Prospect of Using Bacterial Bio-formulation to Suppress Bacterial Leaf Blight of Rice: A Case Study in Cianjur, West Java

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Naskah diterima 25 Juli 2012 dan disetujui diterbitkan 24 Juni 2013

ABSTRAK. Prospek Penggunaan Bioformulasi Bakteri untuk Menekan Penyakit Hawar Daun Bakteri pada Padi: Studi Kasus di Cianjur, Jawa Barat. Hawar daun bakteri (HDB) padi yang disebabkan oleh Xanthomonas oryzae pv. oryzae (Xoo) merupakan salah satu penyakit utama padi yang sulit dikendalikan. Sejumlah isolat bakteri antagonis telah diisolasi dan diuji kemampuan antagonismenya terhadap Xoo di laboratorium, dan 6 isolat yang memiliki kemampuan antagonis tertinggi dan berspektrum luas telah dipilih. Bioformulasi bakteri antagonis diramu dalam bentuk formulasi tunggal dan campuran (konsorsium bakteri) dan telah diuji efikasinya terhadap HDB pada percobaan lapangan di Cianjur, Jawa Barat. Efikasi bioformulasi terhadap penyakit HDB bervariasi pada kultivar padi yang berbeda dengan intensitas penekanan berkisar antara 10,5-29,4% pada MH 2010/2011. Konsorsium bakteri antagonis A6-bentonit dan A8-bentonit menurunkan intensitas penyakit HDB >25%. Formulasi A6-bentonit juga melebihi kemampuan formulasi isolat antagonis tunggal seperti Burkholderia spp., E76-bentonit, dan S. marcescens SKM-kaolin. Aplikasi formulasi konsorsium bakteri antagonis tidak meningkatkan tinggi tanaman, jumlah anakan, berat biji, bobot 1000 butir, tetapi meningkatkan hasil padi >8%. Manfaat penggunaan bioformulasi bakteri antagonis untuk menekan penyakit HDB di lapangan baik secara tunggal maupun campuran masih perlu dikaji lebih lanjut.

Kata kunci: Padi, konsorsium bakteri, hawar daun bakteri, bioformulasi.

***ABSTRACT**. Bacterial leaf blight (BLB) caused by Xanthomonas oryzae pv. oryzae (Xoo) is a major disease of rice in Indonesia which remains difficult to be controled. A number of bacterial isolates had been collected and screened for their ability to antagonize Xoo disease. Six isolates with the highest antagonistic potential and wide spectrum were chosen for studies based on their ability to control the disease. Bio-formulations consisted of single or mixtures (consortium) of the bacterial antagonists were formulated and tested for their effectiveness to control BLB under field trials in Cianjur, West Java. Efficacy of the bio-formulations against the disease on different rice cultivars varied significantly with HDB reductions ranging from 10.5% to 29.4% among the cultivars tested in the WS 2010/2011. The mixture of A6 + A8-bentonite showed a good ability to reduce BB infestation by up to 25%. Performance of the A6+A8-bentonite formulation also exceeded those of the individual isolate formulations, such as Burkholderia spp. E76-bentonite and S. marcescens SKM-kaolin. Application of the bacterial mixture formulation did not affect significantly on rice plant height, number of tillers, and weight of 1000 grains, but increased grain yield up to 8%. This study showed that the advantages of single or mixed cultures were apparent and further evaluation for application of bio-formulation need to be done under more intensive field conditions.*

Keywords: Rice, consortium bacteria, bacterial leaf blight, bio-formulation.

Bacterial leaf blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important bacterial disease of rice. The disease is widely spread in Asia, especially in the tropical rice producing countries. It frequently arises in the proportion of severe situation that can reduce rice yield up to 20-30%. Currently, the disease is reported not only infested wetland rice but also upland rice fields (Kadir *et al.* 2007).

Efforts to control BB are mostly emphasized on the use of resistant rice cultivars. These efforts, however, were hampered by occurrence of strains of the pathogen differing in virulence between seasons and locations that cause resistance of the rice cultivars were ineffective. In Indonesia, at least 12 *Xoo* strains differing in their virulences had been reported, which were commonly called virulence groups or pathotypes (Sudir *et al*. 2009). The wide diversity and fluctuation of the dominant *Xoo* strains in one place and another or in one season and another season had been reported in several countries, and these phenomena are closely related to composition of the host genotypes (Shanti *et al.* 2001).

