b (A3) and mix cultures of E65, C32b and E31 (A6) showed high degree of inhibition with the mean average of the inhibition area of 53.32 cm², 33.65 cm² and 30.22 cm², respectively. The untreated control showed no inhibitory activity. In contrast to the standard chemical control (Copper sulphate 56%), *P. oryzae* did not grow at all, which indicates the maximum inhibition.

Dual culture test using culture filtrates of the selected best formulation

Prior to the formulation of biocontrol agents, the inhibition test of further selected bacterial isolates using E65 (A2) and E65, C32b and E31 (A6) on NA medium containing supernatant of both treatments showed the average of *P. oryzae* radial growth of 2.80 cm² and 5.76 cm² with the inhibition

of 95.59% and 91.00%, respectively (Figure 2); while on the control treatment, *P. oryzae* can grow up to 63.59 cm² across the surface of petri dish. This confirms inhibitory activity of bacterial isolates against *P. oryzae*.

Efficacy of biocontrol agents against *P. oryzae* (green house test)

Observation of the effect of carrier formulations application agents was performed at 14 days after inoculation. Rice blast fungus (*P. oryzae*) showed spots with brown edges; white or grayish center patches. The shape and color of spots varied depending on environmental conditions, and the degree of rice resistance. Following the assessment of blast disease based on SES, IRRI (1996); A2 and A6 treatments in the form of a suspension carrier provides slower blast disease progression than other formulas with the percentage of blast severity was 28.68% and 35.67%, respectively; whilst on untreated control, the blast severity was 57.85% (Table 4).

Table 3. Effect of bacterial consortium used as BWC on the pathogen growth inhibition.

Treatment Codes	Radial growth of <i>P. oryzae</i> (cm ²) ± SD	Mean inhibition over control (%) ± SD
A1	63.59 ± 3.1a	0 ± 0 e
A2	10.27 ± 6.3 d	53.32 ± 6.2 b
A3	29.94 ± 12.8 c	33.65 ± 12.9 c
A4	37.27 ± 4.05 bc	26.32 ± 4.06 cd
A5	44.32 ± 6.64 b	19.27 ± 6.6 d
A6	33.36 ± 10.46bc	30.22 ± 10.4 c
A7	44.26 ± 5.29 b	19.32 ± 5.3 d
A8	42.55 ± 6.11 b	21.04 ± 6.1 d
Copper sulphate 56% (chemical control)	0 ± 0 e	63.59 ± 0 a
water (untreated control)	63.59 ± 3.1 a	0 ± 0 e
CV(%)	20.36	

Note: numbers in each column followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at P=0.05.

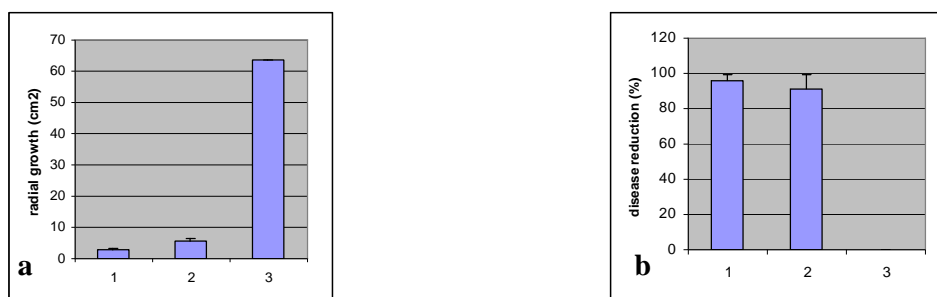


Figure 2. (a) Mean radial growth (±SD) and (b) percentage inhibition (±SD) of *P. oryzae* using bacterial culture filtrate (BCF) after 7 days incubation. 1=A2, 2= A6, 3= water (untreated control).

Table 4. Effect of different formulation to the severity of blast disease in Green House test.

Treatment codes	Mean average of blast severity (%) \pm SD	inhibition over control (%) \pm SD
Healthy plants control	0 \pm 0 f	100 \pm 0
Untreated control	57.85 \pm 8,54 a	0 \pm 0
A2 talc	45.83 \pm 9,79 abcd	20.78 \pm 16.27
A2 bentonite	39.72 \pm 8,92 cde	31.34 \pm 20.39
A2 palm oil	53.89 \pm 9,33 ab	6.85 \pm 3.11
A2 suspension	28.68 \pm 12,23 e	50.42 \pm 24.61
A6 talc	44.17 \pm 7,27 abcd	23.65 \pm 12.58
A6 bentonite	40.49 \pm 11,13 bcde	30.01 \pm 17.60
A6 palm oil	50.76 \pm 13,91 abc	12.26 \pm 9.9
A6 suspension	35.76 \pm 7,77 de	38.18 \pm 18.99
CV (%)	24.09	

Note: numbers in column followed by the same letter are not significantly different according to Duncan's multiple range test (DMRT) at P=0.05.

