BIODIVERSITAS
Volume 24, Number 8, August 2023
Pages: 4209-4217

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240801

The potential of beneficial microbes to suppress the development of bacterial leaf blight in rice plants caused by *Xanthomonas oryzae* pv. *oryzae*

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Manuscript received: 29 November 2022. Revision accepted: 1 August 2022.

Abstract. *Rahma H, Martinius, Khairul U, Rahmi F. 2023. The potential of beneficial microbes to suppress the development of bacterial leaf blight in rice plants caused by* Xanthomonas oryzae *pv.* oryzae. *Biodiversitas 24: 4209-4217. Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is caused bacterial leaf blight in rice plants. One technique for controlling the bacterial leaf blight that currently being developed is using beneficial microbes. The purpose of this study was to isolate and identified beneficial microbes that can suppress bacterial leaf blight development in rice plants. In the present study fifteen isolates of beneficial microbes were investigated for their potential as an antagonist against *Xoo*, the causal agent of bacterial leaf blight of rice plants. Four isolates, namely Act-SK2, Act-Mn2, Act-Hr21, and Act-Pha4, showed the ability to reduce the area under progress curve (AUDPC) values by 51.33, 51.00, 84.00, and 82.33, respectively. The effectiveness of AUDPC suppression ranged between 80%-88%. Two out of four isolates showed potential in reducing the severity of BLB disease by 3.65% (Act-Sk2) and 3.88% (Act-Mn2) as compared to control. The effectiveness of both isolates in reducing disease severity was 86.34% (Act-Sk2) and 85.48% (Act-Mn2). Furthermore, identification based on 16S rRNA gene sequence analysis showed that three isolates, namely Act-Sk2, Act-Hr21, and Act-Pha4, belong to Actinobacteria. Each isolate showed 99.68% similarities with *Streptomyces* sp. strain KS02 (Acc. No. AB373961, 99.84% with *Streptomyces* sp. strains Al-Dhabi-119 (Acc. No.MK675528), and 99.64% with *Streptomyces griseus* strain K 2 (Acc. No. MK811436). Isolate Act-Mn2 showed 100% similarity with *Penicillium janthinellum* strain CMV006C1 (Acc. No. MK450697). The results of this study indicate that the four microorganisms tested have potential to be developed as biological agents.

Keywords: Bacterial leaf blight, diseases suppression, rhizobacteria, induce systemic resistance, rice, *Xanthomonas oryzae*

INTRODUCTION

Indonesia is the world's fourth-largest rice producer. The benefit of rice Indonesia's production was accumulated at 35.4 million metric tons with a land area of 12.16 million hectares, slightly different from Bangladesh, which was 35.85 million metric tons with a land area of 12 million hectares (USDA 2023). Plant-disturbing organisms are one of the limiting factors for increasing rice production. Bacterial leaf blight (BLB) is one of the main diseases of rice. BLB is a vascular disease that causes systemic infection in rice plants. Symptoms of BLB disease are gray to white sores along the vascular tissue. The disease is caused by the bacterium *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (EPPO 2022; Xu et al. 2022), and it ranks fourth position of the ten most plant-pathogenic severe bacteria (Bai et al. 2022). Infections of defenseless plants directly affected by *Xoo i*n rice crops can lead to significant yield losses of up to 50% in tropical Asia (Kim and Reinke 2019; Fiyaz et al. 2022). However, somewhere at maximum tillering, crop losses result in a 20-40% decrease, whereas in the early infection stage, yield losses are approximately 50% (Yasmin and Hafeez 2017). In Indonesia, yield loss due to BLB disease reach 70-80%, while only 6-60% in India, and around 20-50% in Japan (Safrizal et al. 2020).

Various efforts have been made to control bacterial leaf blight, including planting resistant varieties and using bactericides. However, controlling the disease using resistant varieties has not given good results because *Xoo* bacteria have a high diversity of pathotypes and gene mutability. This condition makes *Xoo* easily able to break host plant resistance genes (Nafisah et al. 2019). Control of BLB using bactericides can potentially suppress BLB disease in the field. However, the continuous use of bactericides harms the environment, triggers resistance to pathogens, and impacts human health (Ooi et al. 2022).