Since the last decade, the System of Rice Intensification (SRI) has been promoted as an agronomic management practice to improve yield in rice cultivation that reduces water requirement, raises input productivity, accessible to small holders, and is more favorable to the environment than the conventional practice with its continuous flooding of the crop and heavy reliance on inorganic fertilization (Uphoff and Satyanarayana 2006). SRI has attracted considerable farmers interests, particularly in the Asian countries. Soil nutrient supplies need to be augmented, preferably with compost made of any available biomass with better

quality such as animal manure that can give additional advantages in terms of good soil structure and active microbial communities in the rhizosphere than does the inorganic matter. In a previous experiment at Pusakanagara Experiment Station, West Java, using the Integrated Crop Management (ICM) and SRI practices, application of bacterial consortium decreased the occurrence of rice diseases such as sheath blight, red stripe, and BB (Suryadi *et al*. 2013a).

The SRI included conventionally recommended methods of rice cultivation, proposed the use of single young seedlings, drastically lowered plant densities, keeping fields unflooded, and use of a mechanical weeder that enable to aerate the soil. The minimum of water is applied during the vegetative growth, the flowering and grain filling stage. All these are aimed at providing optimal growth conditions to the plant, hence giving better performances in terms of yield and input productivity (Anitha and Chellappan 2011). In a current field study Cianjur (West Java) more complex disease progression may occurred, since the SRI practice that used excessive organic soil amendment (cow-manure) as the basal fertilizer might induced chemical and physical changes of the soil and changes in population the soil microflora.

Many species of bacteria and fungi produce phytohormones (auxins, cytokinins, and ethylene) in the rhizosphere that regulate and promote root growth. When soils are alternately flooded and drained, certain bacteria as well as mycorrhizal fungi are able to double the size of plant root systems by their activity to contribute on plant growth, increased biological N fixation and P solubilization (Turner and Haygarth 2001). In addition it could changes in microbial population associated with SRI practices (Anas *et al*. 2011).

Currently, considerable attentions have been given on use of biological agents to control plant diseases. Studies on BB control using rice associated bacteria in India had been reported and reviewed (Velusamy *et al*. 2006). Gram negative bacteria such as *Lysobacter* spp. was reported to inhibit a fungal pathogen *Bipolaris sorokiniana* in the field (Kilic and Yuen 2004). The bacteria suppressed the pathogen by various mechanisms such as the production of chitinase and β 1-3 glucanase (Zhang and Yuen, 2000), antibiotic (Nalisha *et al*. 2006), and by induction of systemic resistance (Saikia *et al*. 2006). In line with the SRI practices, biological control using local microorganisms can be applied to contribute its effectiveness in the field.

In Indonesia, research on control of BB using microbial agents such as *Bacillus* spp., *Serratia* spp., *Pseudomonas aeruginosa,* and *Corynebacterium* spp. had been done extensively (Agustiansyah *et al*. 2010, Suryadi *et al.* 2013a, Baskoro, *pers. com*.). Agustiansyah *et al*. (2010) reported that the combination of matriconditioning plus a biological control agent (isolate A6) reduced *Xoo* population in rice plants and improved viability and vigor of rice seeds in the glasshouse. In the previous study, it was reported that applications of an individual antagonistic bacterium such as E 65, C 32a, C 32b, E 31 isolates suppressed BB lesion length in the screen-house test. Several saprophytic indigenous bacteria such as *B. cereus* II.14, *B. firmus* E65, and *P. aeruginosa* C32b produced chitinase and growth hormone (IAA) that suppress ShB and blast disease (Suryadi *et al*. 2011). Inconsistent performances of the microbes in the field, however, had limited their commercial uses; hence combining several modes of actions against the pathogen could improve their effectiveness.

Currently, the uses of bio-formulations of bacterial mixture are gaining great interests in the biological control method, and the products are used as supplement or as an alternative to the chemical control (Gnanamanickam *et al.* 2004). Bentonite (a type of montmorillonite clay), talc, kaolin, other materials that have similar physical appearances in terms of fine powder and ability to absorb liquids in water had been used as carriers for the microbial agents, so that they can be easily used for plant spraying in the field (Ting *et al*. 2009; Ardakani *et al*. 2010). In addition, their potential effects combined with varying degree of plant resistances in the disease control need also be investigated. The present study aimed to identify the prospect of using bio-formulation of consortium bacteria as components in the BB control under the SRI technique.

MATERIALS AND METHODS

Evaluation of Bacterial Consortium *in vitro* and Preparation of Bio-formulation

The preparation of the consortium bacteria included selection of bacterial isolates, evaluation of the antagonistic potential against *Xoo*, and mass production of the formulation. An *Xoo* isolate 27d of Sukamandi and five isolates of saprophytic bacteria representing different species and ecological status were used in this study (Table 1).

