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The potential of endophytic bacteria to suppress bacterial leaf blight in rice plants

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Abstract. *Rahma H, Nurbailis, Busniah M, Kristina N, Larasati Y. 2022. The potential of endophytic bacteria to suppress bacterial leaf blight in rice plants. Biodiversitas 23: 775-782.* Endophytic bacteria are potential as biocontrol agents against bacterial leaf blight (BLB) disease caused by *Xanthomonas oryzae* pv. *oryzae* in rice to support sustainable agriculture. This study aimed to select and characterize 22 endophytic bacteria isolated from healthy rice, determine their ability to promote plant growth and suppress bacterial leaf blight disease in rice and also identify potential endophytic bacterial isolates. The study was arranged in a Completely Randomized Design with 24 treatments and three repetitions. The treatments used in the current study consisted of *Xanthomonas oryzae* infected plants and treated with endophytic bacterial isolates; infected plants without endophytic bacteria treatment (positive control), non-infected plants (negative control). Identification of potential endophytic bacteria was performed based on 16S rRNA sequences. Three out of 22 bacterial isolates, i.e., LmB1, LmA6, and LmB2 were able to suppress bacterial leaf blight disease with severity levels of 35.82%, 23.78%, and 23.78%, respectively. Based on the rice plant growth parameters, three bacterial isolates (LmA6, LmB1, and LmB35) were able to increase the growth of rice plants with an average value of 69.56%, 56.51%, and 47.82%, respectively. Two bacterial isolates, i.e., LmB 1 and LmA6 suppress the development of bacterial leaf blight disease and increase the growth of rice plants. Based on DNA sequence comparisons of DNA fragments amplified by 16S rRNA related marker of the selected bacterial isolates and database, then LmA6, LmB2, LmB1, and LmB35 had similarities with *Bacillus cereus* MD152 (96.87%), *Bacillus thuringiensis* ATCC 10792 (98.20%), *Ochrobactrum intermedium* strain OI1 (97.52%), and *Stenotrophomonas maltophilia* strain A1w2 (97.92%), respectively. Our study revealed that the indigenous endophytic bacteria from rice plants could be potential biological agents for controlling bacterial leaf blight disease and increasing plant growth.

Keywords: Biocontrol, endophytic bacteria, sustainable agriculture, *Xanthomonas oryzae* pv. *Oryzae*

INTRODUCTION

Rice (*Oryza sativa* L) is the staple food for the majority of Indonesian people (Susanto et al. 2003). According to the Ministry of Agriculture of the Republic of Indonesia (2019), the national rice production in the 2015 to 2017 period tends to decline with total rice production of 5.34 tons/ha, 5.23 tons/ha, and 5.15 tons/ha, respectively. However, rice production was slightly increased to 5.19 tons/ha in 2018, but this rice production is still below their potential production of 6-9 tons/ha (Suprihatno et al. 2009).

The decline of national rice production is affected by pests or diseases caused by pathogens. *Xanthomonas oryzae* pv. *oryzae* (Xoo) is a pathogen that causes bacterial leaf blight (BLB), which has attacked approximately 39,565 ha of Indonesia's rice-growing area in 2018 covered the third-largest area of an attack after stem borer and rat. Meanwhile, in 2019, the rice-growing area attacked by *Xanthomonas oryzae* has decreased to 26,998 ha (Directorate of Food Crop Protection 2019). Bacterial leaf blight is when the leaves look curled, folded, and the leaves are colored gray to yellow. Under critical conditions, all leaves are wither and die (Sopialena et al. 2019). Rice yield loss caused by the Xoo attack is majorly determined by the stage of plant growth. According to Suparyono et al. (2004), the symptoms that occur in rice plants at the vegetative phase are called kresek, and symptoms occur in the generative phase called blight. Infection by *Xanthomonas oryzae* reduces the photosynthetic ability and disrupts the grain filling process so that the infected plants produce more empty grains than healthy crops. Yield losses due to disease vary between 15 and 80% depending on the harvest stage when the disease occurs (Sudir and Yuliani 2016).