The biological control of plant diseases is done by suppressing plant pathogen populations by living organisms utilizing beneficial microbes (Köhl et al. 2019; Montoya-Martínez et al. 2022). Some beneficial microbes, such as plant growth-promoting rhizobacteria (PGPR) (Mohanty et al. 2021) and plant growth promoting Fungi (PGPF) (Jahagirdar et al. 2019), are essential members of the plant microbiome. PGPR are non-pathogenic microorganisms that colonize the host plant root area, compete and suppress the growth of pathogenic organisms, so that PGPR has the potential as an antagonist (biopesticide) and biofertilizer (Mohanty et al. 2021). Many of these bacteria have been classified into the genera *Azoarcus, Azospirillum, Azotobacter, Arthrobacter, Bacillus, Paenibacillus, Klebsiella, Enterobacter,*

Pseudomonas, *Serratia* and *Actinomyces* (Backer et al. 2018; Lahlali et al. 2022). Actinomyces are a group of prokaryotic microorganisms considered an intermediate between bacteria and fungi, so their morphology is similar to that of filamentous fungi (Kaari et al. 2022). At the same time, some varieties of PGPF have been studied, including those belonging to the genera *Trichoderma*, *Phoma*, *Fusarium*, and *Penicillium* (Hossain and Sultana 2020; Myung et al. 2020; Choudhary and Nayak 2023). PGPR or PGPF is reported to have the ability to inhibit the growth of various pathogenic bacteria and fungi (Jalmi SK and Sinha 2022). According to Ilsan et al. (2016), eight actinomycetes from rice's phyllosphere could suppress *Xoo* in vitro and stop bacterial leaf blight's severity by 25.87%. Streptomyces has been shown to have the potential to both enhance plant development and generate plant resistance in oak (Kurth et al. 2014) and rice (Suárez-Moreno et al. 2019). A previous study reported that actinobacteria in rice's rhizosphere have an antibacterial activity to *Xoo* (Rahma et al. 2023). Hossain et al. (2014) reported that *Penicillium* spp. GP15-1 inhibited the development of damping off disease by *Rhizoctonia solani* on cucumber plants. These beneficial microbes can dissolve phosphate (Mohanty et al. 2021), bind nitrogen, and produce phytohormones Bizos et al. (2020); Chen et al. (2018); Sharma et al. (2013) and as a biocontrol agent (Jiao et al. 2021) and induction of plant resistance (Zhu et al. 2022); Hossain and Sultana (2020); Mitra et al. (2021). The purpose of this study was to isolate and identified beneficial microbes that can suppress bacterial leaf blight development in rice plants.

MATERIALS AND METHODS

Research site

This research was conducted in the Microbiology Laboratory and Experimental Garden of the Faculty of Agriculture, Universitas Andalas, Padang, Indonesia, from March to October 2022.

Beneficial microbes

Fifteen beneficial microbes isolates (Act-SK2; Act-LB3; Act-Ph 2.1; Act-Mn 2; Act-Pha 3.4; Act-Pha 2.3; Act-Pha 3.3; Act-Pha 4; Act-Pha 3.5; Act-Krj 21; Act-Hr 24; Act-Hr 21; Act-Hr 56; Act-Hr 49; Act-Hr 47) were used in the present study. These isolates were obtained from the Microbiology Laboratory, Faculty of Agriculture, Andalas University, Padang, Indonesia (Table 1).

Preparation of beneficial microbes

Beneficial microbes were cultured on International Streptomyces Project 2 (ISP-2) medium (composition g/l: Yeast extract 4 g, Malt extract 10 g, Dextrose 4 g, Agar 20 g, and Aqua dest 1 L) using the quadrant scratch method to obtain single colonies, and incubated for 2 x 24 hours at room temperature. The microbes were then propagated using ISP-2 broth media on a rotary shaker for 14 days for further testing.

Table 1. Beneficial microbes used in the study

Name of isolates	Isolation sources	Location	Year
Act-SK_2	Rhizosphere (Corn)	Padang, West Sumatra	2020
Act-LB_3	Rhizosphere (Corn)	Padang, West Sumatra	2020
Act-Ph 2.1	Rhizosphere (Corn)	Padang, West Sumatra	2020
Act-Mn 2	Rhizosphere (Corn)	Padang, West Sumatra	2020
Act-Pha 3.4	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Pha 2.3	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Pha 3.3	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Pha 4	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Pha 3.5	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Krj 21	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Hr 24	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Hr 21	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Hr 56	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Hr 49	Rhizosphere (Rice)	Padang, West Sumatra	2020
Act-Hr 47	Rhizosphere (Rice)	Padang, West Sumatra	2020