Isolates of the bacteria were obtained from collections of the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), Bogor (E 76, E 65, C 32b, E 31 and SKM isolates), and the Bogor Agriculture

Table 1. Bacterial isolates used as consortium bacteria representing of different species and ecological status that were used in the study.

Code of isolates	Species	Host	Origin	Year	IAA (ppm)
E 76	*Burkholderia* spp	Rice	Sukabumi, West Java	2004	6.5
E 65	*Bacillus firmus*	Rice	Sukabumi, West Java	2004	21.0
C 32b	*Pseudomonas aeruginosa*	Soil-mud	Sidoarjo, East Java	2007	12.1
E 31	*Seratia marcescens*	Rice	Sukabumi, West Java	2004	20.2
II.14	*Bacillus cereus*	Chili pepper	Bogor, West Java	2007	70.8
SKM	*Seratia marcescens*	Insect-brown plant hopper	Sukamandi, West Java	2010	20.0
Xoo 27d	*Xanthomonas oryzae* pv. *oryzae*	Rice	Sukamandi, West Java	2010	-

IAA = capable of producing a growth regulator Indole Acetic Acid; *Xoo* = *Xanthomonas oryzae* pv *oryzae* (target pathogen).

University Culture Collection, Bogor (II.14 isolate) and further used as a source of mixed cultures (consortium). These isolates have been previously tested effective against *Xoo* under *in vitro* and green house assay (Suryadi, *unpublished data*).

Bacterial stock cultures obtained from ampoules. The bacterial isolates were revived by streaking onto nutrient agar (NA) plates and incubated at room temperature for 48-72 hours. A loopful of each culture was then transferred into an Erlenmeyer flask (250 ml) containing nutrient broth (NB) medium and incubated 24 h at room temperature on a rotary shaker (Stuart Scientific, SI 50) using constant shaking at 125 rpm. The *Xoo* isolate 27d was cultured by plating on the Wakimoto medium (EPPO 2007). Concentrations of the bacterial cultures were measured using a spectrophotometer Hitachi-150-20 and adjusted to OD_{600nm} = 0.5 or approximately equal to 9 x 10^8 colony forming units (CFU)/ml.

In vitro evaluation for potential of the bacterial cultures as biocontrol agents against *Xoo* was conducted following the paper disc method (Ting *et al*. 2009). The treatments consisted of a single or mix cultures of the five isolates tested, namely: A0 = *Xoo* (control); A1 = *B. cereus* II.14; A2 = *B. firmus* E65; A3 = *P. aeruginosa* C32b; A4 = *S. marcescens* E31; A5 = *B. firmus* E65 + *P. aeruginosa* C32b; A6 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b; A7 = *B. cereus* II.14 + *P. aeruginosa* C32b + *S. marcescens* E31; and A8 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31; A9 = copper sulphate (2 g/l); A10 = water (control).

Prior to the evaluation, lawns of *Xoo* were prepared by plating the pathogen on the Wakimoto's medium. Sterilized filter paper discs (1 mm diameter) were dipped in suspension of each of the tested bacterial isolate and then placed carefully on the *Xoo* plates (4 paper discs/ plate). The plates were incubated at room temperature, and antagonistic potential of the isolates were determined by measuring diameter of growth inhibition zones of the *Xoo* 5-7 days after incubation. The experiment was arranged in a completely randomized design with three replications. Data were analyzed using Sirichai statistic V6.

Preparation of bio-formulations using talc, bentonite or kaolin were as follows: each of the selected candidates of biological control agent for *Xoo* were cultured on NA plates and incubated at 37°C for 24 h. Bacterial colonies grown in flask (1:1, v/v) was prepared under continuous cultures, incubated for 24 hours on a rotary shaker with constant shaking (125 rpm). After the incubation, 10% (v/v) of the samples were sub-cultured in a fresh NB medium and incubated as described above. To produce a bioformulation, 10 ml of a bacterial suspension at concentration +10^8 CFU/ml was transferred into a 1,000 ml flask containing 300 ml of NB medium and incubated by shaking at 125 rpm on a rotary shaker for 48 h at room temperature. The carrier materials used in this study were talc, bentonite, and kaolin. The 300 ml of bacterial suspension was then mixed with 1 kg of each carrier material supplemented with 10 g carboxymethyl cellulose (CMC) and 15 g $CaCO_3$. The formulation was then air dried and kept in a moist room temperature until 20% moisture content. Bio-formulation was wrapped on 2kg plastic bag and kept under cool dry place for three month storage. Viability the biological control agents on each bio-formulation material were checked by serial dilution at initial application and final observation following the method of Ardakani *et al*. (2010).

