**BIODIVERSITAS**
Volume 24, Number 4, April 2023
Pages: 2072-2079

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240417

# Screening of phyllospheric and endophytic bacteria as biocontrol agents of *Xanthomonas oryzae* pv. *oryzae*

**TITA WIDJAYANTI[1,♥], RESTU RIZKYTA KUSUMA[1], LUQMAN QURATA AINI[1], CINDY DIAH AYU FITRIANI[1,2], ANTOK WAHYU SEKTIONO[1], MOCHAMMAD SYAMSUL HADI[1], YOGO SETIAWAN[1]**

[1]Department Plant Pests and Diseases, Faculty of Agriculture, Universitas Brawijaya. Jl. Veteran, Malang 65145, East Java, Indonesia.
Tel./Fax.: +62-341-580106, ♥email: widjayanti_tita@ub.ac.id
[2]Wilmar International Plantation. Jl. Jend.Sudirman Km 62, Kotawaringin Timur 74361, Central Kalimantan, Indonesia

Manuscript received: 14 April 2022. Revision accepted: 9 April 2023.

**Abstract.** *Widjayanti T, Kusuma RR, Aini LQ, Fitriani CDA, Sektiono AW, Hadi MS, Setiawan Y. 2023. Screening of phyllospheric and endophytic bacteria as biocontrol agents of* Xanthomonas oryzae *pv.* oryzae. *Biodiversitas 24: 2072-2079.* The use of phyllospheric and endophytic bacteria as biocontrol agents may be an eco-friendly alternative strategy to control bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo). This study aimed to evaluated the antagonistic potential of phyllospheric and endophytic bacteria against *X. oryzae* pv. *oryzae*. The phyllospheric and endophytic bacteria were isolated from organic rice fields in Tumpang Sub-district, Malang District, East Java, Indonesia. The pathogenicity of *X. oryzae* pv. *oryzae* was tested on rice plants by the leaf clipping inoculation method. In this study, eight combinations of phyllospheric bacteria (PB) and endophytic bacterial (EB) isolates were chosen based on the highest percentage of inhibition. Streptomycin (positive control) and distilled water (negative control) were also used as a treatment. Identification of selected phyllospheric and endophytic bacteria was carried out through morphological and molecular characteristics. In total, 181 of PB and 76 of EB were isolated from rice plants. Of these, 11 of PB and 13 of EB isolates had potential as antagonists, shown by inhibition zone. Among all treatments, P55+E15 combination showed the highest inhibition zone (83.49%), followed by P55+E27 (82.33%) and P55+E18 (80.98%). Based on morphological and molecular identification, P55, E15, E27, and E18 isolates were identified as *Delftia lacustris*, *Bacillus cereus*, *Escherichia coli*, and *Lysinibacillus fusiformis*, respectively. The combination of *D. lacustris* (P55) and *B. cereus* (E15) can be used as a biocontrol agent against *X. oryzae* pv. *oryzae* based on in vitro assay.

**Keywords:** *Bacillus cereus*, bacterial consortium, bacterial leaf blight, *Delftia lacustris*, rice, *Xanthomonas oryzae*

## INTRODUCTION

Rice is the main staple food and one of the most important agricultural products in Asia, including in Indonesia (Redfern et al. 2012). Rice is cultivated in several countries, as it is regarded as a strategic crop for food security (Mohanty et al. 2013). However, the susceptibility of rice to insect pests and diseases is a major problem that may reduce yield. Several reports show that 25% of the annual rice yield loss is caused by insect pests and diseases (Mondal et al. 2017). Bacterial leaf blight (BLB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is the most economically important disease in rice production, causing yield loss up to 80% (Sudir and Yuliani 2016; Banerjee et al. 2018). Several strategies are used for the management of BLB, as the usage of resistant varieties to limit the disease below the level that causes economic detriment as well as treatment with quick-acting and readily available chemicals (Khan et al. 2012, 2014). Several broad-spectrum bactericides have been used to control BLB. However, these methods are costly to implement practically and are not eco-friendly (Kim et al. 2016).

Biological control of BLB is an emerging alternative strategy that has been widely considered for its low cost, sustainable, and eco-friendly characteristics (Yang et al. 2017). Phyllosphere bacteria (PB) are bacteria that live on leaf surface and have potential as disease control by protecting host against pathogen infection (Sivakumar et al. 2020; Lestari et al. 2022). PB has been reported to suppress the growth of leaf pathogens through direct competition or antagonism and provide antimicrobial metabolites to several pathogens, such as *Ralstonia solanacearum* and *X. oryzae* pv. *oryzicola* (Barrera et al. 2019; Zhou et al. 2012; Krishanti et al. 2015). Endophytes are plant-associated microbes that grow symptomless within plants as an integral part of host metabolism and function (Khare et al. 2018). Further, microbial endophytes also become an interesting area for study, mainly to understand plant-microbial interactions that enhance plant growth and protect plants from environmental stress. Recent studies highlighted the potential of endophytes for the synthesis of bioactive compounds, promotion of plant growth, and enhanced resistance to various pathogens (Sharma et al. 2016; Kandel et al. 2017; Patel and Archana 2017). In the past decade, endophyte bacteria have been looked into as a resource in the biocontrol of plant diseases and promotion of plant growth. The potential endophyte bacteria as biocontrol agents, such as *Bacillus methylotrophicus*, *B. amyloliquefaciens*, and *B. subtilis*, were reported to have been isolated from rice plants and to show antimicrobial activities against rice plant pathogens such as *Burkholderia glumae* and *Rhizoctonia solani* (Shrestha et al. 2016). The use of phyllosphere and endophyte bacteria as biocontrol

agents may be an alternative ecofriendly strategy to control BLB.