Confirmation of beneficial microbes

A hypersensitivity reaction test on tobacco leaves was conducted to confirm that the microbes were not plant pathogenic. Beneficial microbe's isolates were grown in ISP2 broth media with an incubation period of 7 days at 37°C in a shaking incubator at 150 rpm. The density of liquid culture cells was calculated using a hemocytometer. The culture injected into tobacco has a minimum density of 10^6 CFU mL^{-1}. The culture was injected into the underside of leaf (the part of leaf between two major veins) using a sterile syringe (Rahma et al. 2022). The necrosis in the tissue of injected leaves was observed. If no symptoms of necrosis were observed in the treated leaves 2 x 24 hours after injection, this indicated that microbe did not have the potential as plant pathogens. As a control, sterile distilled water was injected with the same method. The distilled water did not cause necrotic symptoms on the injected leaves.

Source of *Xoo* isolates

Xoo phototype III was obtained from the Microbiology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Andalas. The isolates were cultured by the quadrant scratch method on Wakimoto Agar medium (g/L composition: $Ca(NO_3)_2$ 0.5 g, $Na_2HPO_4.12\ H_2O$ 2 g, peptone 5 g, Sucrose 15 g, $FeSO_4.7\ H_2O$ 0.5 g, Agar 15 g, distilled water 1 L), and incubated for 2 x 24 hours at room temperature. A single colony of *Xoo* strain was used as a source of inoculum and purified on the same medium. After incubation, *Xoo* was diluted with sterile distilled water. Cell suspensions were adjusted to 10^8 CFU/mL using a McFarland solution scale 8.

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

Pathogenicity test was performed to confirm that *Xoo* strain cause bacterial leaf blight in rice. *Xoo* bacteria were inoculated on 21-day-old rice seedlings by cutting the rice leaves' tips using sterile scissors dipped in *Xoo* suspension (population 10^7 CFU/mL) for ±10 seconds. Disease symptoms were observed daily until 14 days after inoculation (Ke et al. 2017).

Capability of beneficial microbes to inhibit disease development of BLB in rice plant

Research design

The experiment was conducted in a completely randomized design (CRD) with 16 treatments and three replications. The treatments used in this study consisted of 15 isolates of beneficial microbes inoculated with *Xoo* and control (infected plants without beneficial microbes treatment).

Preparation of planting media

The planting medium was a mixture of soil and manure as much as 2: 1 (v/v). First, the soil was put into a heat-resistant plastic measuring 5 kg, then sterilized using an autoclave for one hour at a temperature of 100°C (Rahma et al. 2022). After the soil cooled, it was put into a tube of sprouts and polybags covered with plastic.

Propagation and application of beneficial microbes

Isolates of beneficial microbes were propagated in the ISP-2 broth medium. A single colony of beneficial microbes on ISP-2 media was put into 100 mL of ISP-2 Broth medium in 250 mL Erlenmeyer flask, and incubated for 14 x 24 hours on a rotary shaker at 70 rpm. A population density of 10^6 spores/mL was used for application. Rice seeds were surface sterilized using 2% NaOCl for 1 minute, then rinsed with sterile distilled water for 1 minute. Next, seed treatment of rice in each beneficial microbes suspension for 15 minutes and air-dried for 5 minutes. After soaking, rice seeds were planted in a 25 x 20 x 5 cm seedling tub containing sterile soil and manure (2: 1) media. The seedling was kept for 21 days. First, 21-day-old rice seedlings were removed. Next, roots were cleaned from the remaining soil and then immersed in each beneficial microbes isolate (prepared as a seed immersion experiment) for 30 minutes. For control, roots were immersed in sterile distilled water at the same time (Mitra et al. 2021). After soaking, one seedling was planted in polybags containing clean soil and manure (2:1) in 20 cm x 20 cm polybags, and each experiment was repeated three times.

Inoculation of *Xanthomonas oryzae* pv. *oryzae* after application of beneficial microbes in rice plant

Xoo bacterium was inoculated on rice seedlings after being treated with beneficial microbes was done 21 days after planting. *Xoo* bacteria were inoculated on rice leaves using the leaf-cutting method. The leaves are cut using sterile scissors and dipped in *Xoo* suspension (population 10^7 CFU/mL) for ±10 seconds. The incubation period was determined when the first symptoms appeared after the inoculation of *Xoo* bacteria. Disease symptoms were observed daily until 14 days after inoculation (Ke et al. 2017). The effectiveness of suppressing the incubation period of bacterial leaf blight symptoms is calculated using the formula: Effectiveness of suppression of incubation period = (incubation period of beneficial microbes treatment - incubation period of control)/incubation period of control) x 100%. Development of bacterial leaf blight was observed by measuring the symptom's length of the sign and comparing it to the size of the leaf. Symptoms of BLB disease were classified into the following grades based on the percentage of leaf area covered by infection (Table 2).