Biological control agents such as endophytic microorganisms residing in rice plants have widely been developed to control BLB disease. The use of endophytic bacteria as biological control agents is considered more effective than other free-living microorganisms (Sholikhin 2014; Yanuar 2016). Some endophytic bacteria were reported to have the ability to stimulate growth, such as *Burkholderia cepacia*, *Pseudomonas fluorescens*, and *Bacillus* sp. (Kloepper et al. 1999). *Burkholderia* sp. stimulates the growth of grapes (*Vitis vinifera* L.) (Compant et al. 2005). Similarly, *Pseudomonas pseudomallei*, *Bacillus mycoides*, and *Klebsiella ozaenae* increase the growth of potatoes (Juwita 2010). Meanwhile, endophytic bacteria from upland rice stimulate the growth of rice (Munif et al. 2012).

The ability of endophytic bacteria to suppress disease can be triggered by induced systemic resistance (ISR) mechanisms. A study by Juwita (2010) reported that *P. pseudomallei* and *K.*

ozaenae induces potato resistance to yellow potato cyst nematode *Globodera rostochiensis* (Juwita 2010). In addition, *Micrococcus endophyticus* was previously reported to have the ability to induce potato resistance against bacterial wilt disease through jasmonic and salicylic acid pathways (Akhdiya 2014). Several *Bacillus* groups can induce tomato resistance against Cucumber mosaic virus infection (Zehnder et al. 2000), the resistance against BLB disease caused by *X. axonopodis pv. allii* in shallot increased (Resti et al. 2013) and induce resistance to *Xanthomonas oryzae pv. oryzae* (*Xoo*) in rice (Parida 2016). The study aimed to obtain indigenous endophytic bacterial isolates that can induce rice plant resistance to *Xoo* and increase rice growth and also identify potential endophytic bacterial isolates to increase plant resistance.

MATERIALS AND METHODS

Place and time of the study

The research was conducted from August 2019 to June 2020 at the Biological Control Laboratory and Greenhouse, Faculty of Agriculture, Andalas University, Padang, West Sumatra, Indonesia.

Research design

The study was arranged in a Completely Randomized Design (CRD) with 24 treatments and three repetitions. The treatments used in the current study consisted of *X. oryzae* infected plants and treated with endophytic bacterial isolates (22 endophytic bacteria isolates), infected plants without treatment of endophytic bacteria (positive control), non-infected plants (negative control). The leaves of 40-day-old plants were inoculated with *Xoo* pathogen suspension and covered with plastic for 24 h.

Selection of the ability of endophytic bacteria to suppress bacterial blight disease in rice

Inoculum preparation of endophytic bacteria

Endophytic bacterial colonies (48 hour-old) on nutrient agar were taken with a loop needle and transferred to 25 mL of Luria Bertani medium in a culture bottle and incubated for 24 hours on a rotary shaker at a speed of 150 rpm. After incubation, 1 mL of culture were transferred to a culture bottle containing 49 mL of sterile coconut water as the main culture and incubated in a rotary shaker for 2 x 24 hours at a speed of 150 rpm (Yanti et al. 2017). The population density was determined by comparing the turbidity of bacterial suspension with a McFarland 8 scale solution (estimated bacterial population density was 10^8 cells/mL).

Preparation of planting media

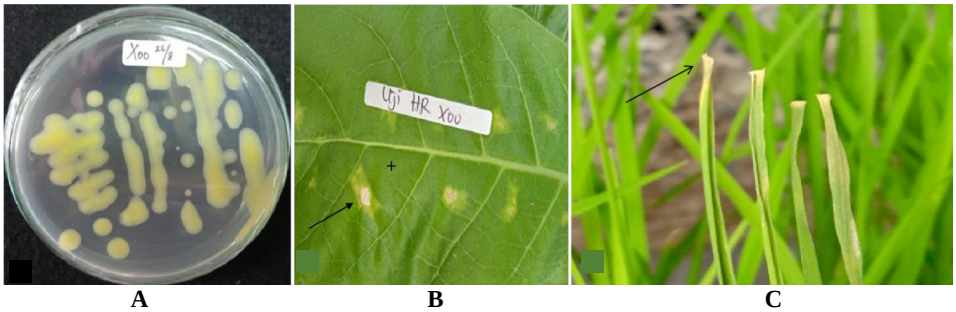
Planting media in the form of a mixture of soil and manure with a ratio of 2: 1 were sterilized at a temperature of 100ºC for 1 hour. Planting media was filled into pots (top diameter 30 cm, bottom diameter 20 cm) and incubated for one day.

