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

HALIATUR RAHMA1,*, NURBAILIS2, MUNZIR BUSNIAH1, NILA KRISTINA2, YUMBA LARASATI2
1Department of Plant Protection, Faculty of Agriculture, Universitas Andalas. Jl. Raya Unand, Limau Manis, Padang 25175, West Sumatra, Indonesia. Tel./fax.: +6281374516900, *email: haliiaturrahma@agr.unand.ac.id
2Department of Agroecotechnology, Faculty of Agriculture, Universitas Andalas. Jl. Raya Unand, Limau Manis, Padang 25175, West Sumatra, Indonesia

Abstract. Rahma H, Nurbaillis, 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 bacterial performance 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., LmB1 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 Alw2 (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 (Sopiaiena 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 Juliani 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. (Kloeper et al. 1999). Burkholderia sp. stimulates the growth of grapes (Vitis vinifera L.) (Compact 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 (Akhdhia 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 was 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 100°C 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 $10^6$ 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.

**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
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:

\[
KP = \frac{\sum n_i \times v_i}{Z \times N} \times 100\%
\]

Where:
- KP: Disease severity
- Ni: The number of infected leaves in each category
- Vi: Numerical value (score) 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):

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

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:

\[
\text{Effectiveness} = \frac{\text{AUDPC of Control} - \text{AUDPC of Treatment}}{\text{AUDPC of Control}} \times 100\%
\]

**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 medium 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' AGAGTTTGATCCTGGAAGTCGAG - 3') and 1492R (5'-GGTTA CCTTGATTACGACTT-3') (Galkiewicz and Kellogg 2008). The PCR mixture contained MyTaq HS Red Mix from Meridian Biosoience 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 Days after inoculation | Effectiveness (%) | Disease Severity | Effectiveness (%) | AUDPC** | Effectiveness (%) |
|-----------|----------------------------------------|-------------------|-----------------|------------------|----------|------------------|
| Control + | 7.16 a*                                | 60.47             | 21.29 a         | 68.07            | 0.81     | 76.79            |
| LmA 6     | 4.66 b                                 | 39.27             | 43.51 abc       | 34.73            | 2.66     | 23.78            |
| LmB 2     | 4.33 bc                                | 34.64             | 45.37 abcd      | 31.95            | 2.69     | 22.92            |
| LmB 7     | 4.00 bcd                               | 29.25             | 71.29 d         | 6.95             | 4.84     | 38.68            |
| LmD 13    | 4.00 bcd                               | 29.25             | 58.33 bcd       | 12.50            | 3.91     | 12.03            |
| LmB 8     | 3.83 bcd                               | 26.11             | 54.62 bcd       | 18.06            | 3.11     | 10.89            |
| LmB 21    | 3.83 bcd                               | 26.11             | 61.11 bcd       | 8.33             | 3.97     | -13.75           |
| LmB 1     | 3.66 bcd                               | 22.68             | 37.03 ab        | 44.45            | 2.24     | 35.82            |
| LmB 16    | 3.66 bcd                               | 22.68             | 61.10 bcd       | 8.34             | 3.56     | -2.01            |
| LmB 19    | 3.66 bcd                               | 22.68             | 61.11 bcd       | 8.33             | 4.04     | -15.76           |
| LmB 22    | 3.66 bcd                               | 22.68             | 57.40 bcd       | 13.90            | 3.37     | 3.44             |
| LmA 5     | 3.66 bcd                               | 22.68             | 66.66 cd        | 0.00             | 4.18     | -19.77           |
| LmB 20    | 3.16 bcd                               | 10.44             | 55.55 bcd       | 16.67            | 3.17     | 9.17             |
| LmB 27    | 3.16 bcd                               | 10.44             | 62.03 bcd       | 6.95             | 4.06     | -16.33           |
| LmD 11    | 3.16 bcd                               | 10.44             | 54.63 bcd       | 18.05            | 3.41     | 2.29             |
| LmB 4     | 3.00 bcd                               | 5.67              | 71.29 d         | 6.94             | 5.06     | -44.99           |
| LmB 35    | 3.00 bcd                               | 5.67              | 50.00 bcd       | 25.00            | 2.95     | 15.47            |
| LmD 14    | 3.00 bcd                               | 5.67              | 56.47 bcd       | 15.29            | 3.48     | 0.29             |
| LmD 15    | 3.00 bcd                               | 5.67              | 71.29 d         | 6.94             | 4.44     | -27.22           |
| LmD 16    | 3.00 bcd                               | 5.67              | 55.55 bcd       | 16.67            | 3.08     | 11.75            |
| LmB 6     | 2.83 cd                                | 0.00              | 65.73 cd        | 1.40             | 3.88     | -11.17           |
| LmB 12    | 2.83 cd                                | 0.00              | 57.40 cd        | 13.89            | 3.91     | -12.03           |
| LmB33     | 2.50 d                                 | -13.20            | 64.81 cd        | 2.78             | 4.24     | -21.49           |
| Control - | 2.83 cd                                | 0.00              | 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 phytohorones 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%. Rahman 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 O11 (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 rhizosphere 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. LmA6, 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 thuringensis 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 (%)** | **Seeding height Cm (cm)** | **Number of seedling leaves Blade (%)** | **Root length of seedling Cm (%)** |
| LmA 6 | 100 | 4.66 | 28.06 | -15.43 | 4.43 | 12.72 | 10.28 | -9.82 |
| LmB 2 | 98.88 | 4.39 | 28.55 | -13.95 | 4.46 | 13.49 | 9.65 | -15.35 |
| LmB 13 | 98.88 | 4.39 | 30.41 | -8.35 | 4.60 | 17.05 | 11.60 | -1.75 |
| LmB 35 | 98.88 | 4.39 | 22.12 | -33.30 | 3.93 | 0.00 | 6.30 | -44.74 |
| LmB 11 | 98.88 | 4.39 | 27.40 | -17.42 | 4.23 | 7.63 | 8.10 | -28.95 |
| LmB 14 | 98.88 | 4.39 | 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 10 | 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 |
| LmB 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 | 1.51 | 33.18 | 0.00 | 3.93 | 0.00 | 11.40 | 0.00 |
| LmB 27 | 95.55 | 1.51 | 32.10 | -3.25 | 3.85 | -20.49 | 8.56 | -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 | 93.33 | -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 |
| LmB 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 (%) |
| LmA 6     | 83.67 ab      | 35.68            | 57.00 a           | 85.87          | 13.00 a           | 69.56          |
| 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           |
| LmA 6     | 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           |
| LmA 5     | 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          |
| Control - | 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          |

