

## Capability of *Streptomyces* spp. in Controlling Bacterial Leaf Blight Disease in Rice Plants

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**Abstract: Problem statement:** Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most damaging disease in lowland rice growing areas in Indonesia. *Streptomyces* spp. have been known as a producer of antimicrobial compounds that can be used as biocontrol agents. This study examined the ability of three promising indigenous *Streptomyces* isolates which were previously selected from *in vitro* agar media and greenhouse test to suppress natural infection of *Xoo* during dry and wet season trials in 2009/2010 at the Muara Experimental Research Station, Bogor West Java, Indonesia. **Approach:** *Streptomyces* isolates (PS4-16, LBR-02 and LSW-05) were applied through seed coating in a peat-based carrier followed by seedling soaking, spray treatment, or combination of both methods, either singly or in combination of two or three isolates. The number of *Streptomyces* population in the peat carrier at the time of inoculation was above  $10^7$  cell  $g^{-1}$ . The efficacy of *Streptomyces* was compared to that chemical spray using NORDOX 56 WP (a.i., zinc oxide 56%) and non-treatment. Treated and untreated seeds were grown in plots ( $5 \times 5$  m<sup>2</sup>) and set in a randomized complete block design with four replications. **Results:** In the dry season experiment, application of *Streptomyces* spp. reduced BLB severity when compared to that of untreated plots, although did not reduce BLB incidence. PS4-16, applied singly through seed coating followed by seedling soaking, reduced the Area Under Disease Progress Curve (AUDPC) at 70 Days After Planting (DAP) to 1458, which was equally effective to the chemical spray (AUDPC value 1434) and simultaneously promoted plant height and gave the highest rice yield. In the wet season trial PS4-16 and LBR-02, applied singly or in dual combination through seed coating followed by seedling soaking, suppressed BLB severity, PS4-16 was confirmed as the most effective isolate by reducing the AUDPC to 1923, which was not significantly different to the AUDPC value obtained from chemical spray treatment (1934). **Conclusion/Recommendations:** All *Streptomyces* isolates had a tendency to increase plant and yield compared to the chemically-sprayed and non-treated plots. For successful biological control of rice BLB, further development of a better formulation for long-term storage with an effective population density of *Streptomyces* and an assessment of its field efficacy in multi-location trials are needed.

**Key words:** Bacterial Leaf Blight (BLB), *Xanthomonas oryzae* pv. *oryzae*, *Streptomyces* spp., rice plant

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### INTRODUCTION

Bacterial Leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important disease of lowland rice growing areas in Indonesia. The disease affects rice production quantity of decreasing harvest and weight of 1000 grains and qualitatively by impairing grain filling and increasing

grain vulnerability during milling process. Crop losses due to *Xoo* infection have been reported since 10-95%.

BLB disease is difficult to control effectively. Rice varieties with race-specific resistance have been the most important method to control BLB disease. Unfortunately, race specific resistant can promote the buildup of new *Xoo* race and result in the failure of resistant rice varieties. On the other hand, chemical pesticides which applied

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preventively and creatively can be an effective method to control BLB, but it is toxic to users, consumers and other non-target organism and may persistent in nature thus accumulate in ecosystems. This condition may have a negative impact on the environment in the long term. Application with bactericides such as Kasugamycin, Phenazin and Streptomycin can suppress the intensity of the BLB in the field but farmers will still have an obstacle because the price is expensive and not environmentally friendly. Alternative control methods can be developed for effectively controlling BLB in rice plant.

One component of integrated disease control is biological control using microorganisms from Actinomycetes group. Actinomycetes are a group of filamentous, Gram positive bacteria with a high G+C content in their DNA. These organisms are aerobic, saprophytic and mesophilic forms whose natural habitat in soil. Several members of the Actinomycetes produce important secondary metabolites, including antibiotics, siderophore, enzyme and plant growth-promoting substances which may contribute to their host plants by promoting growth and enhancing their ability of withstanding the environmental stressing (Khamna *et al.*, 2009; Qin *et al.*, 2011). Over 55% of antibiotics have been isolated from genus *Streptomyces* (Embley and Stackebrandt, 1994) and therefore this genus is one of several biological control agents which are widely studied and used to control various plant pathogens (El-Abyad *et al.*, 1993). For instance, Prabavathy *et al.* (2006) reported that the effectiveness of *Streptomyces* sp. PM5 inhibit the mycelia growth of rice blast fungus *Pryricularia oryzae* and the rice sheath blight fungus *Rhizoctonia solani* was related to the production of aliphatic antifungal compounds (SPM5C-1 and SPM5C-2) which have lactone and ketone carbonyl units. Commercial formulation of antibiotics or mycelia and spores of *Streptomyces* that are effective to control *Rhizoctonia solani* on rice and *Fusarium* wilt and *Botrytis* gray mold on vegetable crops or ornamental plants have been sold under the name Jinggaamycin and Mycostop™ in Finland (Fravel, 2005).