Inoculation of endophytic bacteria and planting

The rice seeds used in this study were the Batang Piaman variety. Endophytic bacteria were inoculated twice. The first inoculation of endophytic bacteria was carried out on seeds that have been surface sterilized. They were immersed in a suspension of endophytic bacteria for 15 minutes, then sown in a sprouting bath containing sterile planting media (Rahma et al. 2019). As a control, rice seeds were soaked in sterile distilled water simultaneously. The second inoculation of endophytic bacteria was carried out in rice seedlings at the age of 20 days after planting. Rice roots were cleaned from the remaining soil and then soaked in a suspension of endophytic bacteria for 15 minutes (Khaeruni et al. 2014). As for the control, the seedlings were soaked in sterile distilled water for the same period. After soaking, the rice seeds were planted in pots containing sterile planting media.

Culturing Xanthomonas oryzae *pv.* oryzae, *Hypersensitivity reaction, and pathogenicity test*

Xanthomonas oryzae pv. *oryzae* isolate was obtained from the Indonesian Center for Rice Research, Sukamandi, West Jawa Province. The isolate was retrieved and cultured using the scratch method on Wakimoto Agar medium and incubated for 2 x 24 hours (Figure 1A). *Xoo* isolate was tested for its hypersensitive response and pathogenicity (Figure 1). Routine hypersensitivity test: Approximately 10^8 CFU/mL of freshly cultured bacteria from Wakimoto plates were injected using a syringe onto the abaxial surface of three parts/leaves of Virginia cultivar tobacco. Sterile aqua dest was used as a control. A positive reaction was indicated by the presence of complete collapse of tissue after 24 hours followed by necrosis (Klement et al. 1990). A pathogenicity test was carried out using the clipping method on a 40-day-old Batang Piaman rice cultivar (Khaeruni et al. 2014). Leaf tips were cut into a 5 cm length and immersed in Xoo suspension at a density of 10^8 cells/mL for ± 10 seconds. The number of rice leaves inoculated with Xoo suspension was 5 leaves per tested plant. The inoculation process was performed in the afternoon to avoid the temperature being too high for *Xoo* infection.



A **B** **C**

Figure 1. *Xanthomonas oryzae*. A. Colony Morphology of *Xoo* 72 hours-old culture on Wakimoto media, B. Hypersensitive reaction on tobacco leaves +, C. Pathogenicity on rice leaves

Table 1. Severity scores of BLB disease in rice

Score	Symptoms
0	There are no symptoms.
1	There is a symptom of 1-2 mm long spots around the inoculation point
2	Symptoms form a circular shape like an ellipse with a length of about 2-3 cm.
3	Symptoms begin to elongate to less than ½ of the leaf length.
4	Symptoms widen and begin to coalesce, the top of the leaf begins to experience tissue death, extending approximately ¼ from the lower part of the leaf surface which is the point of inoculation.
5	Symptoms of blight coalesce, the tops of the leaves become dry, the symptoms extend to ½ of the leaf length.
6	Symptoms extend to ¼ from the underside of the leaf.
7	Symptoms extend too close to the underside of the leaf and almost destroy the entire leaf
8	Symptoms of blight destroy the entire leaf blade and extend to about ½ of the leaf midrib
9	All leaves and midribs are infected

Observation during the incubation period was carried out daily after inoculation of the pathogen until the first symptoms appeared. The development of the disease was observed by calculating the length of leaf blight and stopped after blight symptoms reached the base of the leaf. Furthermore, the disease severity was calculated using the following formula:

$$KP = \frac{\sum ni \times vi}{Z \times N} \times 100\%$$

Where:
KP: Disease severity
Ni: The number of infected leaves in each category
Vi: Numerical value (sco re) in each attack category
N: The number of leaves observed
Z: Numerical value (score) for the toughest attack category

The value of disease severity is calculated by the leaf damage score according to Ou (1985), which is shown in Table 1. Data on all disease severity are analyzed using the Area Under Disease Progress Curve (AUDPC) formula (Van der Plank 1963):

$$AUDPC = \sum_{i}^{n-1} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where:
yi: the ith observation data
yi + 1: the ith observation data + 1
ti + 1: time of the ith observation + 1
ti: time of the ith observation

After obtaining the AUDPC value, the level of control effectiveness can be determined by calculating the disease suppression index value with the following formula:

$$Effectivenes = \frac{AUDPC\ of\ Control\ -\ AUDPC\ of\ Treatment}{AUDPC\ of\ Control} x\ 100\%$$

Maintenance of the plants

The maintenance of rice plants includes watering, weeding, fertilizing, and controlling pests mechanically. Fertilization was applied according to the recommendations of the Husnain et al. (2020), namely Urea at a dose of 0.25 grams/bucket and NPK Phonska fertilizer at a dose of 0.75 grams/bucket (equivalent to 100 kg of Urea and 300 kg/ha of NPK Phonska fertilizer).