In the case of foliar pathogens, the disease suppression is achieved through activation of host defense response or by induced systemic resistance (ISR) (Romera et al. 2019). However, the success rate of ISR to manage foliar pathogens under field conditions has not been very promising (Ji et al. 2006). Several studies have reported on the potential of PB in improving plant growth (Sharath et al. 2021) and suppression of foliar phytopathogens (Sowndhararajan et al. 2013; Agbavor et al. 2022). Endophyte bacteria also have been reported to improve chickpea (*Cicer arietinum* L.) plant growth and induce suppression of black root rot caused by *Fusarium solani* (Egamberdieva et al. 2017). The combination of biocontrol agents that occupy different niches and provide different beneficial effects and mechanisms on the host plant is advantageous compared to their individual applications (Ji et al. 2006; Senthilraja et al. 2010). This study aimed to evaluated the antagonistic potential of phyllospheric and endophytic bacteria associated with rice plants against bacterial leaf blight of rice caused by *X. oryzae* pv. *oryzae*.

## MATERIALS AND METHODS

### Isolation of *Xanthomonas oryzae* pv. *oryzae*

*Xanthomonas oryzae* pv. *oryzae* was isolated from leaves of rice plants with indicated symptoms of bacterial leaf blight disease. Symptomatic leaves were cut (into 28×7 mm pieces) with a sterile scalpel. The surface of leaf was sterilized with 1% clorox for three minutes and then washed with distilled water. Then, the symptomatic leaves were crushed with a mortar and pestle before being mixed with 10 mL of distilled water and grown on semi-selective Yeast Dextrose CaCO3 Agar (Yeast extract 10 g; CaCO3 20 g; Dextrose 20 g; agar 20 g, and distilled water 1 L). The plates were incubated for 24 to 48 hours at 28˚C. Single yellow colonies, round and smooth margin, mucoid and a non-flat shape were chosen and transferred to Nutrient agar (NA) medium as pure culture for this study (Fatimah et al. 2014; Chukwu et al. 2020).

### Isolation of phyllospheric and endophytic bacteria

In this study, phyllospheric and endophytic bacteria were isolated from rice fields that were organically cultivated in Tumpang Sub-district, Malang District, East Java, Indonesia. Phyllospheric bacteria were isolated through the leaf washing technique. Five leaves from each plant were washed with 25 mL of saline solution (0.85%). Bacterial colonies on the leaf surface were dislodged using a sterile brush during washing. The suspension was transferred to 50 mL tubes and centrifuged at 6,800 g for 10 minutes. The pellet was dissolved in 1 mL of saline solution and used for bacterial isolation following the serial dilution technique (up to $10^{-7}$) on NA medium. After incubation at 35 ± 2°C for 36 hours, morphologically different bacterial colonies appearing on the medium were selected and pure-cultured on NA slants.

Endophytic bacteria were isolated from roots and stems of healthy rice plants (Hazarika et al. 2021). Samples were washed in running water to remove soil particles and sterilized by sequential immersion in 70% (v/v) ethanol for 5 minutes, followed by soaking in sodium hypochlorite solution (0.9% available chlorine) for 20 minutes and washing of the surface-sterilized samples in sterile water three times to remove surface sterilization agents. The samples were soaked in 10% (w/v) $NaHCO_3$ solution for 10 minutes to retard the growth of endophyte bacteria. Stems and roots were crushed in a mortar with a pestle, and distilled water was added to prepare a bacterial suspension of $10^7$ cfu/mL; the bacterial suspension was then transferred to NA medium with an inoculating loop (Nichrome, Qty/pk-12). Bacterial colonies of phyllospheric and endophytic bacteria were examined under a stereoscope and identified based on morphological characteristics.

### Pathogenicity test of *Xanthomonas oryzae pv. oryzae*

The pathogenicity of *X. oryzae pv. oryzae* was tested on rice plants with the leaf clipping inoculation method (Ke et al. 2017). Xoo was cultured in nutrient broth at 30°C for 24 hours, and $10^9$ cfu/mL bacterial suspension was prepared. Bacterial suspension was infiltrated into leaves using scissors dipped in the test suspension. Virulence of *X. oryzae pv. oryzae* strains was evaluated on 25-day old rice plants (IR64 variety). The virulence was assessed on the basis of lesion length initiated by individual strains at 14 days after inoculation.

### Antagonistic assay of phyllospheric and endophytic bacteria

The antagonistic assay for phyllospheric and endophytic bacteria was performed following the method described by Kawaguchi et al. (2007). Each bacterial colony was suspended in 1 mL of sterile water and mixed. Then, sterile paper discs (5 mm diameter) were placed on the bacterial suspensions; the sterile paper discs were drained for 60 minutes before being placed on NA medium and incubated for 48 hours at 28°C. Each treatment repeated four times and were then placed face down on filter paper moistened with chloroform to inactivated the test strain bacteria with chloroform vapor. After chloroform evaporation for 20 minutes, five plates were obscured by suspension of Xoo pathogenic bacterial cells with a density of $10^9$ cfu/mL. The plates were incubated 48 hours after the drizzle. Phyllospheric or endophytic bacteria that formed clear zones around bacterial colonies possessed the mechanism of antibiotic inhibition of Xoo pathogenic bacteria.

### Synergy test between phyllospheric and endophytic bacteria

The synergism test was conducted to investigate phyllospheric and endophytic bacteria against Xoo according to the method of Kawaguchi et al. (2007) with modifications. For this, a sterile paper disc (5 mm diameter) was dipped into the phyllospheric bacterial suspension. The wet discs were dried for 60 minutes before being placed on NA medium and incubated for 48 hours at 28°C. The plates

were then placed upside down on filter paper moistened with chloroform to inactivated the test of bacterial strains. After evaporation of chloroform, endophyte bacteria with a density of $10^9$ cfu/mL was sprayed using sprayer on the same plates of phyllospheric bacterial. This testing was observed for 48 hours; if no inhibition zones formed around the bacterial colonies, the bacteria can then be developed for the consortium.

