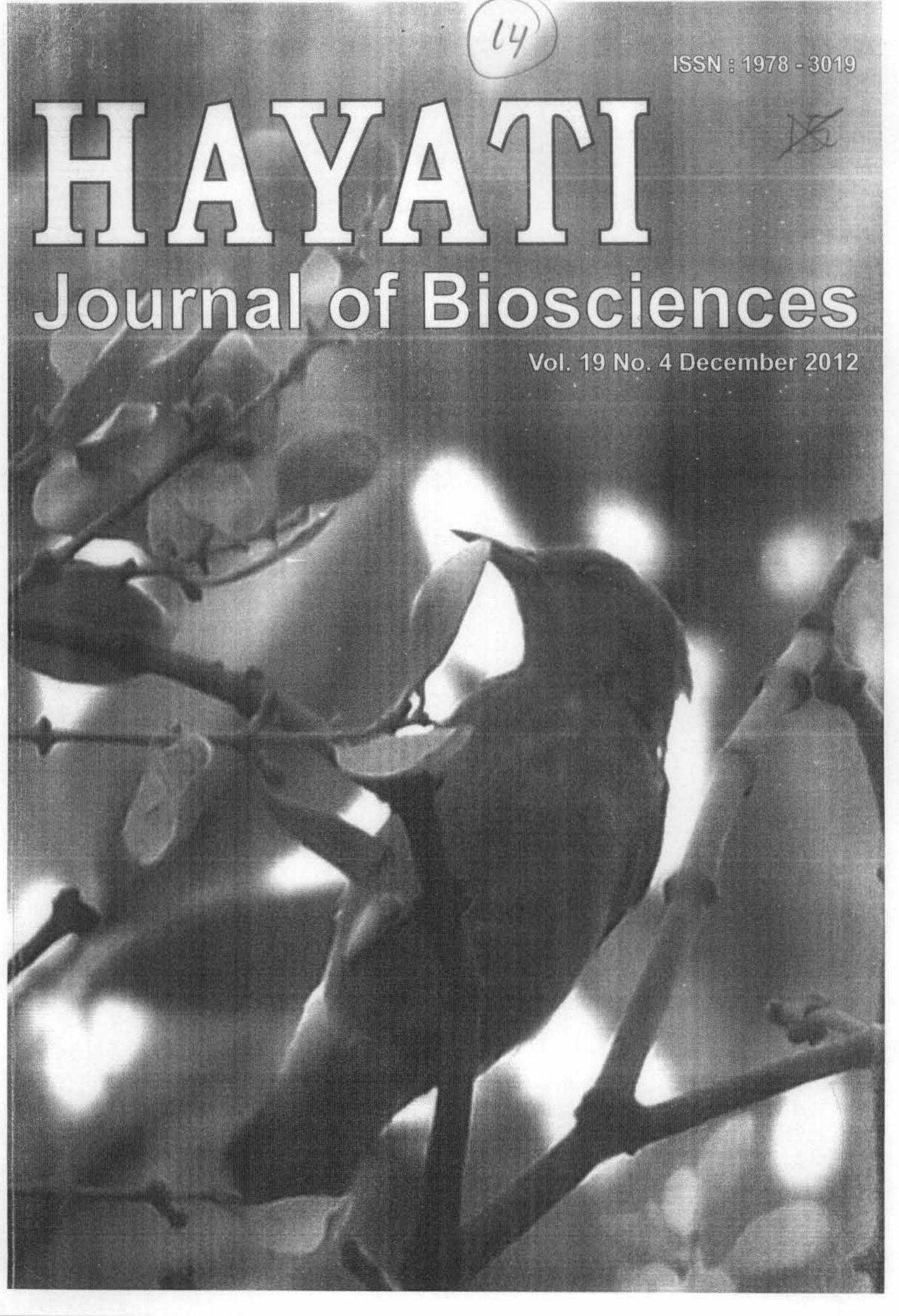
ISSN : 1978 - 3019

HAYATI

Journal of Biosciences

Vol. 19 No. 4 December 2012



ISSN: 1978-3019 (printed)
EISSN: 2086-4094 (electronic)

HAYATI
Journal of Biosciences

Vol. 19 No. 4 December 2012

Accredited by Indonesian Directorate General of Higher Education No. 80/DIKTI/Kep/2012

Granted for Internationalization Programe of Scientific Journal by Directorate General of Higher Education No. 773/D3/LL/2009

PUBLISHER
Indonesian Society for Biology and
Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University

EDITORIAL ADDRESS
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Phone/Fax: +62-251-8421258; E-mail: hayati_j_biosci@cbn.net.id
http://journal.ipb.ac.id/index.php/hayati

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HAYATI Journal of Biosciences December 2012
Vol. 19 No. 4, p 155-162
ISSN: 1978-3019

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.19.4.155

Endophytic *Streptomyces* spp. as Biocontrol Agents of Rice Bacterial Leaf Blight Pathogen (*Xanthomonas oryzae* pv. *oryzae*)

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Received April 18, 2012/Accepted October 23, 2012

***Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a causal agent of bacterial leaf blight (BLB), is one of the most important pathogens of rice. The effectiveness of ten *Streptomyces* spp. isolates in suppressing *Xoo* disease was assessed *in planta* and *in vitro*. *In planta* experiments were carried out in a greenhouse and arranged in a randomized completely block design (RCBD) with three replications. Twenty treatments were tested which included plants inoculated with both *Streptomyces* spp. and *Xoo*, and plants inoculated with only *Streptomyces* spp. Plants inoculated with *Xoo* and sprayed with a chemical bactericide, and plants inoculated with only *Xoo* served as positive controls, whereas plants not inoculated with either *Streptomyces* spp. or *Xoo* were used as negative controls. The results showed that the effect of endophytic *Streptomyces* spp. on BLB disease expressed as area under disease progress curve (AUDPC) was not significantly different to that on control plants ($P > 0.05$). However, plants inoculated with endophytic *Streptomyces* spp. were significantly taller and produced higher tiller number than control plants ($P < 0.05$). *Streptomyces* spp. isolate AB131-1 gave the highest plant height. *In vitro* studies on biocontrol mechanisms of selected *Streptomyces* spp. isolates showed that isolate LBR02 gave the highest inhibition activity on *Xoo* growth, followed by AB131-1 and AB131-2. Two isolates (AB131-1 and LBR02) were able to produce chitinase, phosphatase, and siderophore which included biocontrol characteristics. Morphological and colonization studies under SEM and light microscopy confirmed that the three isolates were endophytic *Streptomyces* spp. from different species. These studies found that the paddy plant which was inoculated with endophytic *Streptomyces* spp. AB131-1 and infected by *Xoo* could increase the height of plant and number of tillers.**

Key words: endophytic *Streptomyces* spp., *Xanthomonas oryzae* pv. *oryzae*, biocontrol character, BLB disease suppression, rice plant

INTRODUCTION

Rice is an important food crop in Indonesia where more than 50% of the population consumes rice as a staple food. One of the major constraints in rice productivity is bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*). In 2011, BLB disease incidences reached 69.633 ha, where the most damaged areas were located in West Java [http://tanamanpangan.deptan.go.id/doc_upload/padi_blb_pdf. (15 April 2012)]. Infection of leaves by *Xoo* results in low plant quality and yield. Chemical bactericides, are routinely used to control this disease in Indonesia. However, excessive dependence on chemical bactericide frequently causes environmental pollution and outbreaks of resistant pathogens. Furthermore, bactericide residues on grain may cause health problems to consumers. Therefore, the use of microbe-based biocontrol agents such as endophytic bacteria belonging to the actinomycetes group has been pursued as an alternative replacement or supplement for chemical bactericides.

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Endophytic bacteria colonize healthy plant tissue without causing symptoms or damages to the host (Hallman *et al.* 1997). They can be isolated from internal plant tissue after thorough surface-disinfection of the plant tissue, either from herbaceous or woody plants (Taechowisan *et al.* 2003b; Cao *et al.* 2004; Inderiati & Franco 2008). Endophytic microorganisms provide advantages to the host plant by enhancing the physiological activity of the plant or through other modes of action and thus may serve as a source of agroactive compounds, biocontrol agents, or plant growth promoters (Shimizu *et al.* 2009; Dombou *et al.* 2002).

Some researchers reported that actinomycetes are capable of suppressing the development of diseases caused by plant pathogenic bacteria or fungi (Hasegawa *et al.* 2006; Lestari 2006). Other researchers also reported that endophytic actinomycetes were able to protect plants from soil borne pathogens such as *Rhizoctonia solani*, *Verticillium dahliae*, *Plectosporium tabacinum*, *Gaeumannomyces graminis* var. *tritici*, *Fusarium oxysporum*, *Aphanidermatum* sp., *Colletotrichum orbiculare*, and *Phythium* sp. (Krechel *et al.* 2002; El-Tarabily 2003; Coombs *et al.* 2004; Cao *et al.* 2005; El-Tarabily *et al.* 2009; Shimizu *et al.* 2009). Furthermore,

seed treatment with endophytic *Streptomyces* sp. and *Micromonospora* sp. strain EN27 and EN43 increased the resistance of *Arabidopsis thaliana* from infection of *Erwinia carotovora* and *F. oxysporum* (Conn *et al.* 2008). Bacon and Hinton (2006) reported that varying levels of disease suppression in the field were positively correlated with those obtained from *in vitro* experiments. The growth inhibition of plant pathogen by endophytic bacteria indicates the presence of antagonistic activities between them, which may act directly, by mechanisms of antibiosis, competition, and lysis, or indirectly, by inducing plant defense and growth promoter substances (Berg & Hallman 2006).

Many convincing evidences of biocontrol activity by endophytic actinomycetes against a variety of plant pathogens have been provided, but there have been very few studies on endophytic actinomycetes isolated from rice and its antagonistic effect on *Xoo* infection.

The objectives of this experiment were to study (i) the effect of endophytic *Streptomyces* spp. on *Xoo* infection of rice plants, (ii) the effect of *Streptomyces* spp. on rice plant growth, and (iii) biocontrol mechanisms of selected endophytic *Streptomyces* spp. *in vitro*.