Observations were made every day after pathogen inoculation until the first symptoms appeared. The severity of disease was determined using the following formula:

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

Where:
DS : 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 most challenging attack category

Furthermore, disease severity was calculated based on an analysis of the area under the disease progression curve (AUDPC), according to the formula of Simko (2012):

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

Where:
Yi : The i^{th} observation data
yi + 1 : The i^{th} observation data + 1
ti + 1 : Time of the i^{th} observation + 1
Ti : Time of the i^{th} observation

The following formula was used to calculate the effectiveness of suppressing the incubation period, disease severity, and AUDPC:

E = ((P - C)/C) x 100%

Where:
E : Effectiveness
P : Treatment
C : Control

Molecular identification of selected beneficial microbes

DNA extraction

For the extraction of DNA, isolated beneficial microbes (50-100 mg) were suspended using distilled water in a 1.5 mL microtube. DNA was extracted using Genomic DNA extraction with Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA) following company recommendation.

Table 2. Scale and severity of BLB disease

Scale	Symptoms in Leaf Area (%)
0	No symptoms
1	Symptoms 1-6%
3	Symptoms >6-12 %
5	Symptoms >12-25 %
7	Symptoms >25-50 %
9	Symptoms >51-100 %

Note: Standard Evaluation System for Rice (SES) (IRRI 2014)

PCR amplification

The amplification of Gene 16S rRNA of isolates Act Pha4, Act LB3, Act Sk2, and Act Hr21 base was carried out by the polymerase chain reaction (PCR) technique using universal primers 27F (5' AGAGTTTGATCCTGG CTCAG-3') and 1492R (5'-GGTTACCTTGTTACGAC TT-3' (Fujiyoshi et al. 2020). PCR mix contained Taq HS Red Mix (Bioline, PCR Biosystems Inc. USA.) 25 L, each primer 1 L (20 M each), 1 L genomic DNA (200 ng), and dH2O so that the total reaction volume is 50 L. The PCR reactions were subjected to the following temperature: cycling profile denaturation of DNA at 95°C for 1 min, primer annealing at 55°C for 1 min, DNA elongation at 72°C for 1.5 minutes, and the final stage at 72°C for 5 minutes. Meanwhile, Act Mn2 isolates were amplified based on the internal transcribed spacer (ITS) using the ITS-1 primer pair (5'-TCCGTAGGTGAACCTGCGG-3') ITS-4(5'TCCTCCGCTTA TTGATATGC-3') (Divya et al. 2013), and PCR reaction following (Ali et al. 2023), Whole genomic DNA was exposed to PCR using the GeneAmp PCR System 9700 equipment model (Applied Biosystems, USA). The amplification results were separated with 1% agarose gel electrophoresis under an ultraviolet transilluminator with a DNA marker size of 1 kb (Geneaid).

Sequencing analysis

DNA sequencing was performed on the amplification results 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/). Phylogenetic analysis was performed using the MEGA X software and the Maximum Likelihood approach (Hall 2013). Then, the phylogenetic tree was constructed using the neighbor-joining method and viewed using the MEGAX software's Tree Explorer. During tree construction, a bootstrap test with 1000 replications was added.

Data analysis

The parameters observed were the incubation period, and disease severity was analyzed using the analysis of variance (ANOVA). If the data were significantly different, followed by the Least Significant multiple t-tests difference (LSD) at the 5% level.

RESULTS AND DISCUSSION

Confirmation of beneficial microbes

Confirmation of beneficial microbes was carried out through a hypersensitive reaction test by inoculating beneficial microbes on the lower surface of tobacco leaves. Beneficial microbes did not trigger necrotic symptoms on inoculated tobacco leaves after 2 x 24 hours. These results indicate that inoculated microbes were not plant pathogenic microbes. Furthermore, it can be used as a biocontrol agent against plant pathogens.