Endophytic bacteria identification based on 16S rRNA sequences

Identification of endophytic bacteria was carried out on LMA6, LMB1, LMB2, and LMB35 isolates. Endophytic bacteria culture on LB + glycerol media was rejuvenated using LB media and incubated for 24 hours at 28°C (room temperature). DNA isolation was carried out using a Genomic DNA extraction with Presto™ Mini gDNA Bacteria Kit (Geneaid). Amplification of 16S rRNA gene was conducted by Polymerase Chain Reaction (PCR) technique using 27F universal primers (5' AGAGTTTGATCCTGGCTCAG-3 ') and 1492R (5'-GGTTA CCTTGTTACGACTT-3' (Galkiewicz and Kellogg 2008). The PCR mixture contained MyTaq HS Red Mix from Meridian Bioscience 12.5 µL, 1 µL for each primer, 1 µL genomic DNA, and 9,5 µL ddH2O, so the total reaction volume is 25 µL. The PCR process was performed under initial denaturation conditions at temperatures of 95°C for 5 minutes, 30 cycles of denaturation at 95°C for 1 minute, primer attachment at 55°C for 1 minute, DNA elongation at 72°C for 1.5 minutes, and the final stage at 72°C for 5 minutes using GeneAmp PCR System 9700 (Applied Biosystems, USA). The PCR product was then separated by 1% agarose gel electrophoresis under an ultraviolet transilluminator with the size markers (1 kb DNA ladder, Geneaid). The size of the 16S rRNA gene was ± 1500 bp in size. DNA sequencing was performed on the results of the amplification by Genetika Science Indonesia. Related sequences were identified using the BLAST search program, National Center for Biotechnology Information (NCBI), National Library of Medicine, USA (http://www.ncbi.nlm.nih.gov/) (Altschul et al. 1997).

Data analysis

The observed data obtained during the incubation period and disease severity were analyzed using analysis of variance (ANOVA). If the data were significantly different, it was continued with the multiple t-test Least Significant Differences (LSD) at the 5% level. The sequence alignments were performed by BioEdit 7.2.1 (Hall 2011), and phylogenetic trees were constructed based on the neighbor-joining method (Saitou and Nei 1987) using MEGA6 software (Kumar et al. 2004).

Table 2. Development of *Xoo* pathogens in rice plant after inoculating with endophytic bacterial isolates

Treatment	Incubation Period		Disease Occurrence (%)	Disease Severity		AUDPC**	Effectiveness (%)
	Days after inoculation	Effectiveness (%)		%	Effectiveness (%)		
Control +	7.16 a*	60.47	100	21.29 a	68.07	0.81	76.79
LmA 6	4.66 b	39.27	100	43.51 abc	34.73	2.66	23.78
LmB 2	4.33 bc	34.64	100	45.37 abcd	31.95	2.69	22.92
LmB 7	4.00 bcd	29.25	100	71.29 d	-6.95	4.84	38.68
LmD 13	4.00 bcd	29.25	100	58.33 bcd	12.50	3.91	12.03
LmB 8	3.83 bcd	26.11	100	54.62 bcd	18.06	3.11	10.89
LmB 21	3.83 bcd	26.11	100	61.11 bcd	8.33	3.97	-13.75
LmB 1	3.66 bcd	22.68	100	37.03 ab	44.45	2.24	35.82
LmB 16	3.66 bcd	22.68	100	61.10 bcd	8.34	3.56	-2.01
LmB 19	3.66 bcd	22.68	100	61.11 bcd	8.33	4.04	-15.76
LmB 22	3.66 bcd	22.68	100	57.40 bcd	13.90	3.37	3.44
LmA 5	3.66 bcd	22.68	100	66.66 cd	0.00	4.18	-19.77
LmB 20	3.16 bcd	10.44	100	55.55 bcd	16.67	3.17	9.17
LmB 27	3.16 bcd	10.44	100	62.03 bcd	6.95	4.06	-16.33
LmD 11	3.16 bcd	10.44	100	54.63 bcd	18.05	3.41	2.29
LmB 4	3.00 bcd	5.67	100	71.29 d	-6.94	5.06	-44.99
LmB 35	3.00 bcd	5.67	100	50.00 bcd	25.00	2.95	15.47
LmD 14	3.00 bcd	5.67	100	56.47 bcd	15.29	3.48	0.29
LmD 15	3.00 bcd	5.67	100	71.29 d	-6.94	4.44	-27.22
LmD 16	3.00 bcd	5.67	100	55.55 bcd	16.67	3.08	11.75
LmB 6	2.83 cd	0.00	100	65.73 cd	1.40	3.88	-11.17
LmB 12	2.83 cd	0.00	100	57.40 bcd	13.89	3.91	-12.03
LmB33	2.50 d	-13.20	100	64.81 cd	2.78	4.24	-21.49
Control -	2.83 cd	0.00	100	66.66 cd	0.00	3.49	0.00