Field Evaluation of Bio-formulation Effectiveness Containing Single and Mix Cultures of Antagonist against BB in the Wet Season 2010/2011

A field experiment was conducted at Cipeuyeum District, Cianjur (West Java, Indonesia), with an altitude of + 287 m above the sea level (asl.) under naturally BB infested field, during the Wet Season (WS) 2010/2011. Based on previous study using differential rice varieties, this site

was predominantly occupied by *Xoo* race VIII (Kadir, *unpublished data*). Rice plants of four cultivars differing in their BB resistance level, i.e. Sintanur (R), Inpari 10 (M), Inpari 13 (M) and Code (R) were grown in 4 m x 5 m plots. The organic SRI practices in this study consist of transplanting of single young seedlings (+ 12 day-old seedling) with 30 cm x 30 cm spacing. The crop was fertilized with animal compost material (chicken manure) at the rate of 4.0 t/ha. Intermittent irrigation system was done. Pest management practice was done without using synthetic pesticides but using local microorganism and plant as natural pesticides.

The experiment was arranged in a factorial experiment with four replications. There were six treatments of bio-formulations with two carrier material (bentonite and kaolin) were tested consisting four formulations with single bacterial isolate (A2: *B. firmus* E 65-bentonite, *Burkholderia* sp.E 76-bentonite, *S. marcescens* SKM-kaolin), one with three bacterial isolates (A6: *B. firmus* E 65; *B. cereus* II.14; *P. aeruginosa* C32b-bentonite) and one consisted of four bacterial isolates (A8: *B. firmus* E 65; *B.cereus* II.14; *P. aeruginosa* C32b; *S. marcescens* E31-bentonite). No biocontrol formulation was given to the control plots (untreated).

Prior to the field test, each bio-formulation was diluted in sterile distilled water to a concentration of 3 g/ml. The BB control was done by spraying the rice plants with suspension of the bio-formulation suspension using a knapsack sprayer four times, at 14, 28, 35 and 45 days after planting (DAP) at spraying volume 500 l/ha.

The BB disease severity was evaluated at 70 DAP based on 0-9 scales following the IRRI Standard Evaluation System for Rice (IRRI 1996). Yield of each rice varieties were observed after harvest. Data were analyzed using analyses of variance (ANOVA) and the differences among the treatments were evaluated with the Duncan' Multiple Range Test (DMRT) at P = 0.05.

RESULTS AND DISCUSSION

Evaluation of Bacterial Consortium *in vitro* and Preparation of Bio-formulation

In the preliminary test, antagonistic potential of the isolates were indicated by diameter of the inhibition zones of *Xoo* (Figure 1). It was shown that effective treatment was observed using A2, A5 and A8 formulation. The diameter of inhibition zones ranged from 0 to 0.6 cm. The growth of *Xoo* was inhibited by A2 = *B. firmus* E65, A5 = *B. firmus* E65 + *P. aeruginosa* C32b, A6 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b and A8 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31 with the highest inhibition zone was observed upon treatment with A2 followed by treatment with A5, A8, and A6. However, inhibitions by the bacterial mixture were better than that by 2% CuSO4 suspension. A1 (*B. cereus* II.14), A3 (*P. aeruginosa* C32b), A4 (*S. marcescens* E31) did not show antagonistic effects. Based on the *in vitro* study formulation A2 containing single isolate was further developed for bio-formulation using talc-based method.