MATERIALS AND METHODS

Effect of *Streptomyces* spp. Inoculation on BLB Disease and Plant Growth. Seven endophytic *Streptomyces* spp. (AImp 6-1, AMbr-1, AFat-1, AB131-1, AB131-2, AB131-3, and DImp6-1) and three *Streptomyces* spp. isolates obtained from the collections ofthe Microbiology Laboratory, Bogor Agricultural University (PS4-16, LBR02, and LSW05) were used in this study. Each isolate was grown on International *Streptomyces* project (ISP) 4 medium using an incubator shaker for 10 days at 29 °C. The supernatant was collected after centrifugation at 10,000 rpm for 5 minutes at 4 °C (Avanti J-E). To prepare inoculants, the pellets were mixed with the supernatant in a ratio of 1:1. Rice seeds (cv. IR64) were surface sterilized by soaking the seeds in 96% ethanol for 1 minute, washed with sterile water three times, immersion in 0.2% $HgCl_2$ for 8 minutes, and finally washed with sterile water six times. To enhance germination the seeds were soaked in sterile water overnight. Seeds were drained and soaked for 15 minutes with the prepared inoculants of each *Streptomyces* isolate. Non *Streptomyces*–inoculated seeds were sown directly on a steam-sterilized mixture of soil and compost (1:1). Twelve days after sowing, seedlings were uprooted and subsequently dipped for 15 minutes in the inoculant preparation of each endophytic *Streptomyces* spp. Seedlings dipped in ISP 4 medium were used as control. Two seedlings were transplanted to a pot filled with 5 kg of steam sterilized soil and fertilized with urea (1.11 g urea/pot), SP-36 (0.69 g SP36/pot), and KCl (0.5g KCl/pot). An isolate of *Xoo* from pathotype IV obtained from the Indonesian Center for Rice Research Sukamandi, West Java, was grown in Potato Sucrose Broth (PSB) Wakimoto medium on a shaking incubator (150 rpm) at 30 °C for 2 days. *Xoo* concentration was adjusted to approximately 10^7-10^8 CFU/ml using a spectrophotometer. Forty-one-day-old plants were inoculated with *Xoo* by cutting the ends of leaves with a scissor which had been dipped in the suspension of *Xoo*. Plants were then sprayed with *Xoo* suspension to increase infection. Plants inoculated with *Xoo* and sprayed with chemical bactericide at a concentration of 2-2.5 g/l were used as positive control.

The experiment was performed in a Completely Randomized Block Design (CRBD) with three replications. The treatments were as follows: (1) without *Streptomyces* and *Xoo* inoculation (negative control); (2) *Xoo* ; (3) *Xoo* + chemical bactericide (positive control); (4) *Xoo* + AImp 6-1; (5) *Xoo* + AMbr1; (6) *Xoo* + AFat-1; (7) *Xoo* + AB131-1; (8) *Xoo* + AB131-2; (9) *Xoo* + AB131-3; (10) *Xoo* + DImp6-1; (11) *Xoo* + PS4-16; (12) *Xoo* + LBR02; (13) *Xoo* + LSW05; (14) AImp 6-1; (15) AMbr-1; (16) AFat-1; (17) AB131-1; (18) AB131-2; (19) AB131-3; (20) DImp6-1; (21) PS4-16; (22) LBR02; (23) LSW05.

The efficacy of *Streptomyces* inoculation was evaluated based on disease severity. Disease severity was evaluated weekly starting from 7 day after *Xoo* inoculation using the Standard Evaluation System of IRRI (Standard Evaluation System for Rice 1988) where: 0 = no symptoms, 1 = 1-5%, 3 = 6-12%, 5 = 13-25%, 7 = 26-50%, and 9 = 51-100% of leaves were infected respectively. The disease scores were used to calculate the disease severity index (DSI) using the formula:

$$DSI = \{(a_1N_1 + a_2N_2 + ... + a_nN_n) / (\text{number of plants scored} \times 7)\} \times 100$$

where a is the score of each plant and N is the number of plants with a certain score. The DSI data from all observation dates were converted to the area under the disease progress curve (AUDPC) using the following formula:

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

where R_i is the DSI on the i-th observation, t is the time of observation, and n is the number of observations.

Plant height and the number of tillers were measured weekly from 27-48 DAP. The data was converted to the area under the plant height progress curve (AUPHC) and the area under the number of tiller progress curve (AUNTC) using the formula for AUDPC calculation, as described previously. Plants were harvested at 115 DAP. Plant dry weight and grain yield data were also collected. Disease parameter and agronomic data were analyzed using the General Linear Model procedure of SPSS 12.0. The mean separation between treatments was done using the Duncan Multiple Range Test (DMRT) at P = 0.05.

***In Vitro* Studies on Biocontrol Mechanisms of *Streptomyces* spp.** The antibiosis and competition mechanism of ten endophytic *Streptomyces* spp. to suppress the *in vitro* growth of *Xanthomonas oryzae* pv. *oryzae* (*Xoo)* were detected using a dual culture assay. One milliliterof *Xoo* (10^7-10^8 CFU/ml), obtained from 24-day-old cultures in PSB Wakimoto media, was added to 10 ml of 0.3% PSA (± 55 °C) and then poured on 10 ml of PSA medium in a Petri dish which had already solidified.

The upper agar medium was allowed to solidify and air dried. A piece of agar disc (diameter 0.5 cm) of each *Streptomyces* spp. isolate was placed on the agar. Petri dishes were incubated at room temperature (± 30 °C) for 24 hours after which the diameter of the inhibition zone was measured. Treatments were replicated three times. The level of *Xoo* growth inhibition was determined by following the method of El-Tarabily *et al.* (2000) by measuring the difference between the clear zone formed (γo) and the diameter of the tested isolate (γ), or by the equation $\Delta\gamma = \gamma o - \gamma$. The level of *Xoo* growth inhibition was divided into four categories: if $\Delta\gamma \geq 20$ mm, it was scored as +++; $\Delta\gamma \geq$ 10-19 mm, ++; $\Delta\gamma \geq$ 5 -9 mm, +; and $\Delta\gamma < 5$ mm, no inhibition activity occured.