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)
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 LmB1 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, and 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.

ACKNOWLEDGEMENTS

This research was funded through a grant of The Directorate of Research and Community Service, Directorate General of Research and Technology Strengthening Ministry of Research, and the Technology and Higher Education, with Research Contract Number: 163/SP2H/AMLD/LT/DRPM/2020 and T/26/UN.16.17/PT.01.03/AM/PD-Pangan/2020.

REFERENCES

Ahkidi A. 2014. Karakterisasi bakteri endofit penghasil Volatile Organic Compounds (VOCs) untuk meningkatkan ketahanan tanaman kentang terhadap penyakit layak buah. [Disertasi]. Sekolah Pascasarjana Institut Pertanian Bogor, Bogor. [Indonesian]

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25 (17): 3389-3402. DOI: 10.1093/nar.25.17.3389

Annan IA, Ludwig W, Schleifer KH. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59 (1): 143-169. DOI: 10.1128/mr.59.1.143-169.1995

Banerjee G, Gorhics, Chattopadhyay P. 2018. Beneficial effects of biocontrolling agent Bacillus cereus IB311 on the agricultural crop production and its biomass optimization through resistance surface. An. Acad. Bras. Ciênc. Anais da Academia Brasileira de Ciências 90: 2149-2159. DOI: 10.1590/0001-37652017201703062

Compart S, Reiter B, Sessitsch A, Nowak J, Clement C, Barka EA. 2005. Endophytic colonization of Vitis vinifera L. by plant growth-promoting bacterium Burkholderia sp. Strain PsJ2, Appl Environ Microbiol 71 (4): 1685-1693. DOI: 10.1128/AEM.71.4.1685-1693.2005