Indigenous *Streptomyces* spp. isolated from Indonesia soil were reported to produce a variety of compounds which inhibiting the growth of Gram-positive bacteria *Bacillus subtilis* and Gram-negative *Xanthomonas axonopodis* (Lestari, 2006). Five *Streptomyces* isolates that inhibited *Xoo* growth in agar medium tests were shown to effectively suppress *Xoo* infection under pot experiment using sterile soil (Unpublished data). However, effective control by *Streptomyces* under controlled environment conditions may not be transferable to the field conditions. Complex interaction present in natural soil among indigenous soil

microorganisms, host, pathogen and a variety of environmental conditions, which can positively or negatively affect the biological control activity of microbes. This suggests that the effectiveness of *Streptomyces* spp. to control BLB disease must be assessed further in the field. To our knowledge, field efficacy of *Streptomyces* to control *Xoo* infection and its simultaneous effects on plant growth and yield has not been well studied in Indonesia. The purposes of this study were to (1) evaluate the ability of *Streptomyces* spp. to control natural *Xoo* infections in the field and (2) study the effect of *Streptomyces* spp. on the rice plant growth and yield.

## MATERIALS AND METHODS

The experiment was conducted in two cropping seasons, dry season (May until September, 2009) and wet season (December 2009 until April 2010) at the Muara Experimental Research Station, Bogor, West Java, Indonesia and Laboratory of Soil Biology, Indonesian Soil Research Institute. A composite soil sample of Latosol surface layer (0-20 cm depth) was collected and analyzed for soil pH, C-organic and selected nutrients according to standard procedures of the Soil Chemistry Laboratory of Indonesian Soil Research Institute (ISRI) in Bogor. Data in Table 1 indicates that the soil is acidic, with low organic content, low Cation Exchange Capacity and P content is very high. Population of soil microbes as prior to planting was  $6.4 \times 10^5$  cfu, actinomycetes  $4.6 \times 10^5$  cfu and fungi  $2.3 \times 10^3$  cfu per gram of soil. Three isolates (PS4-16, LBR-02 and LSW-05) obtained from Microbiology Division, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University were used in these experiments.

**Streptomyces spp. Preparation:** Each *Streptomyces* spp. isolate was propagated in 200 ml of ISP- 4 medium and incubated for 10 days at room temperature. The culture was harvested by centrifuging at 10,000 rpm (Beckman Coulter, Avanti® J-E) for 15 minutes to separate the pellet from the supernatant. For preparation of peat-based formula, 2 g of cell biomass was mixed with 40 g sterile peat and incubated for a week. To prepare a liquid formulation, the pellet and supernatant were mixed in a ratio of 1:1 and 40 mL of this mixture was diluted in 4 L of water containing 1% gum Arabic and 5 drops of Tween 20.

**Planting preparation:** Two hundred grams of rice seed (cv. IR 64, susceptible to *Xoo* race IV) was rinsed with tap water and soaked for 24 hours to stimulate germination.

Table 1: Soil chemical characteristic in Muara Experimental Research Station, Bogor, West Java Province, Indonesia

Soil characteristic	Value
pH (extract 1:5)	
H <sub>2</sub> O	5.40
KCl	4.80
Organic content	
% C (Walkley& Black)	1.71
% N (Kjeldahl)	0.15
C/N	11.00
HCl 25%	
P <sub>2</sub> O <sub>5</sub> (mg /100 g)	147.00
K <sub>2</sub> O (mg/100 g)	19.00
P <sub>2</sub> O <sub>5</sub> (ppm) Olsen	176.00
K <sub>2</sub> O (ppm) Morgan	113.00
CEC (NH <sub>4</sub> -Acetat 1N, pH7)	
Ca (cmol (+) /kg)	10.32
Mg (cmol (+) /kg)	2.93
K (cmol (+) /kg)	0.22
Na (cmol (+) /kg)	0.19
Total	13.66
CEC (%)	16.32
BS (%)	84.00
Al <sup>3+</sup> (cmol /kg)	0.01
H <sup>+</sup> (cmol /kg)	0.11

CEC = Cation Exchange Capacity, BS = Base Saturation

Half of the soaked seeds were coated with the *Streptomyces* inoculants at a dose of 200 grams ha<sup>-1</sup> and amended with Gum Arabic 2% as an adhesive. Both coated and uncoated seeds were planted in separate seedbeds. After 21 days in the nursery, seedlings were uprooted. Seedlings grown from uncoated seeds were directly transplanted to experimental plots, whereas the pre-coated seedlings were soaked for 15 m in liquid formulation of *Streptomyces* prior to planting.