**In vitro assay of consortium phyllospheric and endophytic bacteria against *Xanthomonas oryzae* pv. *oryzae***

The phyllospheric and endophytic bacteria were suspended in 1,000 µL of sterile water and mixed using a vortex (suspensions for phyllospheric and endophytic bacteria were made separately). Then, 500 µL of each phyllospheric and endophytic bacteria suspensions were transferred to a 1.5 mL of eppendorf tubes and mixed with a vortex. Four filter paper sheets were soaked in bacterial consortium suspension for 1 minutes. Then, sheets were dried or 60 minutes and placed on NA medium, then incubated for 48 hours at 28°C. The plates were then placed upside down on filter paper moistened with chloroform to inactivated the test of bacterial strains. After evaporation of chloroform, the pathogenic bacteria Xoo was sprayed at $10^9$ cfu/mL using sprayer. The plates were examined after 24 hours incubation at 28°C. Phyllospheric and endophytic bacteria that formed clear zones around bacterial colonies possessed the mechanism of antibiotic inhibition of Xoo pathogenic bacteria. The ability of bacterial consortium to inhibit the growth of Xoo was investigated based on the diameter of clear zones. The zones formed in each treatment indicated the inhibition index of these bacteria. The following formula was used to calculate the inhibitory index (Dias et al. 2014):

$$\text{Inhibition index (\%)} = \frac{\emptyset \, Clear\ zone\ (Cm) - \emptyset \, bacterial\ colony(Cm)}{\emptyset \, Clear\ zone\ (Cm)}$$

**Morphological and molecular identification of selected phyllospheric and endophytic bacteria**

Morphological characterization was performed by observing bacterial colonies, including colony shape, differences in cell shape, elevation, and edges of colonies. The bacterial colonies were observed under a stereoscope microscope (OLYMPUS, SZX-ILLB2-200) with a magnification of 40x. Molecular identification was carried out at PT. Genetica Science Indonesia, City of Tangerang, Banten. Molecular identification involved several steps, such as isolation of bacterial genome DNA, amplification of the 16S rRNA gene by the PCR method, agarose gel electrophoresis, and sequencing. The DNA isolation process was carried out to obtain genomic DNA that can then be used in detection tests by PCR and for sequencing. DNA isolation was carried out using the PrestoTM Mini gDNA Bacteria Kit. Isolated genomic DNA was amplified by the PCR technique using the universal 16S rRNA primers of 63f (5'- CAGGCCTAACACATGCAAGTC 3') and 1387r (5'-GGGCGGWGTGTACAAGGC-3') with a target amplicon of 1500 base pairs.

**Data analysis**

The data of inhibition zone diameter and inhibition index were analyzed by using analysis of variance (ANOVA), and if the results were significantly different among the treatments, a *post-hoc* test was used with the Duncan Multiple Range Test (DMRT) at an error level of 5%. Data were analyzed with the SPSS program, version 22. The phylogenetic tree was made by using MEGA X (Molecular Evolutionary Genetics Analysis) program.

## RESULTS AND DISCUSSION

**Morphological characteristics of *Xanthomonas oryzae* pv. *oryzae* from infected rice leaves**

Bacteria isolated from rice leaves were yellowish in color, fluidal, and round with equal border colonies (Figure 1A-B). *Xanthomonas oryzae* pv. *oryzae* bacterial isolates showed BLB symptoms on IR64 rice varieties. BLB symptoms appeared at the tips of inoculated leaves, which turned them from green to dull, yellowish, and dry, and occurred two days after inoculation (Figure 1C). The pathogenic bacteria isolated from rice leaves was identified as *X. oryzae* pv. *oryzae*. The bacterium was gram-negative, showed hypersensitive reactions on tobacco leaves, oxidative, not producing fluorescent pigment on King's B agar, and accumulated Poly-β-hydroxybutyrate in cells (Table 1).

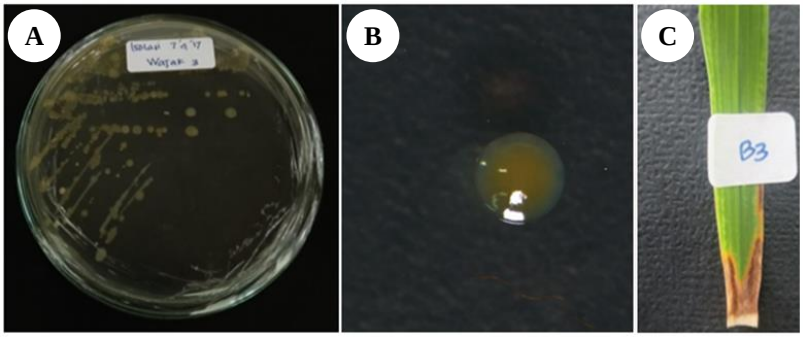


**Figure 1**. Morphological identification of *Xanthomonas oryzae* pv. *oryzae*: A. colony of *Xanthomonas oryzae* pv. *oryzae* in YDCA medium; B. single colony of *Xanthomonas oryzae* pv. *oryzae*; and C. BLB symptoms on rice leaf

**Table 1.** The characteristic of bacterial leaf blight caused by *Xanthomonas oryzae* pv. *oryzae*

| Characteristic | Reactions |
|---|---|
| Hypersensitive Reactions (HR) | + |
| Pathogenicity (wilt symptom on potato plant) | + |
| Gram | - |
| Catalase | + |
| Oxidative/Fermentative | O |
| Nitrate Reduction | - |
| Gelatin Hydrolysis | + |
| Fluorescence pigment production | - |
| Arginine Hydrolysis | + |
| Poly-β-hydroxibutirate accumulation | + |
| Levan production | + |
| Starch hydrolysis | + |
| Soft rot on potato | - |
| Acid Production from: | |
| a. Glycerol | + |
| b. Sorbitol | + |
| c. Mannitol | + |
| d. Glucose | + |
| e. Galactose | + |
| f. Sucrose | + |
| g. Lactose | + |
| h. Maltose | + |