Directorate of Food Crop Protection . 2019. Laporan Serangan OPT dan DPI. http://deptan.go.id/. Diakses pada 21 November 2020. [Indonesian]

Faisal M. 2013. Inoculation of plant growth promoting bacteria Ochrobactrum intermedium, Brevibacterium sp. and Bacillus cereus induce plant growth parameters. Appl Biotechnol Rep 1 (1): 45-53. DOI: 10.5296/jab.v1i1.3698

Faisal M, Hasnain S. 2006. Colonization potential of Ochrobactrum intermedium, Bacillus cereus and Brevibacterium sp. on Triticum aestivum and Helianthus annuus roots. J Plant Sci 1 (1): 36-41

Faltin F, Lottmann J, Grosch R, Berg G. 2004. Strategy to select and assess antagonistic bacteria for biological control of Rhizoctonia solani. Kuhn. Can J Microbiol 50 (10): 811-820. DOI: 10.1139/W04-063

Galkiewicz JP, Kelloo CG. 2008. Cross-kingdom amplification using bacteria-specific primers: Complications for studies of coral microbial ecology. Appl Environ Microbiol 74 (24): 7828-7831. DOI: 10.1126/AM1.01303-08

Hall T. 2011. BioEdit: An important software for molecular biology. GERF Bull Biosci 2 (1): 60-61

Hallmann J. 1999. Plant Interaction with Endophytic Bacteria. Ed. CAB Publishing. New York.

Husnain, Widowati LR, Las I, Sarwani M, Rochayati S, Setyorni, Hartatik, Subokha, IGMB, Sussitik IW. 2020. Rekomendasi Pupuk N, P, dan K Spesifik Lokasi untuk Tanaman Padi, Jagung dan Kedelai pada Lahan Sawah (Pemakanan). Buku I: PADI. Badan Penelitian dan Pengembangan Pertanian, Kementerian Pertanian, Subang. [Indonesian]

Indonesian Ministry of Agriculture. 2019. Data Lima Tahun Terakhir Produksi, Luas Panen, dan Produktivitas Padi di Indonesia. https://www.pertanian.go.id/. Diakes pada 27 November 2019. [Indonesian]

Juwita. 2010. Potensi bakteri endofit dalam ketahanan tanaman kentang terhadap penyakit layak daun bakteri pada tanaman padi di lapangan menggunakan rizobakteri indujen. Jurnal HPT Tropika 14 (1): 57-63. [Indonesian]

Klement Z, Rudolph K, Sand DC. 1990. Methods in Phytochemistry. Academia Kiado Budapest.

Kloeper JW, Zablотовicz RM, Tipping EM, Lishitsh R. 1999. Plant root bacterial interactions in biological control of soilborne disease and potential extension to systemic and foliar diseases. Australas Plant Pathol 28: 21-26

Kumar S, Tamura K, Nei M. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings Bioinformatics 5 (2): 150-163. DOI: 10.1093/bib/5.2.150

Malfanova N. 2011. Characterization of Bacillus subtilis HCS, a novel plant beneficial endophytic strain from giant hogweed. Microbiol Biotechnol 4 (4): 23-32. DOI: 10.1111/j.1751-7915.2011.00253.x

Mehnaz S, Mirza MS, Haurat J, Bally R, Normand P, Bano A, Malik KA. 2001. Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. Can J Microbiol 47: 110-117. DOI: 10.1139/cjm-47-2-110

Messiha NAS, van Diepening AD, Farag NS, Abdallah SA, Janse JD, van Bruggen AHC. 2007. Stenotrophomonas maltophilia: a new potential biocontrol agent of Ralstonia solanacearum, causal agent of potato brown rot. Eur J Plant Pathol 118: 211-225. DOI: 10.1007/s10658-007-9136-6

Munif A, Wiyono S, Suwarno. 2012. Isolasi bakteri endofit asli padi gogo dan potensinya sebagai agens biokontrol dan pemacu pertumbuhan. J Fitopatol Indonesia 8 (3): 57-65. [Indonesian]