Table 3. Effect of propagation and application of beneficial microbes to suppression of incubation periods of *Xoo* cause bacterial leaf blight in rice plants

Isolates code	Incubation periods (*dai*)	Suppression effectiveness (%)
Act-LB_3	4.33	62.78
Act-Pha 3.5	4.33	62.78
Act-SK2	3.66	37.59
Act-Mn2	3.66	37.59
Act-Pha 2.3	3.66	37.59
Act-Krj 21	3.66	37.59
Act-Hr 56	3.66	37.59
Act-Ph 2.1	3.33	25.18
Act-Pha 3.4	3.33	25.18
Act-Pha 3.3	3.33	25.18
Act-Hr 24	3.33	25.18
Act-Hr 21	3.33	25.18
Act-Hr 49	3.33	25.18
Act-Hr 47	3.33	25.18
Act-Pha 4	3.00	12.78
Control	2.66	0

Note: *dai* = days after inoculation

Capability of beneficial microbes to inhibit disease development of BLB in rice plant

Propagation and application of beneficial microbes

The results showed that seed treatment beneficial microbes had no significant effect on the incubation period of the inoculation of *Xoo* bacteria causing BLB disease in rice plants. The application of beneficial microbes can suppress incubation period of BLB disease symptoms. The incubation of periods of BLB disease in the treatment of beneficial microbes ranged from 3.0 to 4.33 days after inoculation (dai), while in the control it was 2.66 dai. The symptom appeared to be a characteristic gray-yellow color at the tips of injured leaves. Isolates Act-LB3 and Act-Pha 3.5 suppressed the most prolonged incubation period of *Xoo* compared to other beneficial microbes isolates, with a suppression effectiveness value of 62.78% (Table 3).

Disease severity and area under the disease progress curve (AUDPC)

Beneficial microbes treatment had a significantly different effect on the severity of BLB disease, with an average range of 3.65-26.10% and effectiveness of 2.35-86.34% compared to negative control. The moderate disease severity in beneficial microbes treatment ranged from 3.65-26.10% with an effectiveness of 2.35-86.34%. Not all treatments of beneficial microbes isolates were able to potentially suppress the severity of BLB disease. Two isolates, namely Act-MN2 and Act-SK2, were able to reduce the disease severity with an effective range of disease suppression of 85.48-86.34% (Table 4).

The application of beneficial microbes had a significantly different effect on the AUDPC value of BLB disease compared to controls. BLB disease developed from the initial appearance two days after injection. The AUDPC value and condition suppression index in each treatment showed disease progression. AUDPC of BLB disease in rice after beneficial microbes isolate treatment ranged from

51.333-446.33 with negative control of 418.33, while the percentage of AUDPC suppression ranged from -6.69 - 88%. The lowest AUDPC value of BLB disease in rice was observed by Act-SK2 isolate at 51.333, with a disease suppression percentage of 88%. The highest (446.33) AUDPC value of BLB disease was found in Act-Krj 21 isolate treatment, with -6.69% disease suppression (Table 4). This showed that if the AUDPC number was lower, the treatment was more effectively control pathogens.

On the other hand, the higher the AUDPC number, the less affected the pathogen infection. Four beneficial microbes isolates, namely Act-Ak2, Act-Mn2, Act-Hr21, and Act-Pha4 showed the lowest AUDPC values which ranged between 51-82.33. The four isolates were able to suppress the development of bacterial leaf blight compared to other beneficial microbes isolates. This study indicated that the AUDPC value could indicate the resistance of rice plants after the application of beneficial microbes isolates. The AUDPC observed over a certain period illustrates the disease development rate over time in each beneficial microbes isolate treatment. According to Sanchez-Gonzalez et al. (2019), AUDPC value shows a genotype's resistance level, where the high value is regarded as susceptible and resistant if the AUDPC value is low. Plant disease often begins at a low level and steadily increases in frequency and severity during the period. During disease epidemics, disease progression in plants is frequently detected numerous times. According to Simko and Piepho (2012), measuring disease progression is critical for understanding plant-pathogen interaction in quantitative resistance, where distinctions in the resistance level are usually less evident.

Table 4. Disease of severity and AUDPC of BLB disease in rice plants after inoculated by beneficial microbes

Isolates code	Disease severity (%)	Disease suppression effectiveness (%)	AUDPC	AUDPC suppression effectiveness (%)
Control	26.73 a*	0.00	418.33 a*	0
Act-Hr 47	26.10 ab	2.35	427.33 a	-2.15
Act-Krj 21	25.89 ab	3.14	446.33 a	-6.69
Act-Pha 2.3	20.93 abc	21.69	366.33 ab	12.43
Act-Pha 3.3	20.37 abcd	23.79	431.00 a	-3.03
Act-Pha 3.4	9.38 bcd	44.25	127.67 bc	69.48
Act-Hr 56	8.95 cd	64.9	136.33 bc	67.41
Act-Pha 3.5	8.92 cd	66.51	139.67 bc	66.61
Act-LB3	7.93 cd	66.62	111.00 bc	73.47
Act-Hr 49	7.02 cd	70.33	109.67 bc	73.78
Act-Hr 24	6.88 cd	73.73	113.00 bc	72.99
Act-Pha 4	5.96 cd	74.26	82.33 c	80
Act-Ph 2.1	5,706 cd	78,653	83.00 c	80
Act-Hr 21	4.95 cd	81.48	84.00 c	80
Act-Mn2	3.88 d	85.48	51.00 c	88
Act-SK2	3.65 d	86.34	51.33 c	88