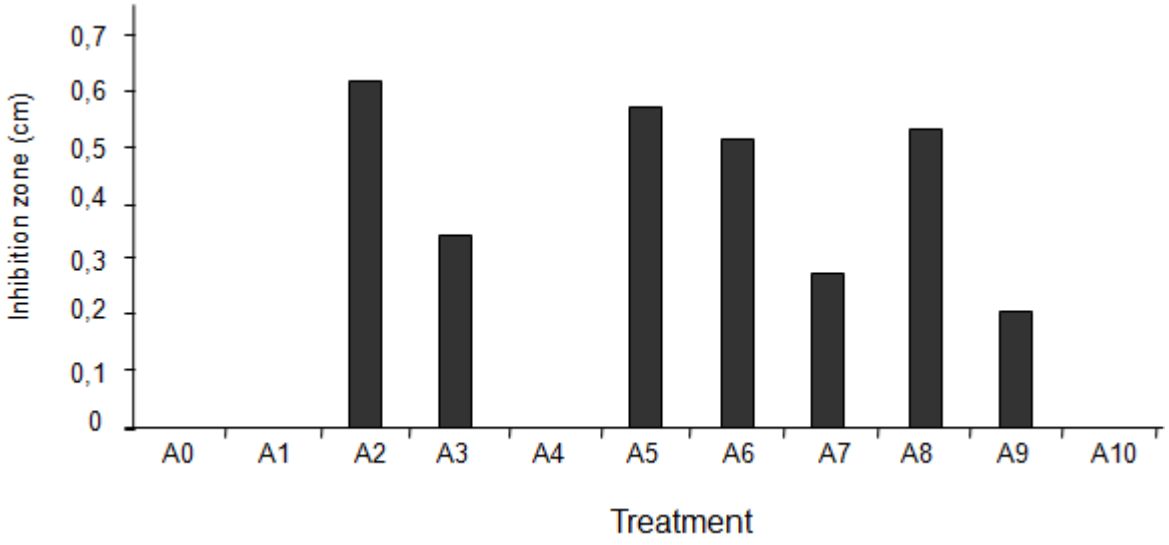

Figure 1. Diameters of growth inhibition zones of *Xoo* by single or consortium of antagonistic bacteria on agar plates. A0 = *Xoo* (control); A1 = *B. cereus* II.14; A2 = *B. firmus* E65; A3 = *P. aeruginosa* C32b; A4 = *S. marcescens* E31; A5 = *B. firmus* E65 + *P. aeruginosa* C32b; A6 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b; A7 = *B. cereus* II.14 + *P. aeruginosa* C32b + *S. marcescens* E31; A8 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31; A9 = 2% copper sulphate (Control); A10 = water (control).

Table 2. Effect of different BB bio-formulations on BB severity and BB suppression of rice cultivars. Cianjur, Wet Season 2010/2011.

Treatment	BB severity (%)		Decrease of severity (%)	Bio-control efficacy (%)*
	With formulation	Without formulation (Control)		
Code + A2-bentonite	24.8 ^{d}	28.5 ^{d}	3.7	13.0
Sintanur + A2-bentonite	40.7 ^{bc}	57.7 ^{a}	17.0	29.4
Inpari 10 + SKM-kaolin	52.6 ^{a}	63.2 ^{a}	10.6	16.7
Inpari 13 + E76-bentonite	56.3 ^{a}	62.9 ^{a}	6.6	10.5
Inpari 10 + A6-bentonite	39.3 ^{bc}	52.8 ^{a}	13.5	25.6
Sintanur + A8-bentonite	32.2 ^{cd}	43.2 ^{b}	11.0	25.3
Mean	40.9^{A}	51.4^{B}	-	-

* Calculated using formula DS = (C-T)/C x 100%, where DS = disease suppression, C = BB severity in control (without formulation), T = BB severity in treatments (with formulation). Numbers in each column followed by the same letter are not significantly different by the Duncan's Multiple Range Test at P = 0.05. A2 = *B. firmus* E65; A6 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b; A8 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31.SKM = *S. marcescens*.

Field Evaluation of Bio-formulation Effectiveness Containing Single and Mix Cultures of Antagonist against BB in the Wet Season 2010/2011

In the WS 2010/2011 two bacterial consortiums (A6-bentonite and A8-bentonite) were developed using combinations of three to four bacterial isolates. These consortium inhibited BB up to 25% compared with other treatments (<20%); whilst single A2-bentonite treatment showed 29.4% BB suppression (Table 2).

Bacterial consortium containing more than one bacterial isolates capable of suppressing BB severity. Consortium A8-bentonite (consisted of four potential isolates identified belonging to *B. firmus, P. aeruginosa, B. cereus and S. marcescens)* exhibited good ability in reducing BB infection under field test. The performance of consortium A6-bentonite also exceeds the performance of the individual isolates such as *Burkholderia* sp E76-bentonite and *S. marcescens* SKM-kaolin. It was observed that the presence of *B. firmus* E 65 isolate in some of the selected formulations improved the suppressing ability of the mix culture. These were observed at Sintanur (A8-bentonite) and Inpari 10 (A6-bentonite) cultivar treatments. A single culture of *B. firmus* isolate E 65 (A2) using bentonite combined with cv. Code (Resistant to BB) was found capable of reducing BB pathogen most efficiently (24.8% severity) compared to the other treatments. The BB disease reduction compared with untreated cultivars ranged from 10.5% to 29.4% (Table 2).

The data presented here showed the performance of combining treated of different rice cultivars with bacterial formulations raised under SRI practices in the field having the same soil, and climatic conditions. Bio-control efficacy of consortium bacteria varied significantly among different rice cultivars with disease reduction ranging from 10.5% to 29.4% in 2010/2011 WS across the cultivars tested. Jochum *et al*. (2006) reported application of bio-control agent among different wheat cultivars in the green house showed varying degree in reducing head blight, suggesting biocontrol effects was not directly related to the reported levels of resistance. It was suggested that plant morphology such as leaf surface structure (hydatodes, mesophyl) of different rice cultivars might have affected colonization of bio-control agent.