Chitinase production was determined using the methods described by Taechowisan *et al.* (2003a). An agar disc of each isolate obtained from a 7-day-old culture grown on yeast malt extract medium was placed on a Petri dish containing chitin agar medium (20 g colloidal chitin; 0.1 g K_2HPO_4; 0.1 g $MgSO_4 \cdot 7H_2O$; 1 g NaCl; 2.5 g $(NH_4)_2SO_4$; 1 g yeast extract; 20 g agar and 1000 ml distilled water). Petri dishes were incubated at 30 °C for 6 days. Observations were conducted by measuring the clear zone around the colony (halo) which indicated chitin solubilization by chitinase producing bacteria.

Phosphate Solubilization was determined by using a Pikovskaya medium. Each isolate was grown in a yeast soluble starch (YSA) medium for 7 days. An agar disc (diameter 5 mm) from each isolate was placed on a Petri dish containing Pikovskaya medium (5 g $Ca_3(PO_4)_2$; 0.5 g $(NH_4)_2SO_4$; 0.2 g NaCl; 0.1 g $MgSO_4 \cdot 7H_2O$; 0.2 g KCl; 10 g glucose; 0.5 g yeast extract; 20g agar; 0.0025 g $MnSO_4$; 0.0025 g $FeSO_4$; 1000 ml distilled water). Petri dishes were incubated at 28 °C in the dark for 2 weeks. Treatments were repeated three times. Solubilizing activity was indicated by the formation of a clear zone around the agar disc. Isolates producing a clear zone with a diameter of more than 20 mm were considered as having high phosphate solubilization activity.

Siderophore production was determined through the methods described by Macagnan *et al.* (2008). Selected isolates were grown on King's B (KB) broth medium for 10 days. The culture was centrifuged at 10,000 rpm for 10 minutes at 4 °C (Avanti JE). One milliliter of the supernatant was added with 1 ml of Chromo-azurol S (Aldrich 199 532), according to the method of Schwyn and Neylands (1987), and then mixed. Siderophore production was indicated by a change in color of the mixture from bluish-red to brown in 15 minutes. Medium containing 2 µmol L^{-1} Fe^{3+} from a sterile solution of FeSO4·$7H_2O$ was used as control. All treatments were repeated three times.

Hydrogen cyanide production by endophytic actinomycetes was detected by the alkali picric method described by Ramette *et al.* (2003). Each isolate was transferred into individual slants of YSA medium supplemented with glycine (4.4 g/l). A piece of filter paper impregnated with 0.5% of picric acid and 2% of Na_2CO_3 solution was placed on the upper medium. The test tubes were incubated at room temperature for 3 to 5 days. The assay was done with two replications. A change in color from yellow to orange-brown on the filter paper indicated the production of cyanide.

Morphological Characterization and Colonization of Selected *Streptomyces* spp. The morphologies of three selected *Streptomyces* spp. isolates were characterized based on growth and colony appearances on four media: yeast extract malt extract Agar (YMA), oatmeal agar (OA), yeast extract starch agar (YSA), and glycerol asparagine Agar (GAA), as described by Miyadoh (1997). Scanning electron microscopy (SEM, Jeol Type JSM-5310LV) with a magnification of 3500x was used to observe the formation of chain spores.

Colonization was determined by using plants which have been grown in pots filled with sterile soil for four weeks, inoculated with *Streptomyces* spp., uprooted, surface sterilized, and stained with the reducing tetrazolium method (Patriquin & Dobereiner 1978). The roots and stems of rice plants were cut by Microtom Freezing (Yamato RV-240) and Yamato Electro Freezer MC-8, put on the object glass with a drop of glycerin 50%, and then observed under a light microscope with a magnification of 40 x 10.

RESULTS

Effect of Endophytic *Streptomyces* spp. Inoculation on BLB Disease and Plant Growth. *In planta* experiments showed that the area under the disease progress curve (AUDPC) of the plants inoculated with *Streptomyces* spp. and infected by *Xoo* was not significantly different to the control. Isolate AB131-1 was able to achieve the plant height with value of AUHPC 1546.3 and dry weight of 22.9 g/pot. This value was compared with the control (*Xoo*, *Xoo* and chemical bactericide, and uninoculated) in Table 1. The growth of rice plants inoculated with only *Streptomyces* spp. was lower than the rice plants inoculated with *Streptomyces* spp. and infected with *Xoo*. However, the density and the spectrum of bacterial root endophytes was increased. This data was similar with the result reported by Hallman and Berg (2006).

The effect of endophytic *Streptomyces* spp. on the number of tillers during observation was shown by the calculation of the area under the number of tillers curve. Accumulation of the number of tillers at 48 DAP was significantly different. The plants inoculated with Dimp6-1 had a higher number of tillers than the other treatment groups. Although the effect of endophytic *Streptomyces* spp. inoculation on increasing grain yield and plant dry weight was not significantly different, the measurements were still higher compared with the control group, the group infected with *Xoo*, and chemical bactericide application.