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

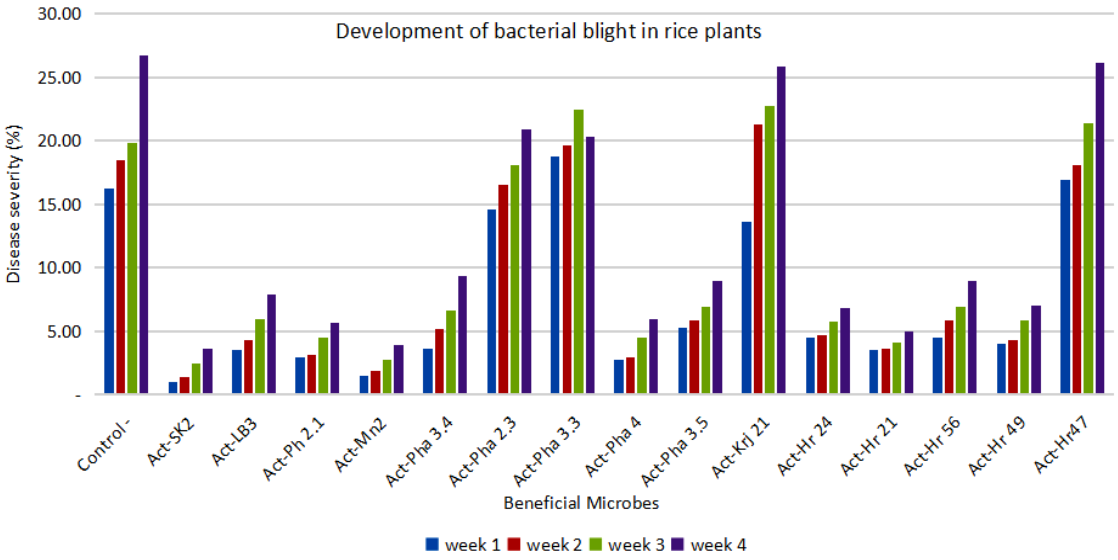

Figure 1. Development of bacterial leaf blight in rice plants after inoculation by beneficial microbes

The development of symptoms of bacterial leaf blight can be seen in Figure 1. Fifteen beneficial microbes isolates could suppress the growth of BLB disease compared to the control, while the other four isolates did not conceal the development of BLB disease. The best suppression of the development of BLB symptoms was shown by Act-Sk2, Act Mn2, and Act-Hr21. The low severity of BLB disease indicated beneficial microbes isolates ability to increase rice plants resistance compared to control. According to Franco-Correa et al. (2016), beneficial microbes have direct and indirect interaction mechanisms in influencing the growth of host plants. Natural methods include generating essential crop growth factors, such as growth hormones and supportive actions on nitrogen fixation, phosphate solubilization, and iron acquisition. The indirect mechanism occurs through the induction of plant resistance by suppressing the development of plant diseases and producing antimicrobial secondary metabolites. A previous study by Rahma et al. (2022) reported that the beneficial microbes isolate Act-LB3, Act Mn2, and Act-Pha4 could suppress *Xoo* growth in vitro. The beneficial microbes isolates have the activity of phosphatase, protease, and amylase enzymes.

Identification of selected beneficial microbes

The result showed that obtained sequence length of beneficial microbes isolates ranged from 1157 - 1535 bp. Beneficial microbes sequester Act-Hr21 had 99.84% similarity with *Streptomyces* sp. strain Al-Dhabi-119 (accession number MK675528). Isolate Act-Pha4 had 99.64% similarity with *Streptomyces griseus* strain K 21 with accession number MK811436, and Act Sk2 isolate had 99.68% similarity with *Streptomyces sp* strain KS02 (accession number AB373961) (Table 5). Moreover, Act-Mn2 isolate was amplified using a genetic marker based on the internal transcribed spacer (ITS), resulting in a sequence length of 858 bp. Act Mn2 isolate was similar to *Penicillium janthinellum* strain CMV006C1 (MK450697) by 100% (Table 5). Srinivasan et al. (2015) reported the most commonly utilized molecular marker for bacterial classification is 16S ribosomal RNA (rRNA) genes. According to Kai et al. (2019), 16S rRNA gene in bacteria is about 1500 bp long and comprises both conserved and variable sections that change at various rates. The sluggish evolution rates of the former areas allow the invention of universal primers that amplify genes across taxa.