Most bio-control agents using local microorganism application showed varied performance in different environmental conditions which attributed to differences in mode of action under natural environments. It was reported that the isolation and screening of several microorganisms from rice seed samples collected from the field also capable of reducing BB infection (Agustiansyah *et al*. 2010). In the previous experiment the suppressive ability of *Bacillus* and other microbial community against several plant pathogens showed varied effect (Kita *et al.* 2005). *B. subtilis* was used effectively to control fungal and bacterial plant pathogens (Nalisha *et al.* 2006).

Endophytic bacterial isolates *viz.* E 31, E 65 and E76 isolates used in this study were obtained from rice nearby endemic rice disease area at Cikembar, Sukabumi, West Java. Isolates C 32b was isolated from mud soil near Sidoarjo region in E. Java; while isolate *B. cereus* II.14 was isolated from chili pepper in Bogor area. These isolates probably have acquired natural adaptation to survive in the presence of formulative media used in the study. The initial population observed prior field application was approximately 2.1 x 10^{9} CFU/ml; whilst at final observation the population reaches

Table 3. Effect of different bio-formulations on number of panicles per hill of rice cultivars. Cianjur, Wet Season 2010/2011.

Treatment	No. of panicle per hill		
	With formulation	Without formulation (control)	Mean
Code + A2-bentonite	17.0^{ab}	16.5^{abc}	16.8
Sintanur + A2-bentonite	15.8^{abc}	16.5^{abc}	16.1
Inpari 10 + SKM-kaolin	15.5^{bc}	17.7^{a}	16.6
Inpari 13 + E76-bentonite	13.3^{d}	13.1^{d}	13.2
Inpari 10 + A6-bentonite	14.8^{cd}	16.5^{abc}	13.9
Sintanur + A8-bentonite	14.5^{cd}	13.3^{d}	15.7
Mean	15.1	15.6	-

Numbers in each column followed by the same letter are not significantly different by the Duncan's Multiple Range Test at P = 0.05. A2 = *B. firmus* E65; A6 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b; A8 = *B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31. SKM= *S. marcescens*.

Table 4. Effect of different bio-formulations on rice yields of rice cultivars. Cianjur, Wet Season 2010/2011.

Cultivar	Yield (t/ha) w.c 14%		
	With formulation	Without formulation (control)	Mean
Code + A2-bentonite	3.7^{bc}	3.0^{c}	3.3
Sintanur + A2-bentonite	4.2^{ab}	4.7^{ab}	4.5
Inpari 10 + SKM-kaolin	4.4^{ab}	4.9^{a}	4.7
Inpari 13 + E76-bentonite	3.8^{bc}	2.9^{c}	3.3
Inpari 10 + A6-bentonite	4.1^{ab}	4.3^{ab}	4.2
Sintanur + A8-bentonite	4.6^{ab}	3.0^{c}	3.8
Mean	4.1	3.8	-

* Rice grain yield was measured at 14% seed water content. Numbers in each column followed by the same letter are not significantly different by the Duncan's Multiple Range Test at P = 0.05.A2= *B. firmus* E65; A6 = *B. cereus* II.14 + *B. firmus* E65 + *P.aeruginosa* C32b; A8 = *B. cereus* II.14 + *B. firmus* E65 + *P.aeruginosa* C32b + *S. marcescens* E31. SKM = *S.marcescens*.

up to 4 x 10^7 CFU/ml, meaning that viability was gradually decreased after three month storage.

SRI increases rice production and raises the productivity of land, labor, water and capital through different practices for managing very young seedlings in transplanting; singly or only one per hill instead of 3-4 together to avoid root competition and used widely spaced to encourage greater root and canopy growth. Under SRI cultural practices use of soil amendment with organic manure of livestock could affect physical and biological soil content. In addition, the supplement of local microorganisms practice by farmer may also improve microbial community that promote plant growth as well as suppress plant diseases (Anita and Chellappan 2011).

It was demonstrated in the previous study that several bacterial isolates have relatively high potent to inhibit multiple plant pathogens both *in vitro* and *in vivo* test (Suryadi *et al.* 2011). The result indicates that BB disease reduction may not dependent on bio-formulation itself, but also related to the bacterial species used in the formulations. It was shown that although the percentages of BB reduction did not reach maximum inhibition, some of the consortium still provided substantial reduction in development of BB disease relative to the control. Among the two bacterial consortium used in this study, the consortium A8–bentonite treated on cv. Sintanur showed similar inhibition with that of cv. Code. These results compared favorably with previous results, when it was reported that mixed microbial consortium A8-bentonite showing 37.5% sheath blight disease reduction after four weeks observation (Suryadi *et al*. 2013b).