Table 5. Cell viability of consortium bacteria during period of storage.

Bacterial consortium codes	Formulated carrier	Month- 1 Cells number (CFU/ml)	Month -3 Cells number (CFU/ml)
A2	Talc	8.1 x 10 ⁸	4.5 x 10 ⁸
	Bentonite	6.2 x 10 ⁸	9.2 x 10 ⁸
	Palm oil	3.3 x 10 ⁸	6.6 x 10 ⁸
	Suspension	4.4 x 10 ⁸	9.6 x 10 ⁸
A6	Talc	1.1 x 10 ⁹	4.4 x 10 ⁷
	Bentonite	1.56 x 10 ⁹	1.6 x 10 ⁸
	Palm oil	7.15 x 10 ⁸	1.6 x 10 ⁸
	Suspension	1.75 x 10 ⁹	1.9 x 10 ⁸

The effective formulation of A2 and A6 using talc and bentonite were also reduced blast severity over 20% inhibitions; whereas the inhibition of oil formula ranged from 6.85 to 12.26%. At the initial formulations process, the bacterial isolates had an average of 4.0 x 10⁹ CFU /ml; whereas single cultures of *B. cereus* II. 14, *B. firmus* E65, *P. aeruginosa* C32b and *S. marcescens* E31 had viability of 2.5 x 10⁹, 2.6 x 10⁹, 5.1 x 10⁹, and 6.0 x 10⁹, respectively. After the bacterial isolates were mixed in the form of formulations, at month-1 and month-3 the quantity of E65 isolate (A2) decreased in average of 5.5 x 10⁸ CFU/ml. The average of bacterial cells viability of A6 consortium was approximately 3.01 x 10⁸ CFU/ml. The overall cells viability was slightly decreased during period of storage (Table 5).

Discussion

Rice cultivar Inpari 13 used in this study was derived from backcross between OM606 and IR18348-36-3-3 that was introduced in late 2009 by the ICRR Sukamandi, although little was known among farmers about its popularity. This variety has the advantage of high potential productivity of 8.0 tones/ha with an average of 6.59 tones/ha and has 103 days maturity (Darajat and Rojakurniati,

2011). This variety showed resistance to brown plant hopper and bacterial leaf blight, however Inpari 13 are still susceptible to blast disease particularly in Kuningan district, West Java.

P. oryzae the causal agent of blast disease is a type of fungi that is morphologically similar with *P. grisea* known to attack many types of grasses and weeds (Rush, 1992). *P. oryzae* has long conidiophores and rarely branched. At the terminal end of the conidiophores, the conidia had oval-shaped with pointed tip, with the average size of 20-22 x 10-12 μ m. Under light microscope observation, conidia of *P. oryzae* consist of 2-3 cells, with hyaline brownish color. The result of greenhouse experiment under moist condition demonstrated that blast disease symptoms begin to appear at 4 days after inoculation which was characterized by the emergence of small spots. The latent period of blast disease in the tropics occur at 4-5 days after inoculation (Ou, 1985).

In recent years the use of bioformulations as biocontrol product of bacterial origin is gaining great interest in crop protection, and the product may be supplemented as an alternative to chemical control (Fravel, 1988). Dual culture test using bacterial isolates showed the formulation containing *B. firmus* E65 (A2) *B. cereus* II.14 (A3)

and consortium of *B. firmus* E65, *B. cereus* II.14, and *P. aeruginosa* C32b (A6) had the highest inhibitory effect compared to other bacterial isolates. Control treatment using chemical fungicide (Nordox 56WP with 56% copper-sulfate active ingredient) showed maximum inhibition of *P. oryzae*. Further inhibition test using bacterial cells cultures filtrate showed that A2 and A6 consortium could effectively suppress *P. oryzae* with the percentage inhibition of 79.05% and 69.92%, respectively.