Note: *The numbers followed by the same letter in the same row are not significantly different according to the least significantly different (LSD) test at the 5% level. ** AUDPC = Area Under Disease Progress Curve

Effect of endophytic bacteria inoculation on rice plant growth

Several endophytic bacterial isolates were able to increase the germination of rice seeds up to 100% in comparison to that of the germination capacity of the seeds (96.33%), while the germination capacity of control seeds was 95.55% (Table 3). The seedling height of the seeds inoculated with endophytic bacteria was not significantly different from the control, except for LmB 7 isolates (15.91%). Inoculation of endophytic bacteria also had no significant effect on the root length of the seedlings but had a fairly good effect on the number of leaves (LmD 15 with the effectiveness of 20.36%) (Table 3).

The application of endophytic bacteria on rice plant growth after transplanting was significantly different. Inoculation of, LmB 1, LmA 6, and LmB 35 isolates resulted in a higher increase of plant height with the effectiveness of 37.84%, 35.68%, and 20.54% than controls and other treatments (Table 4). The ability of endophytic bacterial isolates to increase the growth of rice plants might be due to the bacteria producing phytohormones and siderophores, as well as the ability to dissolve phosphate. Fatlin et al. (2004) reported that endophytic bacteria isolated from potato plants can suppress *Ralstonia solani* pathogenic infection in the field up to 37% and increase potato production up to 12%. Rahma et al. (2014) also reported that 11 out of 17 potential endophytic bacterial isolates as biological agents are IAA producers and can dissolve phosphate and induce maize resistance with a percentage suppression of severity against Stewart wilt disease around 48.95-55.60%.

Based on the results of partial 16S rRNA sequencing and the similarity of endophytic bacterial species to the GenBank data center using the BLAST-N program on the NCBI website http://www.ncbi.nlm.nih.gov, it showed that sequence length of endophytic bacterial isolates ranges from 1217-1494 base pairs (bp). Endophytic bacterial LmA6 strain had 96.87% similarity with *Bacillus cereus* strain MD152 (accession number MT642947). LmB1 isolate had 97.52% similarity with *Ochrobactrum intermedium* strain OI1 (accession number KT985368). LmB2 isolate had 98.20% similarity with *Bacillus thuringiensis* ATCC 10792 with accession number CP0754, and LmB35 isolate had 97.92% similarity with *Stenotrophomonas chelatiphaga* strain 190306H248 (accession number MT225714) (Table 5). These bacteria have been reported to induce plant resistance to various pathogens and promote plant growth.

A study by Faisal and Hasnain (2006) showed that *Ochrobactrum intermedium*, *Bacillus cereus*, and *Brevibacterium* are bacteria that colonize rhizoplane and the root zone of *Triticum aestivum* plants. Bacterial cells are found in the areas where root exudates are found. The interaction of bacteria and plants shows a mutually beneficial relationship. Colonization of plant roots by bacteria originating from or induced into the soil provides effective bacterial-plant interactions. Faisal (2013) also reported that *Ochrobactrum intermedium* and *Bacillus cereus* are resistant to chromate. Under two different