Some pathogens are more resistant to control by bacterial cells and this may be attributed to their virulence differences. This supports the earlier findings while investigating the suppression of different bacterial isolates against fungus sheath blight *(Rhizoctonia solani*); However, no clear relationship can be observed between the species and the suppression efficiency using the isolates in this study. It was pointed out that strains of *P. aeruginosa* could induce rice resistance against sheath blight by producing different antifungal activity (salicylic acid and peroxidase content) (Saikia *et al.* 2006).

The higher capabilities of consortium A8 and A6 to inhibit BB pathogens within period of observation indicated that mix culture isolates might be capable of reducing BB inoculums. One bacterial isolate may be able to cause an inhibition of one pathogen, which consequently renders it more accessible to another organism that otherwise is unable to reduce BB pathogen. Suryadi *et al*. (2013b) also reported an example of this approach using a mixed culture containing at least four distinct bacterial species for the suppression of rice blast pathogen (*P.oryzae*).

With regards to pathogen reduction probably may take place in anaerobic conditions which indicates that a minimum amount of oxygen present in the facultative anaerobic condition (static condition) was still needed for the consortium to maintain their basic cellular activity. All isolates incubated in the mixed culture could reduce disease severity, suggesting some degree of synergism; Nevertheless the percentage of BB reduction in the 2010/2011 WS by consortium formulation was slightly higher

than those of Inpari 10-(SKM kaolin); Inpari 13-(E76-bentonite); and Sintanur-(A2-bentonite), but lower compare with Code-(A2-bentonite) cultivars treatment. The main factors responsible for the yield enhancement in SRI management were longer panicles with more grains, better grain filling and a significant increase in grain weight (Thakur *et al.* 2010).

The present study indicates that use of formulation bacteria tends to improve rice yield up to 8% compared with that of untreated plot (without formulations). This result may have been due to indirect effect of antagonism as well as competitions with *Xoo* pathogens for essential nutrients. Further study on the use bacterial consortium to BB disease is needed by developing suitable delivery technology specific for certain microorganism use as biocontrol agents.

CONCLUSIONS

1. Bio-control efficacy among different rice cultivars showed BB disease reduction ranging from 10.5% to 29.4% in 2010/2011 WS.
2. The consortium A6 (*B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b) and A8 (*B. cereus* II.14 + *B. firmus* E65 + *P. aeruginosa* C32b + *S. marcescens* E31) with bentonite carrier reduced BB infections up to 25%. The performance of consortium A6-bentonite formulation also gave better effect than the individual isolate, such as that with *Burkholderia* sp. E76 or *S. marcescens* SKM.
3. The use of consortium bacterial formulation increased rice yields up to 8% in the Wet Season 2010/2011 than that of the untreated plot.
4. This study showed that the advantages of single or mixed cultures are apparent and further exploitation of selected bacterial consortium will be beneficial to suppress BB in the field.

ACKNOWLEDGEMENTS

The authors thanks the IAARD-the Indonesian Ministry of Agriculture (APBN 2010), and KKP3T (2011) for financial supports. We also grateful to Mr. Jenal Sobirin for his contribution in preparing the SRI field site at the organic farmer group "Mandiri", Cipeuyeum, Cianjur (West Java).

REFERENCES

Agustiansyah, S. Ilyas, Sudarsono, dan M. Machmud. 2010. Pengaruh perlakuan benih secara hayati pada benih padi terinfeksi *X. oryzae* pv. *oryzae* terhadap mutu benih dan pertumbuhan bibit. J. Agron. Indonesia 38(3): 185-191.

Anas, I., O. P. Rupela, T. M. Thiyagarajan, and N. Uphoff. 2011. A review of studies on SRI effects on beneficial organisms in rice soil rhizospheres. Paddy and Water Environ. 9:53-64.

Anitha, S. and M. Chellappan. 2011. Comparison of the system of rice intensification (SRI), recommended practices, and farmer methods of rice (*Oryza sativa* L) production in the humid tropics of Kerala, India. J. Tropic. Agric. 49(1-2):64-71.

Ardakani, S.S., A. Heydari, N. Khorasani, and R. Arjmandi. 2010. Development of new bioformulations of *Pseudomonas fluorescens* and evaluation of these products against damping-off of cotton seedlings. J. Plant Pathol. 92: 83-88.

EPPO. 2007. *Xanthomonas oryzae*. Diagnostics. 37(3): 543-553.