Parida I. 2016. Isolasi, Seleksi, dan Identifikasi Bakteri Endofit sebagai Agens Penginduksi Ketahanan Tanaman Padi terhadap Penyakit

| Isolate code | Query length | Related species | Similarity (%) | Accession number |
|--------------|--------------|----------------|----------------|-----------------|
| LmA6         | 1494         | Bacillus cereus MD152 | 96.87 | MT642947 |
| LmB2         | 1217         | Bacillus thuringiensis ATCC 10792 | 98.20 | CP027054 |
| LmB1         | 1363         | Ochrobactrum intermedium strain OI1 | 97.52 | KT985368 |
| LmB35        | 1439         | Stenotrophomonas maltophilia strain A1w2 | 97.92 | AY512625 |
Hawar Daun Bakteri. [Thesis]. Sekolah Pascasarjana Institut Pertanian Bogor, Bogor. [Indonesian]

Rahma H, Zainal A, Surahman M, Sinaga MSS, Giyanto. 2014. Potensi bakteri endofit dalam menekan penyakit layu stewart (Pantoea stewartii subsp. stewartii) pada tanaman jagung. Jurnal HPT Tropika 2 (14): 121-137. [Indonesian]

Rahma H, Nurbailis, Kristina N. 2019. Characterization and potential of plant growth-promoting rhizobacteria on rice seedling growth and the effect on Xanthomonas oryzae pv. oryzae. Biodiversitas 20 (12): 3654-3661.

Resti Z, Habazar T, Putra DP, Nasrun. 2013. Skrining dan identifikasi isolat bakteri endofit untuk mengendalikan penyakit hawar daun bakteri pada bawang merah. J HPT Tropika 13 (2): 167-178. [Indonesian]

Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Berg G, van der Lelie D, Dow JM. 2009. The versatility and adaptation of bacteria from the genus Stenotrophomonas. Nat Rev Microbiol 7: 514-525. DOI: 10.1038/nrmicro2163.

Saitou N, Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 4 (4): 406-425.

Sholikhin I. 2014. Keefektifan bakteri endofit sebagai agen hayati terhadap penyakit hawar daun bakteri (Xanthomonas oryzae pv. oryzae) pada padi. [Hon. Thesis]. Institut Pertanian Bogor, Bogor. [Indonesian]

Sopialena, Sofian, Nurdiana J. 2019. Diversity of diseases of rice (Oryza sativa) in Kuta Kertaneagara, Indonesia. Asian J Agirc 3: 55-62. DOI: 10.13057/asianagric/g030204.

Sudir, Yuliani D. 2016. Composition and distribution of Xanthomonas oryzae pv. oryzae pathotypes, the pathogen of rice bacterial leaf blight in Indonesia. AGRIVITA 38 (2): 174-185. [Indonesian]

Suparyono, Sudir, Suprihantro. 2004. Pathotype profile of Xanthomonas oryzae pv. oryzae isolates from the rice ecosystem in Java. Indones J Agric Sci 5 (2): 63-69. [Indonesian]

Suprihantro B, Daradjat AA, Satoto, Baehuki, Widiarta IN, Setyono A, Indrasari SD, Lesmana OS. 2009. Deskripsi Varietas Padi. Balai Besar Penelitian Tanaman Padi, Subang. [Indonesian]

Susanto U, Daradjat AA, Suprihanto B. 2003. Perkembangan pemuliaan padi sawah di Indonesia. Jurnal Litbang Pertanian 22 (3): 125-131. [Indonesian]

Yanti Y, Astuti FF, Habazar T, Nasution CR. 2017. Screening of rhizobacteria from rhizosphere of healthy chili to control bacterial wilt disease and to promote growth and yield of chili. Biodiversitas 18 (1): 1-9. DOI: 10.13057/biodiv/d180101.

Yanuar A. 2016. Potensi agens hayati dalam menekan perkembangan penyakit hawar daun bakteri (Xanthomonas oryzae pv. oryzae) pada padi. [Hon. Thesis]. Universitas Jember, Jember. [Indonesian]

Zehnder GW, Yao C, Murph JF, Sikora EJ, Kloepper JW. 2000. Induction of resistance in tomato against cucumber mosaic cucumovirus by plant growth-promoting rhizobacteria. BioControl 45 (1): 127-137. DOI: 10.1023/A:1009923702103.