Notes: + indicates positive reaction, - indicates negative reaction

**Table 2.** Percentage of inhibition of phyllospheric and endophytic bacteria against Xoo in vitro assay

| Origins | Isolates code | Inhibition (%) |
|---|---|---|
| Phyllosphere | P1 | 59.58 efg |
| | P9 | 33.06 c |
| | P47 | 0.20 a |
| | P53 | 76.59 hi |
| | P54 | 18.27 b |
| | P55 | 79.24 i |
| | P123 | 43.22 cd |
| | P124 | 69.15 ghi |
| | P165 | 76.16 hi |
| | P173 | 65.54 fgh |
| | P174 | 0.40 a |
| | P175 | 0.60 a |
| | P176 | 56.40 ef |
| | Streptomycin 20% | 52.22 de |
| | Aquadest | 0.00 a |
| Endophyte | E15 | 81.03 ef |
| | E18 | 83.68 f |
| | E26 | 54.89 d |
| | E27 | 80.18 ef |
| | E28 | 22.84 b |
| | E29 | 39.76 c |
| | E33 | 73.28 ef |
| | E34 | 74.30 ef |
| | E39 | 73.42 ef |
| | E52 | 54.61 d |
| | E53 | 71.34 e |
| | E54 | 78.90 ef |
| | E55 | 22.84 b |
| | Streptomycin 20% | 55.33 d |
| | Aquadest | 0.00 a |

Note: Means followed by different letters are significantly different in each row is not significantly different by Duncan's test ($P < 0.05$)

**Phyllospheric and endophytic bacteria isolated from rice plants and their antagonistic activity**

In total, 181 phyllospheric bacteria were isolated. Among 181 isolates, 11 isolates showed antagonistic potential to inhibit Xoo. In the dual culture assay, 11 bacteria inhibited the growth of Xoo, ranging from 0.2% to 79.24%. Based on their phylloplane colonization and bacterial growth suppression ability (inhibition percentages), three bacterial isolates, such as P55 (79.24%), P53 (76.59%), and P165 (76.16%) were selected for further studies (Table 2). From the roots and stems of rice plants, 76 of endophytic bacteria were isolated. Of these, 13 showed antagonistic activity against Xoo. The highest antibacterial activity was showed by EA15 (81.03%) and EA18 (83.68%) from roots, and EB27 (80.18%) from the stems of rice plants (Table 2). The phyllospheric and endophytic bacteria that had the highest inhibition percentages were chosen for synergism assay.

**Synergism of phyllospheric and endophytic bacteria against *Xanthomonas oryzae* pv. *oryzae* in vitro**

The phyllospheric and endophytic bacterial isolates showed synergistic relationship, as they did not form inhibitory or clear zones around the paper disc. The P55 isolate had a synergism with E15, E18, and E27 isolates (Figure 2.A). The E27 isolate showed synergism with P124 isolate, as indicated by the absence of a clear zone around the paper disc (Figure 2.B). The EA15 isolates showed synergism with P124 and P165 isolates (Figure 2.C), and E18 isolates showed synergism with PB124 and P165 isolates (Figure 2.D). The presence of synergism among isolates was indicated by the absence of clear zones around the paper disc.

**Effect of the combination of phyllospheric and endophytic bacteria against *Xanthomonas oryzae* pv. *oryzae* in vitro**

The results showed that bacterial consortium had a significant effect on inhibiting Xoo (Table 3). Eight treatments of the bacterial consortium showed clear zones forming around bacterial colonies on NA media. The largest clear zone diameter was found in P55+E15 (3.05 cm) at 24 hours after inoculation (HAI), which decreased to 3.03 cm at 48 HAI. The highest inhibition percentage was found in P55+E15 bacterial consortium treatment, with 83.57% at 24 HAI and 83.49% at 48 HAI (Table 3).

**Identification of selected phyllospheric and endophytic bacteria**

The results showed that all bacterial isolates had the same morphology in form, margin, and elevation (Table 4). However, the bacterial isolates showed various colors (Figure 3).

Based on molecular identification, the visualization of PCR product on agarose gel showed the presence of DNA band encoding the 16S rRNA gene in the sample size of 1200 bp after comparison with the marker (1 Kb DNA ladder Gene DireX) (Figure 4).

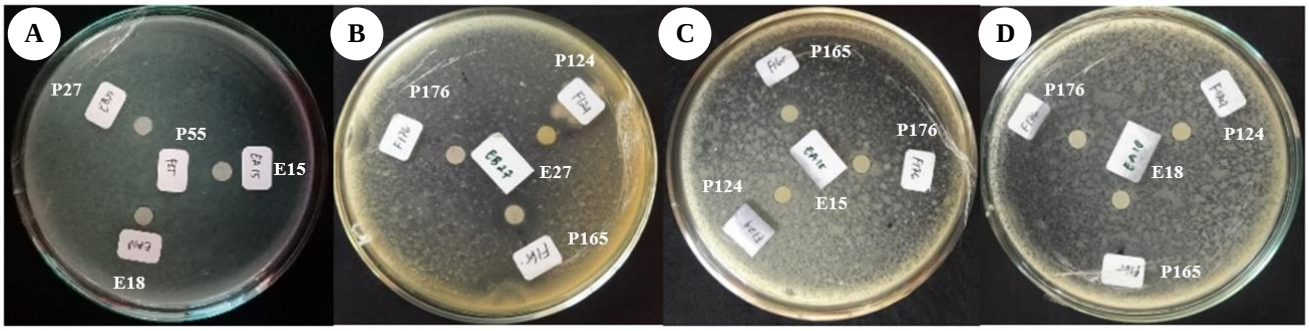


**Figure 2.** Phyllospheric and endophytic bacterial synergism against Xoo at 48 DAI: A. P55 with E15, E18, and E27 isolates; B. E27 with P124 and P165 isolates; C. E15 with P124 and P165 isolates; and D. E18 with P124 and P165 isolates