***In Vitro* Studies on Biocontrol Mechanisms *of Streptomyces* spp.** Based on the level of growth inhibition of pathogens, the inhibition of pathogens by LBR02 was the highest (25 mm) followed by AB131-1 (13 mm), and AB131-2 (12 mm). AB131-1 isolates were able to dissolve phosphate from the Pikovskaya medium. This was

Table 1. Effect of endophytic *Streptomyces* spp. inoculation on bacterial leaf blight disease suppression, plant height, plant tiller number, plant dry weight and grain yield of rice*

Treatment	AUDPC** 69 DAP***	AUHPC 48 DAP	AUNTC 48 DAP	Plant dry weight g/pot	Grain yield g/pot
Uninoculated	-	1414.3f	82.8d	17.1	19.21
Xoo	596	1418.0ef	89.8bcd	19.2	20.76
Xoo + chemical bactericide	575	1481.9a-f	92.2a-d	19.5	22.24
Xoo + AImp 6	646	1509.8ab	101.9a-d	21.2	25.63
Xoo + AMbr 1	628	1511.6ab	102.7a-d	21.4	20.91
Xoo + AFat 1	604	1477.7a-f	89.1bcd	20.3	16.54
Xoo + AB131-1	594	1546.3a	103.4a-d	22.9	23.27
Xoo + AB131-2	490	1506.9abc	112.8abc	21.9	21.71
Xoo + AB 131-3	537	1.443.7b-f	85.2cd	19.9	18.52
Xoo + DImp 6-1	531	1437.4b-f	91.4bcd	19.6	20.76
Xoo + PS4-16	649	1449.3b-f	80.1d	17.4	17.62
Xoo +LBR02	672	1474.2b-f	95.7a-d	22.2	25.69
Xoo + LSW05	566	1508.3ab	113.6abc	21.2	25.95
AImp 6	-	1443.9b-f	102.7a-d	19.2	25.37
AMbr 1	-	1450.2b-f	97.61 a-d	21.0	22.25
AFat 1	-	1488.6a-e	115.1ab	22.5	22.93
AB131-1	-	1489.9a-d	94.9a-d	19.4	22.44
AB131-2	-	1456.5b-f	85.9bcd	17.0	16.55
AB131-3	-	1435.1c-f	100.3a-d	21.6	26.56
DImp 6-1	-	1479.8a-f	121.3a	21.2	20.33
PS4-16	-	1477.1a-f	113.2abc	20.3	26.50
LBR02	-	1433.1def	79.3d	17.5	18.63
LSW05	-	1419.1def	91.4bcd	20.1	19.08

*Average from 2 plants x 3 replications, **AUDPC area under disease progress curve, AUHPC area under plant height progress curve, AUNTC area under number of tillers progress curve. Means followed by the same letter are not significantly different according to Duncan's multiple range tests at the 5% level, ***DAP = Day after planting.

Table 2. Characters of biocontrol and plant growth promoting activity of Endophytic *Streptomyces* spp.

Treatment	Inhibition zone (mm)*	Scored of inhibition zone	Chitinase producer (mm)	Phosphate solubility (mm)	Siderophore producer	Cyanogen
PS4-16	10	++	14.0	5.0	-	-
LSW05	4.5	-	19.5	0	-	+
LBr02	25	+++	17.0	4.5	+	-
AB131-1	13	++	14.5	6.5	+	-
AB131-2	12	++	11.5	0	-	-
AB131-3	7.0	+	16.0	0	-	-
A Imp 6	6.0	+	0	0	-	+
D Imp 6	2.0	-	21.0	2.0	-	-
AMemberamo	8.0	+	19.5	1.0	-	-
A Fat	4.0	-	0	2.0	-	-

$\Delta\gamma \geq 20$ mm (+++); $\Delta\gamma \geq$ 10-19 mm, (++); $\Delta\gamma \geq$ 5-9 mm, (+); and $\Delta\gamma < 5$ mm, (no inhibition activity). +: yes, -: no.

indicated by a clear zone diameter ranging from 1 to 6.5 mm. Most of the tested isolates produced chitinase with a range of 11.5 to 21 mm, while only three isolates (SSW02, LBR02, and AB131-1) produced sideorphone, and only two isolates (LSW05 and AImp6) produced HCN (Table 2, Figure 1).

Morphology Characterization and Colonization of Selected Endophytic *Streptomyces* spp. All isolates grew vigorously on the four tested media (YMA, OA, YSA, and GAA) except isolate AB131-1, which was not able to grow on the GAA medium. Isolate AB131-1 showed the same color of aerial or substrate mycelium on all media, which was brown and cream, respectively (Table 3). The other isolates showed various colors of aerial or substrate mycelia, depending on the growth medium. The mycelium substrates of AB131-2 on media YMA, OA, and YSA were green bluish. On the GAA media, the mycelium arieal were grey, the mycelium substrates were brown, and there were none soluble pigments. LBR02 had brown mycelium aerial and substrates on YMA, OA, and YSA, and grey aerial and substrates on the GAA medium. Meanwhile, there were none soluble pigments in the YMA and GAA medium. Scanning electron microscope observations showed different types of *Streptomyces* spp. spore chain formations. LBR02 formed compact and closed spirals of spore chains, whereas AB131-1 and AB131-2 formed long and stretched spirals in two major types, open and loose (Figure 2).

The three *Streptomyces* spp. (AB131-1, AB131-2, and LBR02) were able to colonize the root tissue of the rice plant (Figure 3), which proved that selected *Streptomyces* spp. were endophytic from different species.