**Field experiment set up:** All trials used plot sizes of 5×5 m<sup>2</sup> and planting distances of 20×20 cm<sup>2</sup> with one seedling per planting hole. Basic fertilizer (200 kg of urea, 100 kg of SP36 and 100 kg of KCl ha<sup>-1</sup>) was provided in accordance with the results of soil analysis before planting or dosage recommendations in the study site.

**Dry season experiment:** Two isolates of *Streptomyces* spp. (LBR-02 and PS4-16) were applied in three ways: A: seed coating followed by soaking seedlings, B: spraying and C: combination of method A and B. Treatments were consisted of six *Streptomyces* combination of isolating and application method which were compared to the standard chemically-sprayed plots with NORDOX 56 WP and non-treated plots as follows:

- Method A + LBR-02
- Method A + PS4-16
- Method B + LBR-02
- Method B + PS4-16

- Method C + LBR-02
- Method C + PS4- 16
- Spray application of NORDOX 56WP (2.0-2.5 g L<sup>-1</sup> of water) and
- Non- treated plots

Spraying application of *Streptomyces* was conducted every two weeks, started at 8 days after planting until 63 DAP. For comparison of *Streptomyces* inoculants treatment, the plant sprayed with NORDOX 56 WP which was widely used by farmers in the northern coastal strip of West Java to control the BLB disease. NORDOX 56 WP was sprayed with frequency and time interval equal to the spraying of *Streptomyces*.

**Wet season experiment:** One additional isolate (LSW-05) was included in this trial. *Streptomyces* was applied using only method A, either singly or in combination with the other isolates. Method A was chosen because it was shown to be the most effective application method (see Results). Six *Streptomyces* treatment combinations were tested and compared to the standard chemically-sprayed and non-treated plots as follows:

- Method A + LBR-02
- Method A + PS4-16
- Method A + LSW-05
- Method A + LBR-02 + PS4-16
- Method A + LBR-02 + LSW-05
- Method A + LBR-02 + PS4-16 + LSW-05
- Spray application of NORDOX 56WP (2.0-2.5 g L<sup>-1</sup> of water) and
- Non- treated plots

**Disease evaluation:** Efficacy of *Streptomyces* was evaluated based on the percentage of infected plants and disease severity. The percentage of infected plants were calculated based on the proportion of infected plants per plot. Ten randomly selected plants from each plot were scored for disease severity using the Standard Evaluation System of IRRI (IRRI, 1996) was: 0 = no symptoms, 1 = 1-5 % infected leaves, 3 = 6-12%, 5 = 13-25%, 7 = 26-50% and 9 = 51-100% infected leaves. The disease scores were used to calculate the score of Disease Severity of Index (DSI) using the formula:

$$DSI = \{(a_1N_1 + a_2N_2 + \dots + a_nN_n) / (\text{number of plants scored} \times 9)\} \times 100$$

where, a is the score of each plant and N was the number of plants with a certain score. The DSI data from all observation dates was converted to the Area

under Disease Progress Curve (AUDPC) using the following formula:

$$AUDPC = \sum_{i=n}^n \{([R_{i+1} + R_i] / 2) \times (t_{i+1} - t_i)\}$$

Here,  $R_i$  is DSI on the  $i$ -th observation,  $t$  is time of observation and  $n$  is the number of observations. In the dry season experiment, disease evaluation was done biweekly starting at 14 until 70 DAP, whereas in the wet season experiment, it was done once a week starting 43 until 77 DAP.

**Agronomic evaluation:** The 10-plant samples scored for disease severity was also observed for plant height, number of tillers, plant biomass and weight of filling the grain. Plant yield parameters which included the weight of dry grain and dry milled grain were obtained per plot. Plant height was measured weekly from 29-56 DAP in dry season and at 20, 32 and 70 DAP in the wet season. Plant height data from all evaluation dates was converted to the Area Under plant Height Progress Curve (AUHPC) using the formula analog to AUDPC calculation as described previously. The numbers of tillers were observed once weeks starting from 35 until 56 DAP in dry season trial and once at 77 DAP in the wet season trial. Plants were harvested at 95 DAP (dry season) and at 105 DAP (wet season).