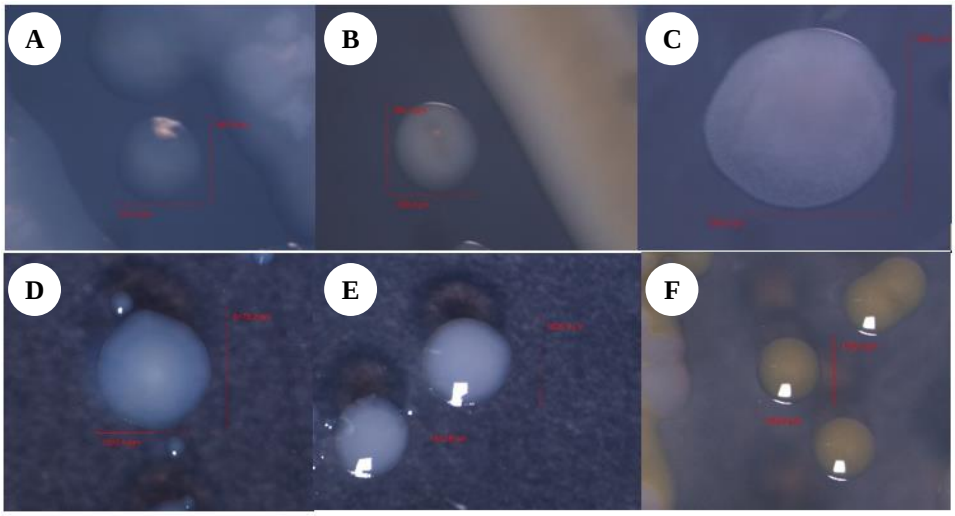


**Figure 3.** Colonies of phyllospheric and endophytic bacteria isolates: A. P55, B. P124, C. P165, D. E15, E. E18, and E27

**Table 3.** Inhibition zone of *Xanthomonas oryzae* pv. *oryzae* by consortium bacteria

| Treatments | Diameter of inhibition zone (cm) | | Inhibition (%) | |
|---|---|---|---|---|
| | 24 HAI (Mean ± SD) | 48 HAI (Mean ± SD) | 24 HAI | 48 HAI |
| Control | 0.00 a | 0.00 a | 0.00 a | 0.00 a |
| P124+E15 | 2.45 d | 2.43 e | 79.53 d | 79.47 d |
| P124+E18 | 1.47 c | 1.42d | 26.21 b | 22.77 b |
| P124+E27 | 0.30 ab | 0.85 b | 14.58 ab | 14.13 ab |
| P165+E15 | 2.32 d | 2.37 e | 78.46 d | 78.87 d |
| P165+E18 | 0.66 b | 1.26 cd | 30.96 b | 19.80 b |
| P55+E15 | 3.05 e | 3.03 h | 83.57 d | 83.49 d |
| P55+E18 | 2.56 de | 2.64 f | 80.45 d | 80.98 d |
| P55+E27 | 2.76 de | 2.83 g | 81.85 d | 82.33 d |
| Streptomycin 20% | 1.16 c | 1.10 c | 56.44 c | 54.45 c |

Note: HAI: Hour after inoculation; Means followed by different letters are significantly different in each row is not significantly different by Duncan's test ($P < 0.05$)

**Table 4.** Colony morphology of phyllospheric and endophytic bacterial isolates

| Isolates | Colony morphology | | | |
|---|---|---|---|---|
| | Color | Shape | Margin | Elevation |
| P55 | Cloudy white | Round | Entire | Convex |
| P124 | Yellow | Round | Entire | Convex |
| F165 | White | Round | Entire | Convex |
| E15 | Milky white | Round | Entire | Convex |
| E18 | Milky white | Round | Entire | Convex |
| E27 | Yellow | Round | Entire | Convex |

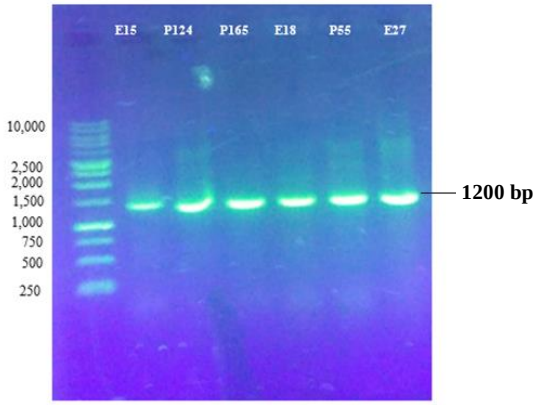


**Figure 4.** Bands of PCR product in agarose gel electrophoresis for 16S rRNA gene

DNA band was firmed and clear on gel when irradiated with ultraviolet light. The bacterial isolates collected from the phyllosphere and endophyte of rice plants were identified as follows: E15 had 98.48% homology with *Bacillus cereus*; E18 had 98.48% homology with *Lysinibacillus fusiformis*; E27 had 98% homology with *Escherichia coli*; P55 had 96.83% homology with the bacterium *Delftia lacustris*; P124 had 84.62% homology with uncultured bacterium clone SN_IN_327; and P165 had 99.39% homology with *Bacillus cereus* (Table 5). The results of bacterial sequences from the 16S rRNA gene were analyzed for their relationship level using the phylogenetic tree approach (Figure 5). The tree showed the position of the sample together with the species to which it belonged.

