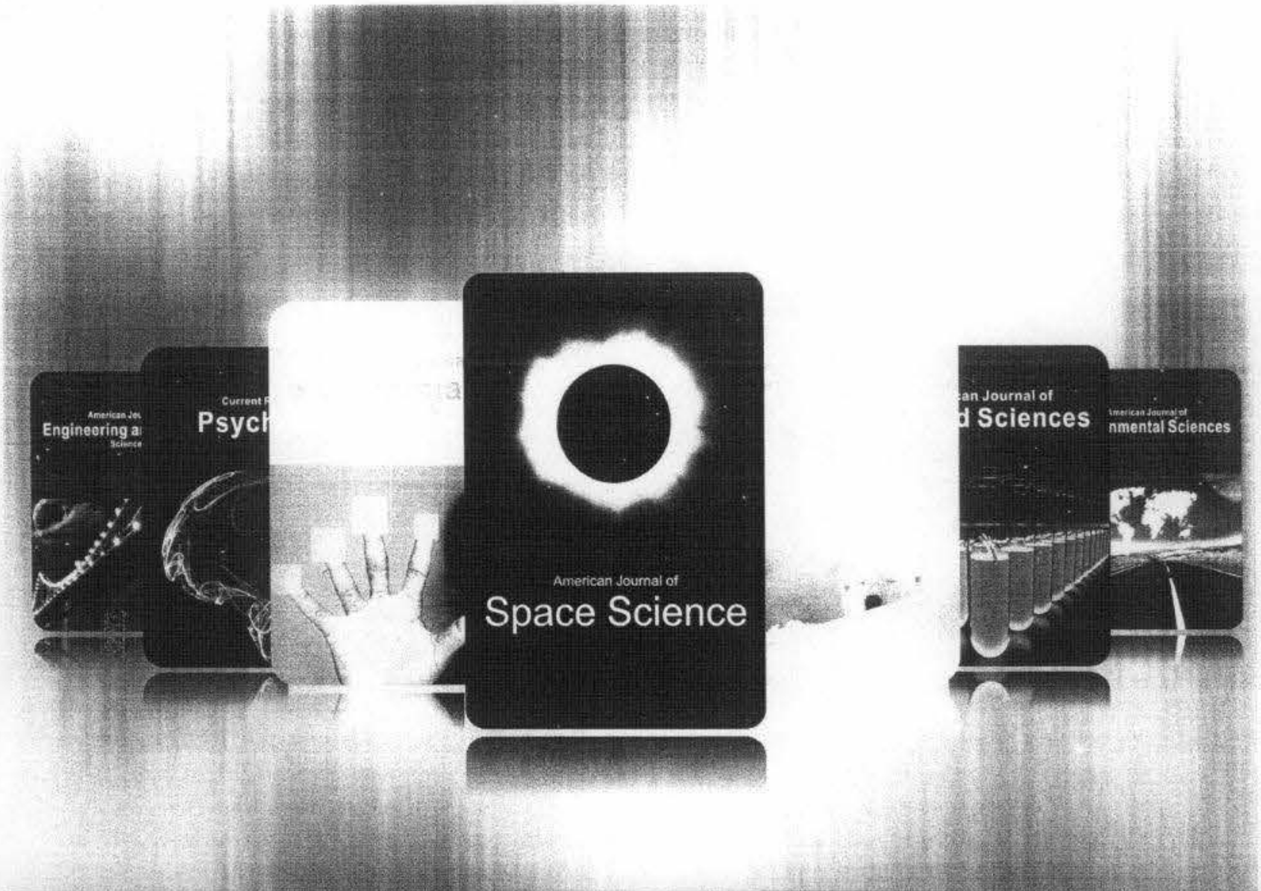

Science Publications

Your Local Publisher

Publish Your Ground Breaking Research with Science Publications

science publications
New York USA

REPRINTS

American Journal of Agricultural and Biological Sciences 7 (2): 217-223, 2012
ISSN 1557-4989

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

[1,2]Ratih Dewi Hastuti, [1]Yulin Lestari,
[2]Rasti Saraswati, [1]Antonius Suwanto and [3]Chaerani
[1]Department of Biology, Faculty of Mathematics and Natural Sciences,
Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia
[2]Indonesian Soil Research Institute, Jl. Ir. H. Juanda 98, Bogor 16123, Indonesia
[3]Indonesian Center for Agriculture Biotechnology and Genetic Resources
Research and Development, Jl. Tentara Pelajar 3A Bogor 16111, Indonesia

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 (5×5 m^2) 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

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

Corresponding Author: Yulin Lestari, Department of Biology, Faculty of Mathematics and Natural Sciences, Bogor Agricultural University, Darmaga Campus, Bogor 16680, Indonesia Tel/Fax: +62-251-8622833