**Statistical analysis:** Both experiments were carried out in a randomized block design with four replications for each treatment. Data were analyzed using the General Linear Model procedure of SPSS 12.0. Mean separation between treatments was done using the Duncan Multiple Range Test (DMRT) at  $p = 0.05$ .

## RESULTS

**Dry season experiment:** *Xoo* infection occurred at 14 DAP and increased with increasing age of the plant. Evaluation on the percentage of infected plants were done four times and terminated at 56 DAP when more than 93% plants have been infected in all experimental plots. At all evaluation dates, application of *Streptomyces* or NORDOX 56 WP did not significantly affect the percentage of infected plants ( $p > 0.05$ ), but significantly affected the AUDPC values at 70 DAP ( $p < 0.05$ ; Table 2). In general, PS4-16 was superior compared to LBR-02 in suppressing BLB disease, especially when applied using Method A (the seed coating followed by seedling soaking). The disease suppression by PS4-16 applied using this method was equally effective (AUDPC value 1458) to the spray application of NORDOX 56 WP (AUDPC value 1434).

Table 2: Effect of *Streptomyces* application on bacterial leaf blight on a CV. IR64 in dry season experiment

Treatment*	Infected plants on 56 DAP (%)**	AUDPC values at 70 DAP***
Method and Isolate		
A + LBR-02	96.3	1694c
A + PS4-16	94.0	1458a
B + LBR-02	94.5	1606abc
B + PS4-16	96.4	1661bc
C + LBR-02	94.2	1602abc
C + PS4-16	94.3	1480ab
Spray application of NORDOX 56WP	93.3	1434a
No treatment	95.1	1711c

The number on the lines indicated by the same letter in same column indicates no significant difference at the 5% level of DMRT. \*: Method A: seed coating followed by soaking seedlings soaking, Method B: spraying and Method C: combination of method A and B. \*\*: DAP = day after planting, averages from 4 replicated plots of 5x5 m<sup>2</sup> size.\*\*\*: AUDPC area under disease progress curve. Average of 10 plants x4 replications

Table 3: Effect of *Streptomyces* application on plant growth of rice CV. IR 64 in dry season experiment

Treatment*	AUHPC ** Values in 56 DAP	Number of tillers	Weight of plant biomass (kg)
Method and Isolate			
A + LBR-02	2475abc	17.2	0.6
A + PS4-16	2639a	16.4	0.6
B + LBR-02	2512abc	16.4	0.6
B + PS4-16	2382c	16.1	0.6
C + LBR-02	2447bc	16.4	0.6
C + PS4-16	2574ab	16.3	0.7
Spray application of NORDOX 56WP	2357c	17.8	0.7
No treatment	2443bc	16.3	0.7

The number on the lines indicated by the same letter in same column indicates no significant difference at the 5% level of DMRT. \* Method A: seed coating followed by seedlings soaking, Method B: spraying and Method C: combination of method A and B. \*\* AUHPC area under plant high progress curve. Average from 10 plants x 4 replications

Table 4: Effect of *Streptomyces* application on rice yield of CV. IR64 in the dry season experiment

Treatment*	Dry grain yield per plot (kg)**	Dry milled grain per plot (kg)	Filled grain (g)***
Method and isolate			
A+ LBR-02	10.4abc	9.2bc	8.1ab
A + PS4-16	12.0a	10.6a	9.3a
B + LBR-02	10.0bc	8.9bc	7.9b
B + PS4-16	9.3c	8.1c	7.4b
C + LBR-02	11.0ab	9.5ab	8.4ab
C + PS4-16	11.0ab	9.7ab	8.5ab
Spray application of NORDOX 56WP	9.9bc	8.8bc	7.8b
No treatment	9.8bc	8.9bc	7.8b

The number on the lines indicated by the same letter in same column indicates no significant difference at the 5% level of DMRT. \*: Method A: seed coating followed by seedling soaking, Method B: spraying and Method C: combination of method A and B. \*\*: averages from 4 replicated plots of 5x5 m<sup>2</sup> size. \*\*\* Average of 10 plants x4 replications

All treatment significantly influenced the AUPHC values at 56 DAP ( $p < 0.05$ ) but did not affect plant tiller number and biomass (Table 3).