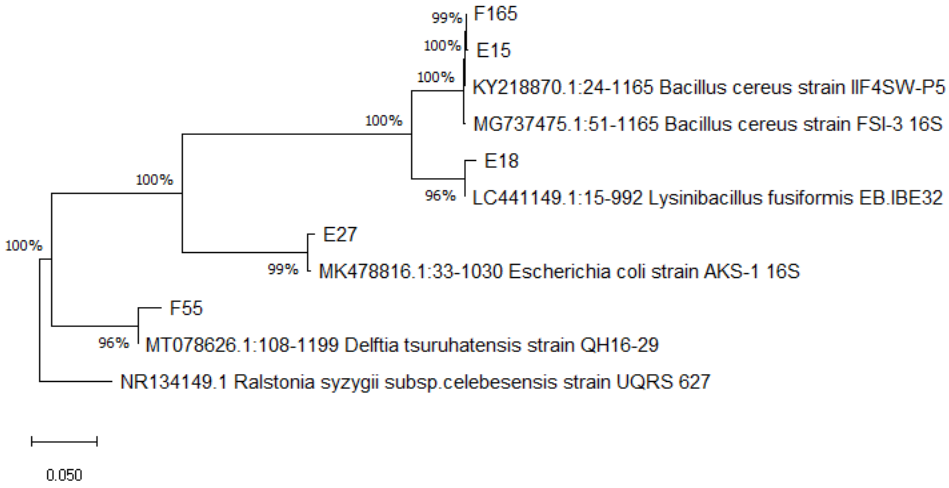


**Figure 5.** Phylogenetic tree of phyllospheric and endophytic bacteria isolates

**Table 5.** Molecular identification of phyllospheric and endophytic bacteria isolates

| Isolates | Taxonomical assignment | Query cover (%) | Similarity (%) | Accession numbers |
|---|---|---|---|---|
| E15 | *Bacillus cereus* | 100 | 98.48 | MG737475.1 |
| E18 | *Lysinibacillus fusiformis* | 92 | 97.55 | LC441149.1 |
| E27 | *Escherichia coli* | 99 | 98.00 | MK478816.1 |
| P55 | *Delftia lacustris* | 97 | 96.83 | KU726262.1 |
| P124 | Uncultured bacterium clone SN_IN_327 | 9 | 84.62 | JN211282.1 |
| P165 | *Bacillus cereus* | 100 | 99.39 | KY218870.1 |

## Discussion

The pathogenic bacteria isolated in the present study showed symptoms of BLB in the IR64 rice variety. Symptoms of BLB appear on the tips of leaves, which turn green to dull and eventually yellow. These symptoms were found to be similar to those reported by Ke et al. (2017). In the present study, BLB symptoms occurred at two days after incubation. The duration of incubation period varies for different diseases. Symptoms of the disease can appear 2 to 4 days after inoculation. However, for most plant diseases, symptoms begin to appear several days to several weeks after inoculation (Leclerc et al. 2014). According to Ahsan et al. (2021), incubation period for 21 rice varieties against bacterial leaf blight ranged from 3-5 days. An influencing factor for the incubation period is the virulence of plant-pathogenic bacteria. According to Leclerc et al. (2014), the incubation period of the disease is one of the factors that indicates the virulence of a pathogen. BLB disease occurs in susceptible rice plants; it is virulent to certain plant varieties and under certain environmental conditions (Niones et al. 2022).

The bacterial consortium consisting of phyllospheric and endophytic bacteria in this study showed a synergistic relationship, as none of the tested bacteria produced inhibition zone around the paper disc. According to Zhang et al. (2018), the microbial consortium is a combination of several microbes that form a community and have cooperative, commensal, and mutualistic relationships. In this study, the phyllospheric and endophytic bacteria have a synergistic relationship as they did not form inhibitory zones, indicating that the bacteria could be developed as a consortium for Xoo. Nurcahyanti et al. (2021) reported that the formation of inhibitory zones indicated a competitive relationship between bacteria. Thomloudi et al. (2019) stated that consortium of two or more strains of bacteria could be carried out if each bacterium does not have a growth suppression effect during contact within in vitro culture or in adjacent growth sites. The results of in vitro synergism test of bacterial strains represent the nature of positive interactions between bacteria to a certain extent, allowing bacterial strain tests to be conserved (Andhare and Babu 2017). Synergistic growth can occur when several species produce complementary enzymes that play a role in forming secondary metabolites between bacterial isolates. The mechanism of synergism between bacterial isolates are thought to be due to the aging of bacteria of certain genera, which can provide some nutrients that cannot be synthesized by bacteria of other genera (Deng and Wang 2016).

The consortium with the highest inhibition against Xoo was P55+E15 isolate treatment. The P55 isolate was collected from the phyllosphere and E15 isolate was collected from the roots of organic rice. Based on the sequence of 16S rRNA, P55 and E15 isolates were identified as *D. lacustris* and *B. cereus*, respectively. Han et al. (2005), reported that *Delftia* sp. possesses nitrogen-fixing trait capable of suppressing the growth of rice pathogens, such as *X. oryzae* pv. *oryzae*, *Pyricularia oryzae*, and *Rhizoctonia solani*. Moreover, species of *Delftia* genus have the ability to solubilize phosphate and have potential as plant growth-promoting bacteria (Woźniak et al. 2019). Whereas, *B. cereus* collected from rice leaves showed antifungal activity against *Magnaporthe oryzae* and contribute to the elucidation of genetic mechanisms of plant growth promotion such as IAA, phenazine, siderophore, and tryptophan (Zhu et al. 2021). Ahmed et al. (2020) reported that *B. cereus* showed strong in vitro antibacterial activity against rice bacterial pathogen Xoo along with an increase in plant growth.

In conclusion, 181 isolates of phyllospheric bacteria and 76 isolates of endophytic bacteria were successfully isolated from infected rice leaves. From these, 11 isolates of phyllospheric bacteria and 13 isolates of endophytic bacteria have the potential as antagonists against Xoo, because they produced inhibition zones. The consortium of P55+E15 isolates showed highest inhibition zone, followed by P55+E27 and P55+E18. Based on morphological and molecular identification, P55, E15, and E27 isolates were identified as *D. lacustris*, *B. cereus*, and *E. coli*, respectively. The combination of *D. lacustris* (P55) and *B. cereus* (E15) can be used as a biocontrol agent against *X. oryzae* pv. *oryzae* based on in vitro assay.

## ACKNOWLEDGMENTS

The authors would like to thank the Young Research Grant from the Research and Community Service Institute, Brawijaya University, as the funder for this research.

## REFERENCES

Agbavor C, Mirza BS, Wait A. 2022. The effects of phyllosphere bacteria on plant physiology and growth of soybean infected with *Pseudomonas syringae*. Plants 11 (19): 2634. DOI: 10.3390/plants11192634.