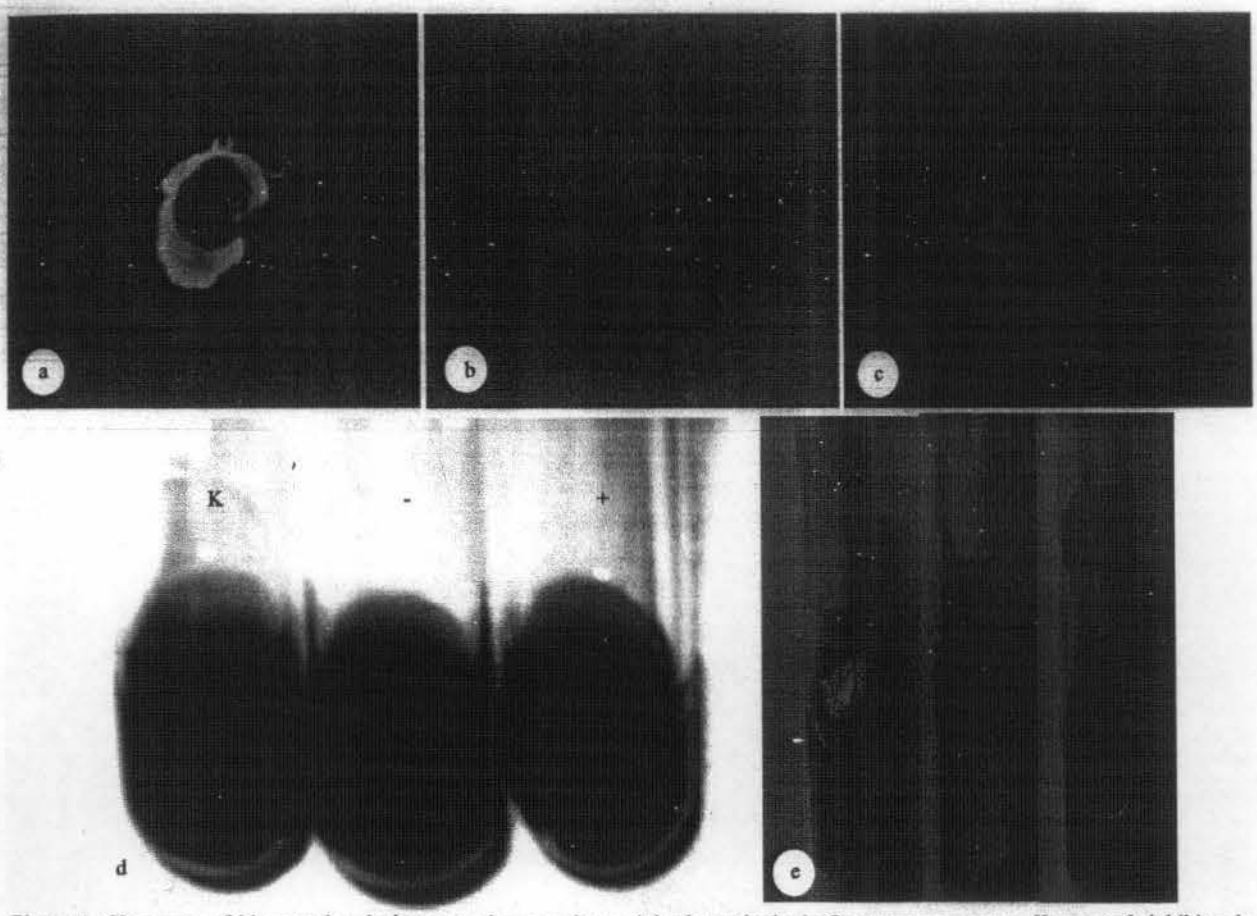

Figure 1. Characters of biocontrol and plant growth promoting activity by endophytic *Streptomyces* spp.: a. *Xoo* growth inhibitor by LBR02 as indicated by clear halo around the colony; b. chitinolytic activity by LBR02 as indicated by clear halo around colony; c. phosphate solubilization activity by AB131-1 as indicated by clear halo around colony; d. siderophore production activity by AB131-1 as indicated by brownish orange color (+); e. HCN production by LSW05 as indicated by orange color (+); K: control and -: indicates no production of the substance.

Table 3. Growth and colony morfology of AB131-1, AB131-2, and LBR02 isolates on four selected media (7 days after incubation at room temperature 28-30 °C)

Medium	Growth	Aerial Mycelium	Substrate Mycelium	Soluble Pigment
YMA				
AB131-1	++++	Brown	Brown	None
AB131-2	++++	Green Bluish	Green Bluish	Green Bluish
LBR02	++++	Brown	Brown	None
OA				
AB131-1	+++	Light Brown	Light Brown	None
AB131-2	+++	Green Bluish	Green Bluish	Green Bluish
LBR02	+++	Brown	Brown Yellowish	Brown Yellowish
YSA				
AB131-1	++++	Light Brown	Light Brown	None
AB131-2	++++	Green Bluish	Green Bluish	Green
LBR02	++++	Brown	Brown	Brown
GAA				
AB131-1	-	Not growth	Not growth	Not growth
AB131-2	+++	Grey	Brown	None
LBR02	+++	Grey	Grey	None

DISCUSSION

This study found that endophytic *Streptomyces* spp. (AB131-1, AB131-2, and LBR02) tend to reduce *Xoo* infection, increase the plant growth, and have a biocontrol characteristic. *In planta* study showed that *Streptomyces* spp. AB131-2 inoculated to rice plants infected with *Xoo* reduced the severity of BLB (AUDPC value 490 unit). Plant growth is a factor that is indirectly involved in pathogen defense. Plant growth promotion mediated by endophytic bacteria may be exerted by several mechanisms, e.g. production of plant growth hormones, synthesis of siderophores, nitrogen fixation, solubilization of mineral such as phosphorous, or via enzymatic activities (Berg & Hallmann 2006). Correlation study was conducted both *in planta* and *in vitro* studies to explain the capability of