Furthermore, quick regions indicate species differences and are helpful for taxonomic classification. At the same time, the Internal Transcribed Spacer (ITS) region of nuclear DNA (rDNA) has evolved into the largest sequenced domain to determine fungal classification at the species and even within species levels. Therefore, this area was recently selected as the fungus kingdom's DNA barcode. The ITS domain is a highly polymorphic non-coding area with a sufficient number of taxonomic units, spanning 450 to 750 bp (Beeck et al. 2014).

Figure 2 depicts the phylogenetic analysis that divided the three actinobacteria isolates into two groups. *Streptomyces griceus*, the closest species to Act-Hr21 and Act-Pha4, was found in the same branch (MK811436.1, MK675528.1, and MK134628.1). The Act-Sk2 isolates were found to be phylogenetically distinct from their nearest relatives. *Streptomyces* is a well-known actinobacteria genus. It is regarded as one of the most helpful rhizosphere bacterium genera because it performs various tasks in soil nutrient cycles and improves plant growth. The ability of *Streptomyces* to enhance plant development and induce plant resistance has been demonstrated in rice (Hata et al. 2021; Ilsan et al. 2016; Suárez-Moreno et al. 2019). The results of this study showed that the identified actinobacterial isolates have potential as biological agents against bacterial leaf blight.

This study revealed that identified actinobacterial isolates have potential as biological agents against bacterial leaf blight. Many reports have demonstrated the potential of *Streptomyces* as a plant disease control agent. *Streptomyces* can potentially increase plant growth and protect plants against various pathogens. According to Ilsan et al. (2016), eight actinomycetes from rice's phyllosphere can suppress *Xoo* in vitro and the severity of bacterial leaf blight by 25.87%. *Streptomyces* has been shown to have the potential to both enhance plant development and generate plant resistance in oak (Kurth et al. 2014) and rice (Suárez-Moreno et al. 2019). According to Vilasinee et al. (2019) and Abbasi et al. (2019), reported that seed treatment of tomato seeds using *Streptomyces* sp. increase tomato plant resistance to *F. oxysporum* f.sp. *lycopersici*. Vergnes et al. (2020) reported treating Arabidopsis leaves with *Streptomyces* sp. AgN23, which results in resistance to *Alternaria brassicicola* infection. According to Olanrewaju and Babalola (2019), Streptomyces is the most proactive microbe, and these microorganisms are a conveniently available natural choice in identifying novel ways to treat plant infections. The following qualities contribute to their ability to combat plant pathogens: antibiotics production (Couillerot et al. 2013), synthesis of plant growth regulators (Goudjal et al. 2013), Siderophore synthesis (Vijayabharathi et al. 2015), Volatile compound secretion (Jones and Elliot 2017) and Nutrient competition.

Table 5. BLAST result of 16S rRNA and ITS gene sequences

Isolates code	Sequence length (bp)	Similarity (%)	Accession numbers	Species
Act-Hr21	1491	99.84	MK675528	*Streptomyces* sp. strain Al-Dhabi-119
Act-Sk2	1157	99.68	AB373961	*Streptomyces* sp. strain KS02
Act-Pha4	1252	99.64	MK811436	*Streptomyces griseus* strain K 21
Act-Mn2	858	98.48	MK450697	*Penicillium janthinellum* strain CMV006C1