Ahmed T, Shahid M, Noman M, Niazi MBK, Mahmood F. 2020. Silver nanoparticles synthesized by using *Bacillus cereus* SZT1 ameliorated the damage of bacterial leaf blight pathogen in rice. Pathog (Basel, Switzerland) 9 (3): 160. DOI: 10.3390/pathogens9030160.

Ahsan R, Ullah S, Yaseen I, Fateh F, Fayyaz M. 2021. Assessment of bacterial leaf blight incidence and severity in rice growing areas of Pakistan. Pak J Agric Res 34: 693-699. DOI: 10.17582/journal.pjar/2021/34.4.693.699.

Andhare P, Babu S. 2017. Compatibility of *Azospirillum brasilense* and *Pseudomonas fluorescens* in growth promotion of groundnut (*Arachis hypogea* L.). An Acad Bras Cienc 89: 1027-1040. DOI: 10.1590/0001-3765201720160617.

Banerjee A, Somnath R, Manas KB, Someswar B, Meera KK, Mandal NP, Arup KM, Dipankar M. 2018. A survey of bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) resistance in rice germplasm from eastern and northeastern India using molecular markers. J Crop Prot 112: 168-176. DOI: 10.1016/j.cropro.2018.05.026.

Barrera SE, Sarango-Flóres SW, Montenegro-Gómez SP. 2019. The phyllosphere microbiome and its potential application in horticultural crops: A review. Rev Colomb Cienc Hortícolas 13 (3): 384-396. DOI: 10.17584/rcch.2019v13i3.8405.

Chukwu SC, Rafii MY, Ramlee SI, Ismail SI, Oladosu Y, Kolapo K, Musa I, Halidu J, Muhammad I, Ahmed M. 2020. Marker-assisted introgression of multiple resistance genes confers broad spectrum resistance against bacterial leaf blight and blast diseases in PUTRA-1 rice variety. Agronomy 10 (1): 42. DOI: 10.3390/agronomy10010042.

Deng YJ, Wang SY. 2016. Synergistic growth in bacteria depends on substrate complexity. J Microbiol 54 (1): 23-30. DOI: 10.1007/s12275-016-5461-9.

Dias C, Aires A, Saavedra MJ. 2014. Antimicrobial activity of isothiocyanates from cruciferous plants against methicillin-resistant *Staphylococcus aureus* (MRSA). Intl J Mol Sci 15 (11): 19552-19561. DOI: 10.3390/ijms151119552.

Egamberdieva D, Wirth SJ, Shurigin VV, Hashem A, Abd_Allah EF. 2017. Endophyte bacteria improve plant growth, symbiotic performance of chickpea (*Cicer arietinum* L.) and induce suppression of root Rot caused by *Fusarium solani* under salt stress. Front Microbiol 8: 1887. DOI: 10.3389/fmicb.2017.01887.

Fatimah, Priyatno TP, Fadlillah SH, Baroya M, Sasongko D, Suryadi Y, Kadir TS. 2014. Isolation and disease assesment of *Xanthomonas oryzae* pv. *oryzae* from Java Island and pathogenic assay on near isogenic lines with different resistant genes. J Biologi Indones 10 (2): 237-224.

Han J, Sun L, Dong X, Cai Z, Sun X. 2005. Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent against various plant pathogens. Syst Appl Microbiol 28 (1): 66-76. DOI: 10.1016/j.syapm.2004.09.003.

Hazarika SN, Saikia K, Borah A, Thakur D. 2021. Prospecting endophytic bacteria endowed with plant growth promoting potential isolated from *Camellia sinensis*. Front Microbiol 12. DOI: 10.3389/fmicb.2021.738058.

Ji P, Campbell HL, Kloepper JW, Jones JB, Suslow TV. 2006. Integrated biological control of bacterial speck and spot of tomato under field

conditions using foliar biological control agents and plant growth-promoting rhizobacteria. Biol Control 36 (3): 358-367. DOI: 10.1016/j.biocontrol.2005.09.003.

Kandel SL, Firrincieli A, Joubert PM, Okubara PA, Leston ND. 2017. An in vitro study of bio-control and plant growth promotion potential of salicaceae endophytes. Front Microbiol 8: 386. DOI: 10.3389/fmicb.2017.00386.

Kawaguchi A, Inoue K, Nasu H. 2007. Biological control of grapevine crown gall by nonpathogenic *Agrobacterium vitis* strain VAR03-1. J Gen Plant Pathol 73: 133-138. DOI: 10.1007/s10327-006-0327-3.

Ke Y, Hui S, Yuan M. 2017. *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. Bio Protoc 7 (19): e2568. DOI: 10.21769/BioProtoc.2568.

Khan J, Siddiq R, Arshad HMI, Anwar H, Saleem K. 2012. Chemical control of bacterial leaf blight of rice caused by *Xanthomonas Oryzae* Pv. *oryzae*. Pak J Phytopathol 24: 97-100.

Khan M, Naeem M, Iqbal M. 2014. Breeding approaches for bacterial leaf blight resistance in rice (*Oryza sativa* L.), current status and future directions. Eur J Plant Pathol 139: 27-37. DOI: 10.1007/s10658-014-0377-x.

Khare E, Mishra J, Arora NK. 2018. Multifaceted interactions between endophytes and plant: Developments and Prospects. Front Microbiol 9: 2732. DOI: 10.3389/fmicb.2018.02732.

Kim SI, Song JT, Jeong JY, Seo HS. 2016. Niclosamide inhibits leaf blight caused by *Xanthomonas oryzae* in rice. Sci Rep 16 (6): 21209. DOI: 10.1038/srep21209.

Krishanti N, Wahyudi A, Nawangsih A. 2015. Non-Pathogenic phyllosphere bacteria producing bioactive compounds as biological control of *Xanthomonas oryzae* pv oryzae. Intl J Pharma Bio Sci 6: B801-B810.