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

Soil characteristic	Value
pH (extract 1:5)	
H_2O	5.40
KCl	4.80
Organic content	
% C (Walkley& Black)	1.71
% N (Kjeldahl)	0.15
C/N	11.00
HCl 25%	
P_2O_5 (mg /100 g)	147.00
K_2O (mg/100 g)	19.00
P_2O_5 (ppm) Olsen	176.00
K_2O (ppm) Morgan	113.00
CEC (NH_4-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^{3+} (cmol /kg)	0.01
H^+ (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^{-1} 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^2 and planting distances of 20×20 cm^2 with one seedling per planting hole. Basic fertilizer (200 kg of urea, 100 kg of SP36 and 100 kg of KCl ha^{-1}) 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^{-1} 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 S*treptomyces* 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^{-1} 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:

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

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

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 the 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

Compant, S., B. Duffy, J. Nowak, C. Clement and E.A. Barka, 2005. Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. Applied Environ. Microbiol., 71: 4951-4959.

El-Abyad, M.S., M.A. El-Sayed, A.R. El-Shansburry and S.M. El-Sabbagh, 1993. Towards the biological control of fungal and bacterial diseases of tomato using antagonistic *Streptomyces* spp. Plant Soil., 149: 185-195. DOI: 10.1007/BF00016608

Embley, T.M. and E. Stackebrandt, 1994. The molecular phylogeny and systematics of the actinomycetes. Ann. Rev. Microbiol., 48: 257-289. PMID: 7529976

Fravel, D.R., 2005. Commercialization and implementation of biocontrol. Ann. Rev. Phytopathol., 43: 337-359. DOI: 10.1146/annurev.Phyto.43.032904.092924

Georgakopoulos, D.G., P. Fiddaman, C. Leifert and N.E. Malathrakis, 2002. Biological control of cucumber and sugar beet damping-off caused by *Pythium ultimum* with bacterial and fungal antagonists. J. Applied Microbiol., 92: 1078-1086. DOI: 10.1046/j.1365-2672.2002.01658.x

Hasegawa, S., A. Meguro, M. Shimizu, T. Nishimura and H. Kunoh, 2006. Endophytic actinomycetes and their interactions with host plants. Actinomycetologica, 20: 72-81. DOI: 10.3209/saj.20.72

IRRI, 1996. Standard Evaluation System for Rice. 4th Edn., International Rice Research Institute, Manila, Philippines, pp: 52.

Khamna, S., A. Yokota and S. Lumyong, 2009. Actinomycetes isolated from medicinal plant rhizosphere soils: Diversity and screening of antifungal compounds, indole-3-acetic acid and siderophore production. World J. Microbiol. Biotechnol., 25: 649-655. DOI: 10.1007/s11274-008-9933-x

Lestari, Y., 2006. Short communication : Identification of indigenous *Streptomyces* spp. Producing antibacterial compounds. J. Mikrobiol. Indones, 11: 99-101.

Patil, H.J., A.K. Srivastava, S. Kumar, B.L. Chaudari and D.K. Arora, 2010. Selective isolation, evaluation and characterization of antagonistic actinomycetes against *Rhizoctonia solani*. World J. Microbiol. Biotechnol., 26: 2163-2170. DOI: 10.1007/s11274-010-0400-0

Prabavathy, V.R., N. Mathivanan and K. Murugesan, 2006. Control of blast and sheath blight diseases of rice using antifungal metabolites produced by *Streptomyces* sp. PM5. Biol. Control, 39: 313-319. DOI: 10.1016/j.biocontrol.2006.07.011

Qin, S., K. Xing, J.H. Jiang, L.H. Xu and W.J. Li, 2011. Biodiversity, bioactive natural products and biotechnological potential of plant-associated endophytic actinobacteria. Applied Microbiol. Biotechnol., 89: 457-473. DOI: 10.1007/s00253-010-2923-6

Sabaratnam, S. and J.A. Traquaira, 2002. Formulation of a *Streptomyces* biocontrol agent for the suppression of Rhizoctonia damping-off in tomato transplants. Biol. Control, 23: 245-253. DOI: 10.1006/bcon.2001.1014

Shimizu, M., S. Yazawa and Y. Ushijima, 2009. A promising strain of endophytic *Streptomyces* Sp. For biological control of cucumber anthracnose. J. Gen. Plant. Pathol., 75: 27-36. DOI: 10.1007/s10327-008-0138-9

Shrivastava, S., S.F. D'Souza and P.D. Desai, 2008. Production of indole-3-acetic acid by immobilized actinomycete (*Kitasatospora* sp.) for soil applications. Curr. Sci., 94: 1595-1604.

Xiao, K., L.L. Kinkel and D.A. Samac, 2002. Biological control of *Phytophthora* root rots on alfalfa and soybean with *Streptomyces*. Biol. Control, 23: 285-295. DOI: 10.1006/bcon.2001.1015