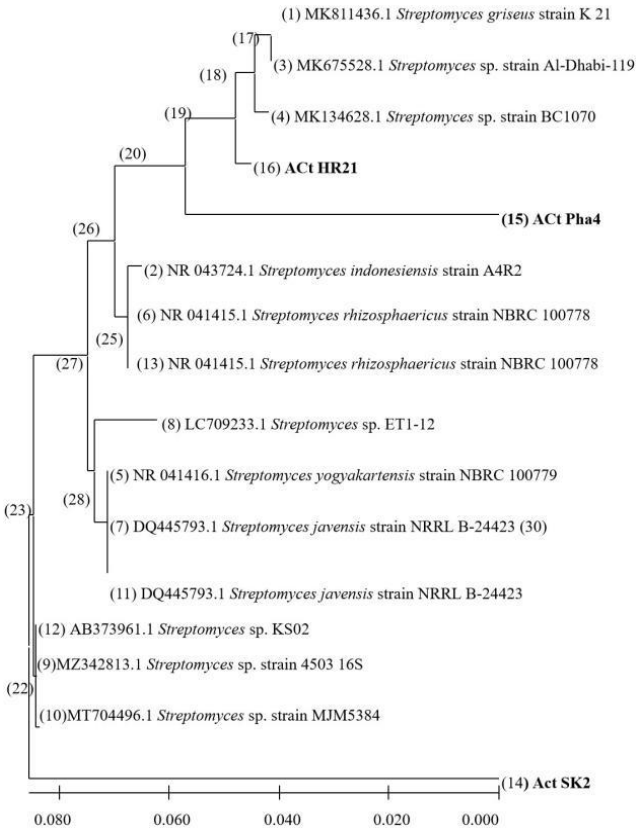

Figure 2. The phylogenetic tree analysis based on the 16S rRNA gene

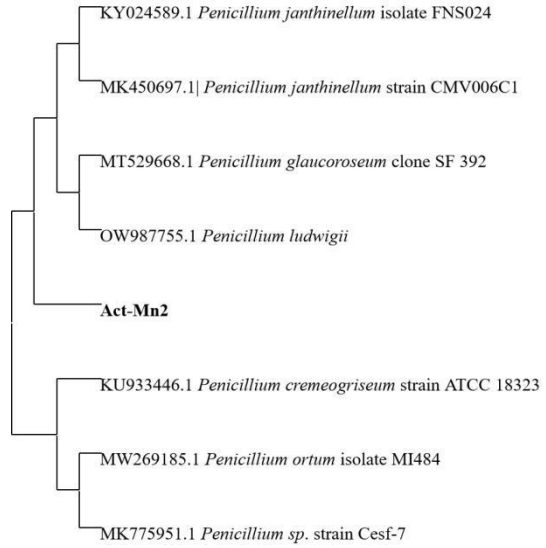

Figure 3. The phylogenetic tree based on the rDNA-ITS region of Act-Mn2 isolate

This study succeeded in identifying Act-Mn2 isolate as *Penicillium janthinellum*. Figure 3 shows that Act-Mn2 isolate showed affinity with the fungal groups *P. janthinellum*, *P. glaucoroseum*, and *P. ludwigii*. The Act-Mn2 isolate has high potential as a biocontrol agent against *Xoo* in vitro (Rahma et al. 2022) and suppressed the development of BLB disease in plants. The fungus can produce protease enzymes and dissolve phosphate. Protease enzyme activity was thought to act as an inhibitor of *Xoo* growth in vitro. According to Nagraj and Gokhale (2018), *P. janthinellum* produces cellulase, amylase, and protease enzymes. It can be a potent disruptor/degrader of the biofilms produced by *Escherichia coli*, *Salmonella enterica*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. According to Khokhar and Bajwa (2014), *Penicillium* species are crucial in agriculture. They recycle vital nutrients like phosphate-solubilizing fungus.

In conclusion, a total of four isolates, namely Act-SK2, Act-Mn2, Act-Hr 21, and Act-Pha 4 showed the potential to suppress the development of bacterial leaf blight, with a percentage of disease suppression effectiveness of 86.34%, 85.48%, 81.48 %, and 74.26, respectively. Furthermore, identification results showed that three isolates, Act Sk2, Act-Hr21, and Act-Pha4, belonged to Actinobacteria group. Each isolate showed similarities with *Streptomyces* sp. strain KS02, *Streptomyces* sp. strains Al-Dhabi-119, and *Streptomyces griseus* strain K 2, respectively. Isolate Act-Mn2 was found similar to *Penicillium janthinellum* strain CMV006C1. The study suggests that *Streptomyces* sp and *Penicillium janthinellum*, which are beneficial microbes, have the potential to act as biocontrol agents against *Xoo* bacteria. Therefore, we recommend further testing these beneficial microbes against the field-scale *Xoo* pathogen to increase rice yields and support sustainable farming systems.

ACKNOWLEDGEMENTS

This research was funded by Andalas University, Padang, Indonesia under the Research Contract for *Penelitian Riset Publikasi Terindeks* (RPT) number: T/82/UN.16.17/PT.01.03/Pangan-RPT/2022, fiscal year 2022.

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