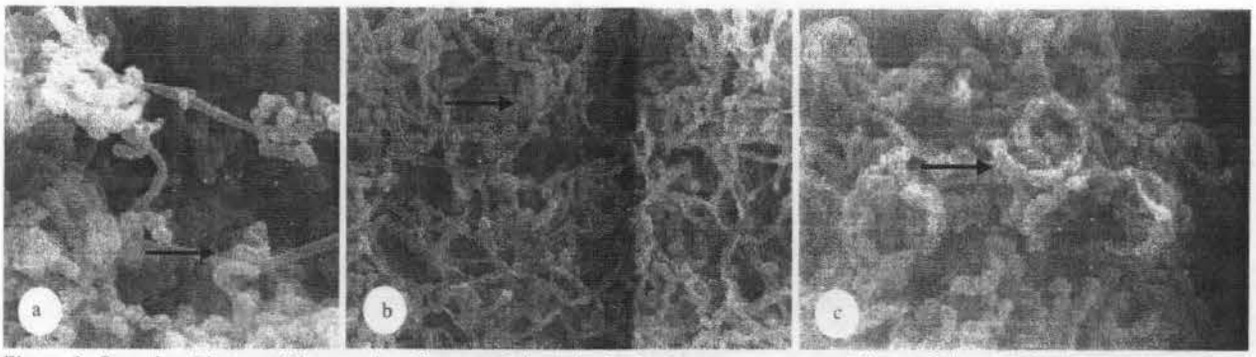

Figure 2. Scanning Electron Micrographs of spore chain endophytic *Streptomyces* spp. (20kv, x3500). a. AB131-1, b. AB131-2, c. LBR02.

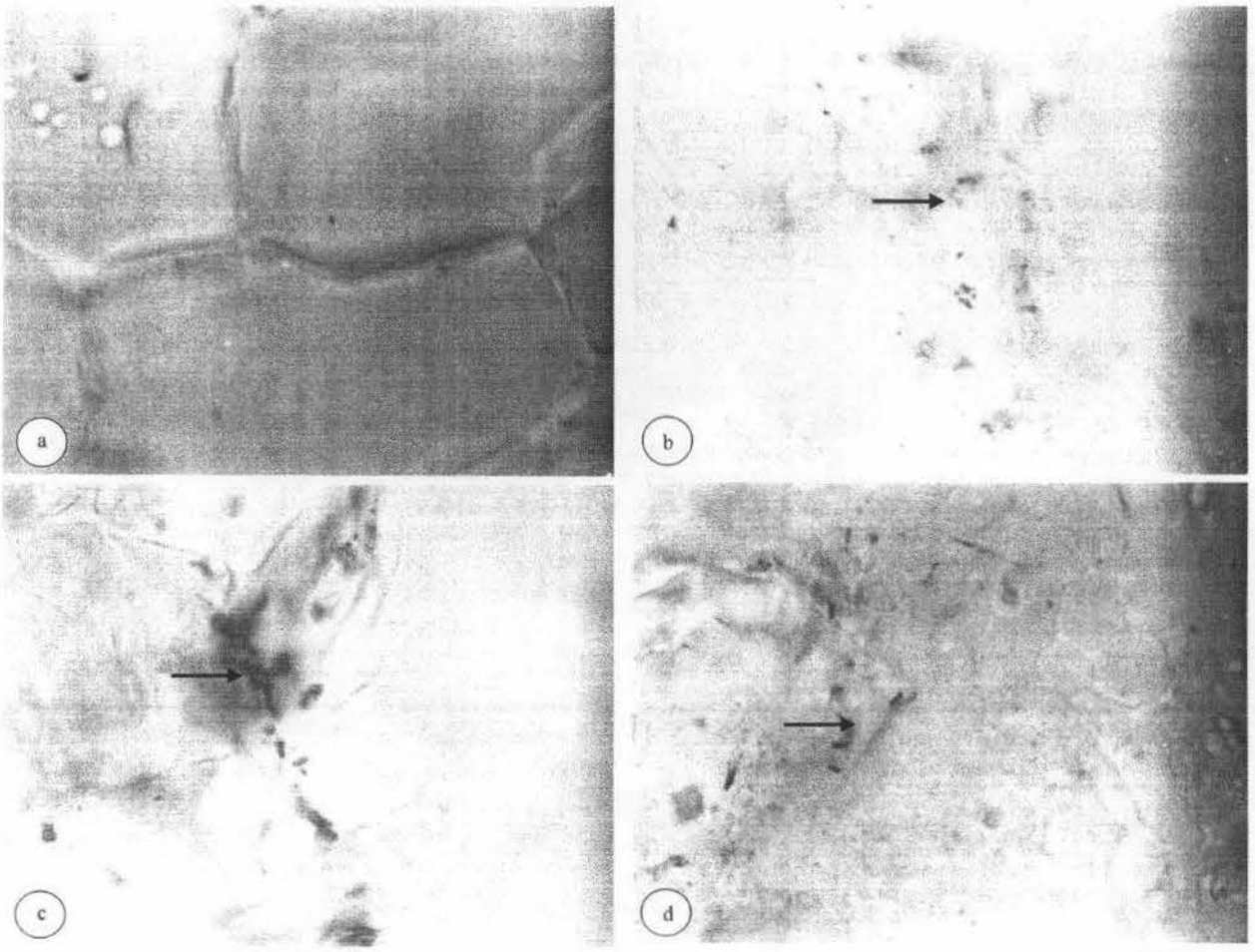

Figure 3. Colonization of endophytic *Streptomyces* spp. in rice stem tissue as indicated by black arrows (a) Control; (b) AB131 -1; (c) AB131-2; (d) LBR02.