Several studies have reported potential microbial biocontrol agents against *P. oryzae* fungus. It was reported that the fungus *Exserohilum monoceras* had the ability to inhibit the growth of the *P. oryzae* fungus by 61.8% - 71%. The use of *Pseudomonas fluorescence* strains 4-15 and 7-14 also showed growth inhibitory activity against *P. oryzae* with a percentage of 59% and 47% (Tsukamoto et al., 1999; Gnanamanickam and Mew, 1992)

The result of bacterial isolate selection through dual culture test has been reported previously using single isolate of *B. firmus* E65, *B. cereus* II.14, and *P. aeruginosa* C32b (Suryadi et al., 2011). Those isolates have activity against *P. oryzae* fungi causing blast disease on rice cv IR 64. *Bacillus* is a genus of Gram-positive bacteria that produce endospores, and can be a potential biological agent due to its resistance to heat and drought conditions, thus suitable for applications in the field (Wayne et al., 2000); whilst *Pseudomonas* is a genus of Gram-negative bacteria that can live in a simple nutrient conditions, and usually colonize the roots of rice.

Similar to the *in vitro* test, the outcome of the greenhouse trial was highly encouraging because the bacterial formulations significantly reduced blast disease. Mechanism of action of biological control agents are generally classified as activities of substances competition, parasitism, and antibiosis (Fravel, 1988; Weller, 1988). In the previous study, it was reported that several bacterial isolates could suppress the growth of pathogenic fungi *P. oryzae* (Suryadi et al., 2011). The mechanisms of bacterial isolates particularly isolates E65, E31, C32b and II.14 produced cell wall degrading enzymes such as chitinase and glucanase that can degrade cell walls of fungi with varying effect. For example the glucanolytic index produced by those bacteria was 2.21; 0.99; 0.50 and 0.63, respectively. In this study, the mechanism of inhibition of bacterial isolates against *P. oryzae* presumably caused by the activity of substance produced by bacteria. *Bacillus* produces a variety of antibiotics that are effective against bacteria and

fungi such as zwittermicin-A (He et al., 1994), kanamisin and lipopeptida of iturin, surfaktin, and fengycin (Stabb et al., 1994). Huang et al. (1993) reported that antibiotic iturin and surfaktin can inhibit the growth of pathogenic fungi. Fengycin produces by *Bacillus subtilis* had inhibitory activity against fungi *P. oryzae* (Joshi and Gardener, 2006). In addition, several species of *Bacillus*, such as *B. cereus* II.14 showed chitinolytic activity (Mubarik et al., 2010). Meanwhile, Hassanein et al. (2009) reported *Pseudomonas* sp. had the ability to produce secondary metabolites such as antibiotics, ammonia, and cyanide. Examples of antibiotics produced by *Pseudomonas* sp. namely pyrrolnitrin effective in suppressing the growth of *Rhizoctonia solani* and pyolutcorin that can suppress the growth of pathogenic fungi *Pythium ultimum* (Howell and Stipanovic, 1980). Siderophore chelating irons (Fe) have the ability to bind iron elements of the environment. Pathogenic fungi do not have the ability to produce the siderophore as *Pseudomonas* sp; hence pathogenic fungi- iron element deficit can lead to stunted growth of pathogens (Neilands and Leong, 1986). The ability of bacteria to produce antibiotic compounds was considered as most appropriate biological control agents compared with other means such as competition and parasitism.

Single bacterial and consortium treatment in this study showed variability of activity. For example, the dual culture test showed that A1 treatment (*B. cereus* II.14) as single bacterium had no inhibitory activity against *P. oryzae*. However, the effect of *B. cereus* was seen on A5 and A6 consortium (mix cultures). Consortium A6 containing *B. firmus* E65, *P. aeruginosa* C32b, and *B. cereus* II.14 had inhibitory activity against *P. oryzae* much better than the A5 consortium containing *B. firmus* E65, and *P. aeruginosa* C32b alone. These results may indicate *B. cereus* II.14 plays synergistic role in the inhibitory activity of *P. oryzae*.

This phenomenon that occurs in bacterial consortium was allegedly a process of quorum sensing (QS). This process is a form of inter-bacterial communication mechanism with the use of chemical signaling molecules called auto inducer (AI). AI is a secreted signaling molecule, accumulated, reabsorbed, and recognized by the bacteria during the process of QS (Rukayadi and Hwang, 2009). QS does not occur by an individual bacterium, but it would be done simultaneously by a large number of bacterial cells and this communication can occur in bacteria or interspecies-intraspecies (Waters and Bassler,

2005). The phenomenon of QS is involved in the regulation of important biological functions such as, production of antibiotics, plasmid transfer, motility, virulence, and expression of genes in the *Pseudomonas aeruginosa* and *Bacillus* sp pathogen (Dong et al., 2002; Zhang and Dong, 2004).