Leclerc M, Doré T, Gilligan CA, Lucas P, Filipe JA. 2014. Estimating the delay between host infection and disease (incubation period) and assessing its significance to the epidemiology of plant diseases. PLoS One 9 (1): e86568. DOI: 10.1371/journal.pone.0086568.

Lestari SR, Choliq FA, Sektiono AW, Hadi MS, Aditya HF, Rahmadhini N, Kusuma RM, Setiawan Y. 2022. Screening of quorum quenching activity of rhizobacteria against *Pectobacterium carotovorum* subsp. carotovorum. Biodiversitas 23 (8): 4336-4342. DOI: 10.13057/biodiv/d230859.

Mohanty S, Wassmann R, Nelson A, Moya P, Jagadish SVK. 2013. Rice and climate change: significance for food security and vulnerability. IRRI Discussion Paper Series No. 49. Los Baños, Philippines: International Rice Research Institute (IRRI).

Mondal D, Ghosh A, Roy D, Kumar A, Shamurailatpam D. 2017. Yield loss assessment of rice (*Oryza Sativa* L.) due to different biotic stresses under system of rice intensification (SRI). J Entomol Zool Stud 5: 1974-1980.

Niones JT, Sharp RT, Donayre DKM. 2022. Dynamics of bacterial blight disease in resistant and susceptible rice varieties. Eur J Plant Pathol 163: 1-17 DOI. 10.1007/s10658-021-02452-z.

Nurcahyanti UD, Wahyuni WS, Masnilah R, Hendranurdika AA. 2021. Diversity of *Bacillus* spp. from soybean phyllosphere as potential antagonist agents for *Xanthomonas axonopodis* pv. *glycines* causal of pustule disease. Biodiversitas 22 (11): 5003-5011. DOI: 10.13057/biodiv/d221136.

Patel J, Archana G. 2017. Diverse culturable diazotrophic endophyte bacteria from Poaceae plants show cross-colonization and plant growth promotion in wheat. Plant Soil 417. DOI: 10.1007/s11104-017-3244-7.

Redfern S, Azzu N, Binamira JS. 2012. Rice in Southeast Asia: facing risks and vulnerabilities to respond to climate change. Building Resilience for Adaptation to Climate Change in the Agriculture Sector, FAO/OECD Workshop Proceedings, 23-24 April 2012.

Senthilraja G, Theerthagiri A, Durairaj C, Kennedy J, Suresh S. 2010. A new microbial consortia containing entomopathogenic fungus, *Beauveria bassiana* and plant growth promoting rhizobacteria, *Pseudomonas fluorescens* for simultaneous management of leafminers and collar rot disease in groundnut. Biocontrol Sci Technol 20 (5): 449-464. DOI: 10.1080/09583150903576949.

Sharath S, Triveni S, Nagaraju Y, Latha PC, Vidyasagar B. 2021. The role of phyllosphere bacteria in improving cotton growth and yield under drought conditions. Front Agron (3). DOI: 10.3389/fagro.2021.680466.

Sharma D, Pramanik A, Agrawal PK. 2016. Evaluation of bioactive secondary metabolites from endophyte fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D.Don. 3 Biotech (2): 210. DOI: 10.1007/s13205-016-0518-3.

Shrestha B, Karki H, Groth D, Jungkhun N, Ham JH. 2016. Biological control activities of rice-associated *Bacillus* sp. strains against sheath blight and bacterial panicle blight of rice. PLoS One (11): e0146764. DOI: 10.1371/journal.pone.0146764.

Sivakumar N, Sathishkumar R, Selvakumar G, Shyamkumar R, Arjunekumar K. 2020. Phyllosphere Microbiomes: Diversity, Ecological Significance, and Biotechnological Applications. Plant Microbiomes Sustain Agric 25: 113-172. DOI: 10.1007/978-3-030-38453-1_5.

Sowndhararajan K, Marimuthu S, Manian S. 2013. Biocontrol potential of phylloplane bacterium *Ochrobactrum anthropi* BMO-111 against blister blight disease of tea. J Appl Microbiol 114 (1): 209-218. DOI: 10.1111/jam.12026.

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. DOI: 10.17503/agrivita.v38i2.588.

Thomloudi EE, Tsalgatidou PC, Douka D, Spantidos TN, Dimou M. 2019. Multistrain versus single-strain plant growth promoting microbial inoculants-The compatibility issue. Hell Plant Prot J 12 (2): 61-77. DOI: 10.2478/hppj-2019-0007.

Woźniak M, Gałązka A, Tyśkiewicz R, Jaroszuk-Ściseł J. 2019. Endophyte bacteria potentially promote plant growth by synthesizing different metabolites and their phenotypic/physiological profiles in the biolog GEN III microPlate(TM) test. Intl J Mol Sci 20 (21): 5283. DOI: 10.3390/ijms20215283.

Yang Q, Wang H, Zhang H, Zhang X, Apaliya MT. 2017. Effect of Yarrowia lipolytica on postharvest decay of grapes caused by *Talaromyces rugulosus* and the protein expression profile of *T. rugulosus*. Postharvest Biol Technol 126: 15-22. DOI: 10.1016/j.postharvbio.2016.11.015.

Zhang S, Merino N, Okamoto A, Gedalanga P. 2018. Interkingdom microbial consortia mechanisms to guide biotechnological applications. Microb Biotechnol 11 (5): 833-847. DOI: 10.1111/1751-7915.13300.

Zhou T, Chen D, Li C, Sun Q, Li L. 2012. Isolation and characterization of *Pseudomonas brassicacearum* J12 as an antagonist against *Ralstonia solanacearum* and identification of its antimicrobial components. Microbiol Res 167 (7): 388-394. DOI: 10.1016/j.micres.2012.01.003.

Zhu F, Wang J, Jia Y, Tian C, Zhao D. 2021. *Bacillus subtilis* GB519 promotes rice growth and reduces the damages caused by rice blast fungus *Magnaporthe oryzae*. PhytoFrontiers 1 (4): 330-338. DOI: 10.1094/PHYTOFR-12-20-0041-R.