K2CrO4 concentrations (0 and 300 μg mL^{-1}), *O. intermedium* and *B. cereus* can increase growth, root length, shoot length, number and weight of seeds per pod, and number and value of grain per plant of *Lens esculenta*. According to Banerjee et al. (2018), the soil-borne bacteria *Bacillus cereus* IB311 is antagonistic to plant pathogens such as *Pseudomonas syringae* and *Agrobacterium tumefaciens*) and have a substantial contribution to the prevention of plant diseases. The bacteria *Stenotrophomonas* spp. are promising candidates for biotechnological applications in agriculture. Treatment with *Stenotrophomonas* spp. can enhance plant growth and influence plant development on marginal conditions. *Stenotrophomonas maltophilia* was obtained in association with plants, and also can be isolated from the rhizosphere or the inner plant tissue, especially from the vascular tissue of the roots and stems (Ryan et al. 2009). Messiha et al. (2007) reported that *Stenotrophomonas maltophilia* isolated from the rhizosphere of eggplant in the Nile Delta of Egypt had antagonistic potential against *Ralstonia solanacearum* race three biovars 2, which is the causative agent of potato brown rot in vitro on KB agar medium and in vivo on potato plants.

The phylogenetic tree shows two main groups, namely the first group consists of the Bacillus bacteria group, Gram-positive bacteria; included LmA6 and LmB2. The second group, Gram-negative bacteria, consists of two subgroups; Ochrobactrum, which contains LmB1 isolates, and Stenotrophomonas, consisting of LmB35 isolates (Figure 3). The sequence information of the conserved regions of the 16S rRNA gene is useful for studying phylogenetic relationships and the design of the probe and specific or generic oligonucleotide primers used for identification by hybridization and discriminant PCR-amplification (Mehnaz et al. 2001). The 16S rRNA region variable provides sequence data for developing particular probes and primers to detect bacteria by hybridization or polymerase chain reactions. The availability and use of PCR-based amplification methods and the sequencing of PCR products in automatic sequencers have dramatically expanded the RNA database over the past few years (Amann et al. 1995). This sequence information is now available in public databases to improve the identification of new bacterial isolates by sequence comparisons.

The results indicate that each endophytic bacteria has a different ability in increasing the growth of rice plants and suppressing the development of BLB disease in plants. LmA 6, LmB 1, and LmB 35 are endophytic bacterial isolates that have the potential to increase plant growth. Meanwhile, LmB 1, LmA 6, and LmB 2 are endophytic bacterial isolates that have the potential to suppress the development of BLB through indirect mechanisms. This is in line with Hallmann (1999) who stated that endophytic bacteria can act as biological agents through direct and indirect mechanisms. Endophytic bacteria function as antagonists through direct mechanisms, some of which produce lysis enzymes, antibiotic compounds, phytohormone producers, siderophore producers, and nitrogen fixers (Malfanova et al. 2011). Meanwhile, endophytic bacteria can indirectly induce plant systemic resistance. Identification of endophytic bacteria showed that the potential isolates had high similarity to *Bacillus cereus* MD152, *Bacillus thuringiensis* ATCC 10792 *Ochrobactrum intermedium* strain OI1, and *Stenotrophomonas maltophilia* strain A1w2.

Table 3. Growth of rice seedling inoculated with endophytic bacterial isolates

Treatment	Field emergence capacity		Seedling height		Number of seedling leaves		Root length of seedling	
	%	Effectiveness (%)	Cm	Effectiveness (%)	Blade	Effectiveness (%)	Cm	Effectiveness (%)
LmB 2	100	4.66	28.06	-15.43	4.43	12.72	10.28	-9.82
LmA 5	100	4.66	32.00	-3.56	4.40	11.96	9.73	-14.65
LmD 13	100	4.66	31.00	-6.57	3.66	-6.87	8.28	-27.37
LmB 22	98,88	3.49	28.55	-13.95	4.46	13.49	9.65	-15.35
LmB 33	98,88	3.49	30.41	-8.35	4.60	17.05	11.60	1.75
LmB 35	98,88	3.49	22.12	-33.30	3.93	0.00	6.30	-44.74
LmD 11	98,88	3.49	27.40	-17.42	4.23	7.63	8.10	-28.95
LmD 14	98,88	3.49	31.23	-5.88	4.,00	1.78	10.66	-6.49
LmB 19	97,77	2.32	27.52	-17.06	3.,90	-0.76	8.15	-28.51
LmB 1	96,66	1.16	30.60	-7.78	3.90	-0.76	8.98	-21.23
LmB 12	96,66	1.16	27.55	-16.97	4.36	10.94	9.63	-15.53
LmB 8	96,66	1.16	30.46	-8.20	4.56	16.03	9.66	-15.26
LmD 16	96,66	1.16	33.05	-0.39	4.53	15.27	10.55	-7.46
LmA 6	96,66	1.16	34.18	3.01	3.76	-4.33	10.55	-7.46
Control	95,55	0.00	33.18	0.00	3.93	0.00	11.40	0.00
LmB 27	95,55	0.00	32.10	-3.25	3.85	-20.4	9.56	-16.14
LmB 20	94,44	-1.16	26.90	-18.93	3.80	-3.31	6.25	-45.18
LmB 7	93,33	-2.32	38.46	15.91	4.66	18.58	9.15	-19.74
LmB 16	93,33	-2.32	32.23	-2.86	3.85	-2.04	8.93	-21.67
LmB 21	93,32	-2.33	26.48	-20.19	3.83	-2.54	7.01	-38.51
LmB 6	93,31	-2.34	29.46	-11.21	4.13	5.09	10.93	-4.12
LmB 4	92,22	-3.33	32.57	-1.84	4.36	10.94	10.96	-3.86
LmD 15	92,22	-3.33	36.46	9.89	4.73	20.36	13.15	15.35