Gnanamanickam S., P. Vasudevan, and Velusamy. 2004. Biological control of bacterial blight of rice in India with rice-associated bacteria: insight into mechanism. Centre for Advanced Studies in Botany, Univ. Madras-Gundy Campus, Chennai, India 600035.

IRRI. 1996. Standard Evaluation System for Rice. IRRI, Manila, the Philippines.

Jochum, C.C., L.E. Osborne, and G.Y. Yuen. 2006. Fusarium head blight biological control with *Lysobacter enzymogenes* strain C3. Biol. Control 39:336-344.

Kadir, T.S., Y. Suryadi., and A. Ruskandar. 2007. Bacterial blight of upland rice. Proc. 3rd Asian Conf. Plant Pathol. The Role of Plant Pathology in Rapidly Globalizing Economies of Asia. Faculty of Agriculture, Gajah Mada University, Yogyakarta.

Kilic. E.O and G.Y Yuen. 2004. Comparison of strains of *Lysobacter enzymogenes and* PGPR for induction of resistance against *Bipolaris sorokiniana* in tall fescue. Biol. Control 30:446-455.

Kita, N., T. Ohya, H. Oekusa, K. Nomura, M. Manago, and M. Shoda. 2005. Biological control of damping-off of tomato seedlings and cucumber *Phomopsis* root rot by *B. subtilis* RB 14-C. JARQ 39(2):109-114.

Nalisha, I., M. Muskhazli, and T. Norfarizan. 2006. Production of bioactive compounds by *B. subtilis* against *Sclerotium rolfsii.* Mal. J. Microbiol. 2(2) 19-23.

Saikia, R., R. Kumar, D.K. Arora, D.K. Gogoi, and P. Azad. 2006. *P. aeruginosa* inducing rice resistance against *R. solani*: Production of salicylic acid and peroxidase. Folia. Microb. 51(5):375-380.

Shanti, M.L., M.L.C. George, C.M.V. Cruz, M.A. Bernardo, and R.J. Nelson. 2001. Identification of resistance genes effective against rice bacterial blight pathogen in Eastern India. Plant Dis., 85: 506-512.

Sudir, Suprihanto, dan T.S. Kadir 2009. Identifikasi patotipe *Xanthomonas oryzae* pv. *oryzae*, penyebab penyakit hawar daun bakteri di sentra produksi padi di Jawa. Penel. Pertanian Tan. Pangan 28(3):131-138.

Suryadi, Y., D.N. Susilowati, K.E. Putri, and N.R. Mubarik. 2011. Antagonistic activity of indigenous Indonesian bacteria as the suppressing agent of rice fungal pathogen. J. Internat'l. Environ. and Appl. Sci. 6(4):558-568.

Suryadi, Y., D.N. Susilowati, A. Akhdiya., T.S. Kadir, and W. Baskoro. 2013a. Efficacy of Consortium Bacteria for Control Rice Diseases under System of Rice Intensification (SRI) in West Java-Indonesia. Albanian J. Agric. Sci. 2013; 12(1): 143-147.

Suryadi, Y., D.N. Susilowati, E. Riana, and N.R. Mubarik. 2013b. Management of rice blast disease (*Pyricularia oryzae*) using formulated bacterial consortium. Emir. J. Food Agric. 25(5): 349-357.

Thakur, A.K., N. Uphoff, and E. Anthony. 2010. An assessment of physiological effects of system of rice intensification (SRI) practices compared with recommended rice cultivation practices in India. Expt'l. Agric.46(1): 77-98.

Ting, A.S.Y., M.T. Fang, and C.S. Tee. 2009. Assesment of the effect of formulative materials on the viability and efficacy of *Serratia marcescens* a biocontrol agent against *Fusarium oxysporum* f.sp *cubense* race 4. Amer. J. Agric. & Biol. Sci. 4(4): 283-288.

Turner, B.L. and P.M. Haygarth. 2001. Phosphorus solubilization in rewetted soils. Nature 411: 258.

Uphoff, N. and A. Satyanarayana. 2006. Prospects for rice sector improvement with the system of rice intensification with evidence from India, pp. 131-142. *In* Book 1. Proc. Rice, Industry, Culture and Environment. IAARD, Jakarta.

Velusamy, P., J.E. Immanuel, S.S. Gnanamanickam, and L. Thomashow. 2006. Biological control of rice bacterial blight by plant-associated bacteria producing 2,4-diacetylphloroglucinol Can. J. Microbiol. 52: 56–65.

Zhang, Z. and G.Y. Yuen. 2000. The role of chitinase production by *Stenotrophomonas maltophila* strain C3 in biological control of *B. sorokiniana*. Phytopathol. 90:384-389.