The effect of formulation application of bacteria to the severity of blast disease of rice was carried out during 14 days after inoculation. Treatment combinations of bacterial immersion and spraying formulation of the selected bacteria showed the suppressing activity against blast disease. Suspension-formulations of A2 and A6 showed potential suppressing effect that causing low blast severity than other formulations. Suspension based formulation is a formula containing only NB medium and bacterial isolates. NB is a common medium for bacterial growth that contains complete nutrition elements needed by bacteria. In this study, the most effective suspension might affect the growth and activity of bacteria. Bentonite and talc based formulations also showed the potential suppression of blast disease, although not as good as suspension formulations. CMC serves as an adhesive agent so that the formulation can be attached to the surface of plant organs, whereas CaCO_3 as a nutritional source of calcium for the growth of bacteria and pH medium becomes neutral (Ardakani et al., 2010). Bentonite and talc based carriers have similar physical appearance in terms of fine powder form, lightweight, and good ability to absorb liquids. A difference between the physical colors of bentonite is rather dark gray, whilst talc has pure white color. During the mixing process of the materials used, visible liquid absorption of bentonite was better than talc. Bentonite absorbs liquid containing bacteria more quickly and evenly; while the talc media had a little longer to absorb the NB, hence the structure eventually form large clumps. At this stage of formulation; bentonite applications seems to be more efficient; easily dissolved in sterile water compared with that of talc.

Research on the carrier material bioformulation using talc and bentonite had also been done by Ardakani et al. (2010) that showed biocontrol with carrier formulation of bentonite, CMC, and the bacterium *P. fluorescence* effectively suppresses the growth of pathogenic fungi *Rhizoctonia solani* on cotton plants. Formulation with oil mixture showed less potential for suppression of blast, causing less percentage of blast inhibition. Use of colloids Triton X-100 in this study may serve as oil emulsifier that can be mixed perfectly with NB

medium containing bacteria. The use of oil based formulation should pay more attention due to disturbing effect on the physiological growth of plants. The rice plants-oil applied formulation has shorter than other formulations application.

Observation of bacterial cell viability was performed three times namely at the beginning of mixing process of formulations, one month and three months after the formulation stored at room temperature (solid formulation) and refrigerator ($\pm 4^\circ\text{C}$) for liquid formulations, respectively. At the initial formulations process, the bacterial isolates had an average of 4.0×10^9 CFU /ml). After the bacterial isolates were mixed in the formulations at month-1, the quantity of E65 bacterial isolate decreased in average of 5.5×10^8 CFU/ml. The decrease of viable bacteria at month-1 and month-3 may occur due storage adaptation that allows bacteria to survive or died. A phase of adaptation can occur because bacterial culture was transferred from nutrient-rich media (NB) to the limited nutrient content media.

Development of biocontrol formulation can be successful if microbial agents could survive during storage, as well as competitive and aggressiveness after inoculation process (Beatty and Jensen, 2002; Selim et al., 2005). In this study, although the potential suspension-based formulation showed good effect in blast suppression, but for large scale this formulation is less efficient in terms of packaging and use. Talc and bentonite based formulations should be further developed because of their efficiency and further research needs to be done to increase the suppression of blast disease.

It has been reported that several diseases are caused by exposure to harmful chemicals during spraying (Rola and Pingali, 1993). The most direct benefit from the use of bacterial consortium is the reduction in disease pressure (blast disease) may imply some savings in the use of traditional pesticides and labor for spraying pesticides. The reduced number of sprays was 5 for traditional pesticides compare with only 3 times for bacterial formulations would have a positive effect on health. Because of reduced severity of blast disease farmers may spent only 5\$ US/kg/ha for bacterial formulations compared with farmers expenditure of using pesticides (15\$ US). There is also substantial savings in the amount of labor used for spraying bacterial formulations.

The results obtained here pointed out the possibility use of bacterial consortium such as A6 (*B. firmus* E65, *B. cereus* II.14, and *P. aeruginosa* C32b) in rice fields for blast disease suppression.

However, further research is needed to elucidate in detail the mechanism of action of these strains and their compatibility with other components in integrated management of rice diseases.

Conclusion

Based on *in vitro* test, A2 (*Bacillus firmus* E65) and A6 consortium (*Bacillus firmus* E65, *Bacillus cereus* II.14, and *Pseudomonas aeruginosa* C32b) could suppressed the radial growth of *P. oryzae* fungus with the inhibition of 19.27% and 53.32%, respectively. It was shown that A2 (*Bacillus firmus* E65) suspension revealed good inhibitory producing the smallest of blast severity (28.68%) under greenhouse condition.

Acknowledgements

This research was financially supported by KKP3T-AARD grant Project Number 868/LB.620/I.1/3/2011 to Dr. Nisa Rachmania Mubarik.

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