Note: Germination capacity 95.33%.

Table 4. Growth of rice plants inoculated with endophytic bacterial isolates

Treatment	Plant height		Number of leaves		Number of tillers	
	Cm	Effectiveness (%)	Blade	Effectiveness (%)	Stem	Effectiveness (%)
LmB 1	85.00 a	37.84	53.33 ab	73.91	12.00 abc	56.51
LmA 6	83.67 ab	35.68	57.00 a	85.87	13.00 a	69.56
LmB 16	77.,67 abc	25.95	43.33 abcd	41.30	9.00 abcd	17.39
LmB 2	76.67 abcd	24.32	44.33 abcd	44.56	9.33 abcd	21.73
LmD 14	75.00 abcd	21.62	33.67 cd	9.78	6.67 d	-13.04
LmB 35	74.33 abcd	20.54	50.67 abc	65.22	11.33 abcd	47.82
LmB 33	73.67 abcd	19.46	37.33 bcd	21.74	8.67 abcd	13.04
LmB 20	72.33 abcd	17.30	40.33 abcd	31.52	9.00 abcd	17.39
LmD 15	70.00 abcd	13.51	34.67 cd	13.04	7.67 bcd	0.00
LmB 7	69.00 bcd	11.89	44.67 abcd	45.65	12.33 ab	60.86
LmB 12	68.67 bcd	11.35	37.00 bcd	20.65	9.33 abcd	21.73
LmD 16	68.33 bcd	10.81	44.33 abcd	44.56	10.67 abcd	39.13
LmB 19	67.67 cd	9.73	29.67 d	-3.26	7.00 d	-8.70
LmB 21	67.67 cd	9.73	32.67 cd	6.52	7.67 bcd	0.00
LmB 2	67.33 cd	9.19	42.00 abcd	36.96	12.00 abc	56.51
LmD 11	66.67 cd	8.11	46.33 abcd	51.08	11.33 abcd	47.82
LmD 13	66.67 cd	8.11	36.00 bcd	17.39	9.00 abcd	17.39
LmA 5	66.33 cd	7.57	39.33 abcd	28.26	10.67 abcd	39.13
LmB 27	65.67 cd	6.49	32.00 d	4.35	7.33 cd	-4.36
Control -	64.00 cd	3.78	33.00 cd	7.61	9.67 abcd	26.09
LmB 6	63.33 cd	2.70	31.33 d	2.17	8.67 abcd	13.04
LmB 4	63.00 cd	2.16	32.00 d	4.35	8.33 abcd	8.69
Control +	61.67 d	0.00	30.67 d	0.00	7.67 bcd	0.00
LmB 8	61.33 d	-0.54	38.67 bcd	26.09	10.67 abcd	39.13

Note: The numbers followed by the same letter in the same row are not significantly different according to the LSD test at the 5% level.