Table 5: Effect of single or combined application of *Streptomyces* isolates on bacterial leaf blight severity caused by *Xanthomonas oryzae* pv. *oryzae* on cv IR64 in wet season experiment

Treatment*	Infected plants at 70 DAP (%)	AUDPC values at 70 DAP***
Method A and Isolate		
LBR-02	90.2	2012ab
PS4-16	91.0	1923a
LSW-05	92.3	2180b
LBR-02 +PS4-16	90.7	2092ab
LBR-02 +LSW-05	87.6	2186b
LBR-02 + PS4-16+LSW-05	90.0	2152b
Spray application of NORDOX 56WP	90.9	1934a
No treatment	90.6	2097ab

The number on the lines indicated by the same letter in same column indicates no significant difference at the 5% level of DMRT \*: Method A: seed coating followed by seedling soakings \*\*\*Average from 10 plants ×4 replications

Table 6: Effect of single or combined application of *Streptomyces* isolates on plant growth of rice cv. IR64 in wet season experiment

plant Treatment*	AUHPC values at 71 DAP**	Number of tiller**	Weight of biomass (kg)**
Method A and Isolate			
LBR-02	3867a	15.5ab	0.71ab
PS4-16	3744ab	3.7c	0.73a
LSW-05	3592bc	14.0bc	0.61bc
LBR-02 +PS4-16	3722ab	15.0abc	0.66abc
LBR-02 +LSW-05	3757ab	16.0a	0.70ab
LBR-02 + PS4-16+LSW-05	3775ab	15.6a	0.72a
Spray application of NORDOX 56WP	3538c	15.5ab	0.57c
No treatment	3517c	15.1abc	0.64abc

The number on the lines indicated by the same letter in same column indicates no significant difference at the 5% level of DMRT \* Method A: seed coating followed by seedling soaking \*\*Average from 10 plants ×4 replications

Table 7: Effect of single or combined application of *Streptomyces* isolates on yield of rice cv. IR64 in wet season experiment

Treatment*	Dry grain weight (kg)	Dry milled grain (kg)	Filled grain (g)
Method A and Isolate			
LBR-02	8.2	7.3	6.6
PS4-16	8.5	7.5	6.5
LSW-05	7.6	6.9	6.0
LBR-02 +PS4-16	8.0	6.7	6.1
LBR-02 +LSW-05	8.4	7.5	6.8
LBR-02 + PS4-16+LSW-05	8.7	7.8	7.1
Spray application of NORDOX 56WP	7.6	6.8	6.1
No treatment	8.0	7.2	6.4

The number on the lines indicated by the same letter in same column indicates no significant Difference at the 5% level of DMRT\* Method A: seed coating followed by seedling soaking

With exception of the application of PS4-16 using Method B and LBR-02 using Method C, disease suppression by all *Streptomyces* applications resulted in increased plant height. In contrast, plants received NORDOX 56 WP treatment did not show improved

plant height despite low levels of BLB severity. PS4-16 in general was also superior to LBR-02 in increasing plant height, especially when applied using Method A.

Plant yield, as measured by the weight of dry grain yield, dry milled grains and filled grains, were significantly affected by all treatments ( $p<0.05$ ). The highest yield was obtained from application of PS4-16 using Method A (Table 4).

**Wet season experiment:** BLB disease developed slowly at the onset of the trial. Percentages of infected plants as high as 87% was achieved at 70 DAP. All treatments did not significantly affect the proportion of infected plants ( $p>0.05$ ), but affected AUDPC values at 77 DAP ( $p<0.05$ : Table 5). PS4-16 applied singly was the most effective treatment in suppressing BLB severity with AUDPC value of 1923, which was comparable to that of spray application with NORDOX 56 WP (AUDPC value 1934). LBR-02 applied singly or in combination with PS4-16 was also fairly effective to reduce the disease, with the AUDPC values of 2012 and 2092, respectively. LSW-05 had the lowest effectiveness compared to other isolates. Dual or triple combination of LSW-05 with the other isolates did not always improve its effectiveness (Table 5).

Plant growth as measured by AUHPC values, number of tillers and plant biomass, was significantly affected by all treatment applications ( $p<0.05$ ). Plants received *Streptomyces* treatment not always show significant difference of plant growth compared to the NORDOX 56 WP and non-treated plots (Table 6). Single or combined *Streptomyces* isolates were equally effective in promoting plant growth except for PS4-16, which did not increase the number of tillers and LSW-05, which did not promote the three growth parameters.