endophytic *Streptomyces* spp. in suppressing the BLB disease and increasing plant growth (biocontrol and plant growth promotion characteristics). The results showed that selected endophytic *Streptomyces* spp. (AB131-1, AB131-2, LBR02) have biocontrol characteristics. Plants treated with AB131-1 and AB131-2 have reduced *Xoo* infection compared with the control (*Xoo*). Besides being able to reduce the intensity of the BLB disease, some *Streptomyces* spp. isolates were also able to improve the growth of the plants, compared with the control group. The treatments were able to improve the growth of rice seedlings. Inoculation of AB131-1 increased plant height and dry weight of the rice plant infected with *Xoo*. Treatment with AB131-2 also produced higher number of tillers compared with the control group. Yusepi (2011) reported that AB131-1 and AB131-2 produced indole acetic acid (IAA), therefore, the two isolates presumably also act as plant growth promoter. Besides IAA, Feng *et al.* (2006) reported that endophyte bacterial activity produced three other types of plant growth substance (abscisic acid, giberellic acid, and cytokinin). Endophytic *Streptomyces* spp. contribute to the growth of its host plant as a plant growth promoter and increase the ability of its host to be able to live in environmental stress conditions. Interactions between the IAA-producing bacteria and host plants play an important role to the diversity of plants. As

Baldani *et al.* (2000) reported, endophytic bacteria inoculated into rice seeds increased the weight of the rice straw and the grain yield.

In vitro study showed that the inhibition zone of LBR02 was higher (25 mm) than the other isolates. However, this isolate was not yet able to suppress the BLB disease. One important character of endophytic bacteria which will make it a successful biocontrol agent is the fast colonization of host xylem vessel (Nawangsih *et al.* 2011). The AUDPC value of LBR02 inoculation with infected *Xoo* was not significantly different compared with the *Xoo* control group because *Xoo* bacteria, which was injected into the plant by cutting the leaves and spaying, can be directly occupy the xylem vessel and the nutrient in the medium. This means that *Xoo* began to infect the plants through the wounds and continue to infect the plant tissue. Symptoms began generally with a graying of the edge of the leaf. Furthermore, Compant *et al.* (2005) reported that induction of plant defense mechanisms or induced systemic resistance (ISR) is influenced by endophytic bacteria living on plants tissue which produce secondary metabolites that can enhance plant resistance to pathogens. Reiter *et al.* (2002) stated that endophytic bacteria can become better biocontrol agents compared with rhizosphere bacteria because they do not compete for nutrition and/or niche in the apoplast and are also more adapted to environmental influences (Rosenblueth & Romero 2005).

The plant disease suppression mechanism by endophytic actinomycetes is presumably caused by the production of bioactive compounds which can act as antibiotics, and/or function as cell wall degrading enzymes in the decision-nutrient competition (El-Tarabily & Sivasithamparan 2006). The six isolates used were phosphate solubilizing endophytic *Streptomyces* spp. These isolates were able to release soluble phosphate from tricalcium phosphate in the Pikovskaya medium. Hamdali *et al.* (2008) reported that the most active rock phosphate-solubilizing strains had the highest stimulating effect on the production of plant biomass. Isolate AB131-1 and LBR02 produced siderophore. The ability to produce siderophores is one of the characters that make microorganisms successful competitors in several environments because, in sufficient quantities, it may limit Fe^{3+} availability to the pathogen and influence the induction of host resistance against the pathogen (Meziane *et al.* 2005). *Streptomyces* spp. LSW05 can produce hydrogen cyanide (HCN), a gas known to have a negative effect on the metabolism and growth of roots. It is often used to control weeds naturally. The *in planta* and *in vitro* experiments conducted provide an explanation for synergistic biocontrol and plant growth promotion. Baldani *et al.* (2000) reported that endophytic bacteria which were inoculated into rice seeds increased the weight of rice straw and grain yield higher than the control. Recently, Patil *et al.* (2010) found that the application of *Streptomyces* sp. in tomato seeds could control damping off diseases caused by *Rhizoctonia solani* by supressing the percentage of disease up to 53.33%. This result is also supported by Bacon and Hinton (2006) which reported that field data also indicated a variety of suppression of plant diseases that correlated with *in vitro* experiments.

Based on their spore chain formation (observed under the scanning electron micrograph), isolate AB131-1 and AB131-2 belong to different species of the *Streptomyces* genus. Therefore, it can be deduced that the endophytic *Streptomyces* spp. have the potential to be biocontrol agents for BLB in rice plant. However, further fieldwork is required to confirm their control efficacy in different climatic regions and under different growth conditions. Formulation and applications that meet common farming practices still need to be developed. For promising biological control agents, strategies that enhance overall control efficacy should be explored.

ACKNOWLEDGEMENT

This study was partly supported by a research grant from the Collaborative Research Project of KKP3T between the Agency for Agricultural Research and Development (AARD), Indonesian Ministry of Agriculture and Bogor Agricultural University (IPB) with contract number 593/LB.620/I.1/2/2009, 20 February 2009 a.n. Yulin Lestari, and partly supported by a Graduate Research Grant from the Directorate General of Higher Education, National Education Ministry awarded to Yulin Lestari.

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