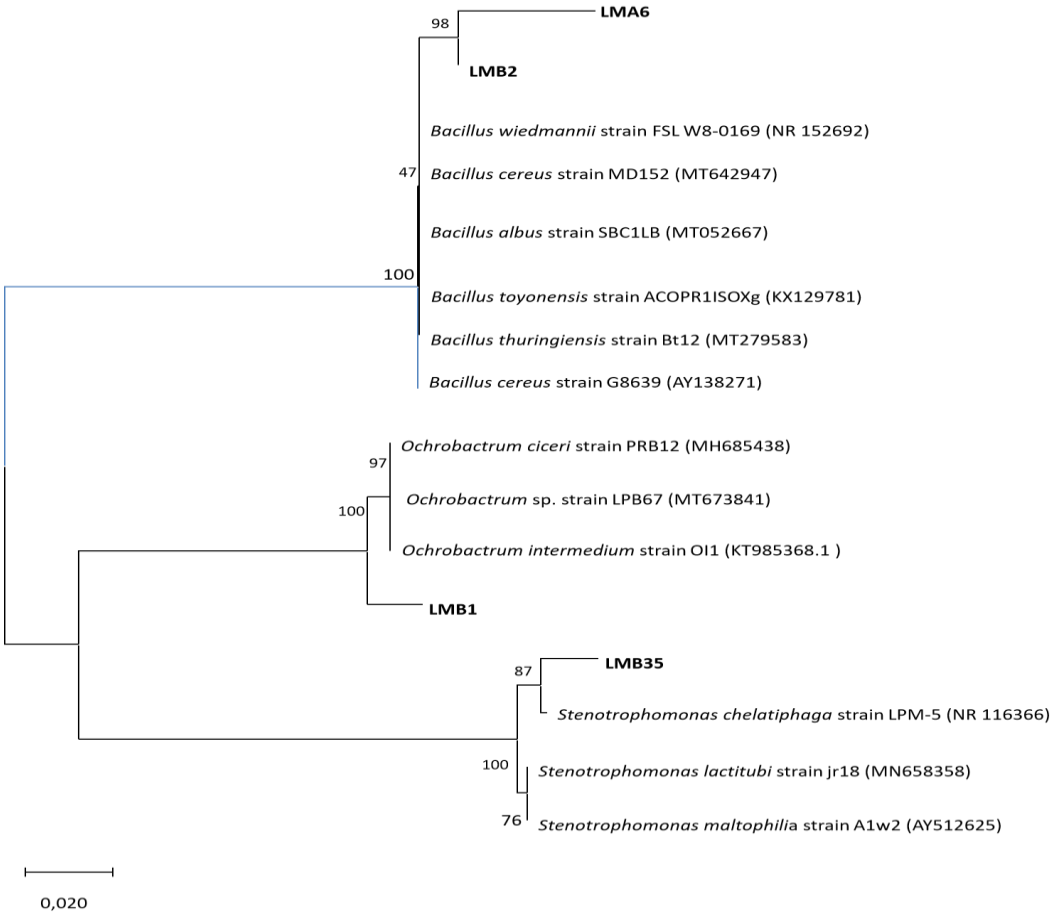


Figure 3. The phylogenetic tree is based on sequencing of the 16S rRNA gene of potential endophytic bacteria. The tree was reconstructed using MEGA6 software (Kumar et al. 2004)

Table 5. The partial sequence analysis of the 16S rRNA gene of bacterial isolates compared to the data in the GenBank data center

Isolate code	Query length	Related species	Similarity (%)	Accesion number
LmA6	1494	*Bacillus cereus* MD152	96.87	MT642947
LmB2	1217	*Bacillus thuringiensis* ATCC 10792	98.20	CP020754
LmB1	1363	*Ochrobactrum intermedium* strain OI1	97.52	KT985368
LmB35	1439	*Stenotrophomonas maltophilia* strain A1w2	97.92	AY512625

In conclusion, bacterial isolates LmB1, LmA6, and LmB2 can suppress the severity of bacterial leaf blight by 35.82%, 23.78%, and 23.78%, respectively. The isolates LmA6, LmB1, and LmB35 could increase the growth of rice plants by 69.56%, 56.51%, and 47.82%, respectively. Furthermore, isolates LmB 1 and LmA6 can suppress the development of bacterial leaf blight and increase the growth of rice plants. Based on 16S rRNA gene identification, the potential isolates LmA6, LmB2, LmB1, LmB35 were similar to *Bacillus cereus* MD152, *Bacillus thuringiensis* ATCC 10792, *Ochrobactrum intermedium* strain OI1, and *Stenotrophomonas maltophilia* strain A1w2, respectively. Our study revealed that the original endophytic bacteria from rice plants could be potential biological agents for disease control and promoting growth.

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