In contrast to the dry season experiment results, *Streptomyces* application in the wet season trial had no significant effect on rice yield as measured by the weight of dry grain, dry milled grain and filled grain (Table 7). However, PS4-16 and LBR-02, either given singly or in dual or triple combination with the other isolates, tended to increase rice yield compared to that of NORDOX 56 WP or non-treated plants.

## DISCUSSION

In this study, BLB disease control was successfully achieved by the application of *Streptomyces* through seed coating, followed by seedlings soaking during two planting seasons (wet season and the dry season). A single application of *Streptomyces* PS4-16 inoculant was effective in controlling BLB disease with the value of AUDPC 1923 and 1458 in the wet season and dry season

respectively. The disease suppression by PS4-16 was equal to that obtained from the chemical spray with NORDOX 56 WP. LBR-02 was less effective than PS4-16, whereas LSW-05 was least effective compared to both isolates. These results partly confirmed the results of *in vitro* studies by Papuangan (unpublished data) in which the isolates used in this study were able to inhibit the growth of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) to form a clear zone of 4 mm (PS4-16) and 5.5 mm (LBR-02). Furthermore, the result also confirmed the effectiveness in a greenhouse experiment by using sterile soil (Hastuti, unpublished data) which showed that these isolates could suppress the BLB disease in IR64 Rice plants. This shows that under natural conditions many factors may inversely affect the effectiveness of *Streptomyces* and therefore field studies are needed.

The ability of rhizosphere bacteria such as *Streptomyces*, when applied as a seed dressing, to reduce foliage pathogen infection was also documented by other workers. Sabaratnam and Traquiaria (2002); Shimizu *et al.* (2009) and Patil *et al.* (2010) reported that seed application of *Streptomyces* successfully control damping off by *Rhizoctonia solani* in tomato seed with maximum disease suppression 53.33% and cucumber anthracnose on cucumber (*Cucumis sativus* L.) caused by *Colletotrichum orbiculare*.

As biological control agents, *Streptomyces* could indirectly act through increased plant fitness whereas the disease suppression may occur through the induction of systemic resistance of plants and production of bioactive compounds, such as antibiotic compounds, siderophore and some lytic enzymes such as glucanase, chitinase and cellulase (Compant *et al.*, 2005; Hasegawa *et al.*, 2006). It could be seen from Table 3, where the inoculation of *Streptomyces* can suppress the severity of the disease has a positive effect on plant height, while giving NORDOX 56WP only able to suppress the severity of the disease course. *Streptomyces* can inhibit *Xoo* through antagonistic mechanism and it, also capable of producing growth regulators or Indole Acetic Acid (IAA). Another mechanism by which disease suppression occurs in foliage by rhizosphere bacteria is induced systemic resistance or ISR (Choudhary *et al.*, 2007). Plant resistance mechanism in this way involves the translocation of plant signal from roots to foliage that activates plant resistance. Application of *Streptomyces* through seed coating may induce systemic resistance. Early colonizing roots of germinated seeds may activate natural plant resistance mechanisms that will increase the capacity of plant defenses against multiple pathogens that will attack later. Soaking seeds in *Streptomyces* suspension further increase the population of *Streptomyces* that can colonize the roots.

Although *Streptomyces* seems to be very promising as *Xoo* biocontrol agents from our point of view, their development into commercial formulation poses several difficulties. The carrier material used must provide favorable conditions for long shelf-life and survival in soil when applied (Georgakopoulos *et al.*, 2002). Addition of selective substrates such as calcium-alginate was reported by Shrivastava *et al.* (2008) to increase the effectiveness of *Streptomyces*. Many factors can determine the viability and effectiveness of biological control agents by *Streptomyces*, among others; environmental conditions such as soil temperature, soil water status, soil nutrient availability and population density of pathogens (Xiao *et al.*, 2002) can certainly influence the biological function of microbial inoculants. Therefore, further studies are needed to develop a better formulation for long-term storage with an effective population density of *Streptomyces* and assess its field efficacy in multi-location trials.

## CONCLUSION

*Streptomyces* spp. Generally suppressed BLB disease severity although it did not reduce the percentage of infected plants. *Streptomyces* PS4-16 which was applied through seed coating and seedling soaking techniques was consistently the most effective *Streptomyces* isolate in controlling BLB disease during dry and wet season trials. An AUDPC value obtained from this treatment was 1458 and 1923, for dry and wet season respectively, which are comparable to those obtained from the standard chemical spray. *Streptomyces* isolates, especially PS4-16 tended to increase plant growth and yield.

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