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Characterization and biocontrol of *Pantoea ananatis*, the causal agent of rice bacterial leaf blight in Malang, Indonesia

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Abstract. *Andriani LT, Poromarto SH, Supyani, Purwanto E, Hadiwiyono. 2025. Characterization and biocontrol of* Pantoea ananatis, *the causal agent of rice bacterial leaf blight in Malang, Indonesia. Biodiversitas 26: 1537-1544.* Disease outbreaks pose a significant obstacle to sustainable agricultural production. Two major diseases affecting rice crops are bacterial leaf blight and blast disease. Bacterial leaf blight caused by *Pantoea ananatis* is considered new, based on field observations, the cause of bacterial leaf blight is still *Xanthomonas oryzae* pv. *oryzae*. Research on *P. ananatis* is important to provide new knowledge about the causes of bacterial leaf blight other than *X. oryzae* pv. *oryzae*. The symptoms of bacterial leaf blight include yellowing and browning of the leaf, followed by drying, resulting in death of the leaf cells. The objectives of this study were to isolate the bacteria, identify them morphologically and molecularly, determine physiological characteristics and explore their biocontrol potential using rhizobacteria. Bacterial characterization was performed both morphologically and physiologically. This study determined the hypersensitive response of tobacco leaves, physiological and biochemical characteristics, pathogenicity tests, and molecular identification via 16S rRNA gene sequencing. The bacteria formed yellow colonies on yeast chalk dextrose agar at 36°C. Isolate was positive for indole and Levan tests, used citrate as a carbon source, and liquefied gelatin at low temperatures. The bacteria showed positive results in tobacco hypersensitivity and pathogenicity tests on paddy rice. Molecular identification results showed that the bacterial pathogen was identified as *P. ananatis*. The result of biocontrol potential test was shown that bacteria with the code UNS-R2 can control the pathogenic bacteria *P. ananatis* using spray method. These findings can be used as new knowledge about the types of leaf blight pathogens other than *X. oryzae* and their control alternatives to determine tactical steps in integrated crop management toward sustainable agriculture. This is the first report on the identification and characterization of *P. ananatis* in rice in Indonesia and the biocontrol potential using rhizobacteria.

Keywords: Bacterial leaf blight, biocontrol, molecular identification, *Pantoea ananatis*, phytopathogen

INTRODUCTION

The agricultural sector makes a significant contribution to achieving the Sustainable Development Goals (SDGs), which aim to minimize global hunger, promote positive well-being and health, ensure sanitation and clean water, and mitigate climate change. The agricultural sector can produce agricultural products and food needed to provide for human nutritional needs and alleviate hunger, and good agricultural products can also have an impact on good health. Climate change and agriculture are interlinked. Crops can be part of the solution to global warming and climate change. Plants use photosynthesis to extract carbon dioxide from the atmosphere, which helps reduce the greenhouse effect and keeps the Earth's temperature stable. However, climate change can also have a negative impact on plants, as increased rainfall leading to flooding and extreme temperatures (drought) can suppress plant growth, damage crops, and reduce yields (US EPA 2015). Moreover, climate change can impact the severity of plant diseases and food security. Plant diseases can be caused by biotic and abiotic factors. Biotic causes of disease can be fungi, bacteria, viruses, mycoplasmas, parasitic higher plants, or nematodes (Agrios 2016). It is important to understand climate change's impact, especially concerning disease and pest prevalence and severity that food security. Climate change is an important factor in the emergence and re-emergence of new pests and diseases, as well as the quality and yield of crops. It can also affect the pathogen's growth, how host plants resist them, and how pathogens and host plants interact. The emergence of resistant pathogens and pests is an indirect consequence of the overuse of synthetic pesticides (Mwangi et al. 2023).

The risk of re-emergence and emergence of new diseases is due to not only climate change but also several other factors, including (i) the movement of pathogens in agricultural production systems, (ii) the development of new varieties that give rise to new resistance to an important pathogen but also the new strains and genetic variability, (iii) the movement of agricultural products or seeds from one place to another that allows pathogens to also move to other areas (Singh et al. 2023). In the last decade, rice research centers in Africa suspected the presence of bacterial pathogens causing leaf blight other than *Xanthomonas oryzae* pv. *oryzae*. These pathogens are identified as member of the genus *Pantoea* (Kini et al.

2017; Kini et al. 2017). In India, the genus *Pantoea* causes bacterial leaf blight, which manifests as lesions at the leaf tips that spread downwards (Mondal et al. 2011). Yellow leaves with brown streaks symptoms are caused by *Pantoea* in Japonica rice in Turkey (Aksoy and Boluk 2019). *Pantoea* spp. is a Gram-negative bacteria that infects rice and causes diseases, such as seed discoloration, leaf blight, and inhibition of seed germination (Cother et al. 2004; Azizi et al. 2019).

However, there have been studies of bacterial leaf blight in rice in Indonesia caused by *Pantoea* pathogens, despite observations of *Pantoea* in other countries, but they are still limited. The suspicion of *Pantoea* as the cause of a new type of leaf blight means that references to its control are still limited. Integrated crop management is a control alternatives farming system that is site-specific and consists of technological components, including the use of varieties that suit local preferences and environmental conditions, the use of quality seeds, the addition of organic materials, spacing in a Legowo pattern, fertilization according to soil and plant needs, and environmentally friendly pest control (BSIP 2009). The objective of this system is to reduce chemical pesticide use and enhance natural enemies. Plant Growth-Promoting Rhizobacteria (PGPR) serves as an effective method for plant disease management, effective biocontrol agents for plant diseases, and natural antagonists against such diseases (Reddy 2014). The objectives of this study were to isolate the bacteria, identify them morphologically and molecularly, determine physiological characteristics and explore their biocontrol potential using rhizobacteria. The results of this study are expected to be a scientific reference for the discovery of new disease-causing agents in paddy rice and the ability of rhizobacteria to control these new diseases, as well as reference for further research on integrated control of plant diseases.

MATERIALS AND METHODS

Procedures

Isolation of pathogen

Rice leaves showing bacterial leaf blight symptoms, were collected from Kalianyar Village, Lawang District, Malang, Indonesia. Parts of leaves were taken between the brownish spots and healthy parts of the leaves. Isolation was done using direct plating method on Nutrient Agar (NA) media. The media was sterilized using an autoclave at 121°C, 1 atm pressure for 25 minutes. The sterilized media was poured into Petri dishes and left to solidify. The leaf samples from symptomatic rice plants were cut into small pieces (± 1 cm^2) with a scalpel and then soaked in 70% alcohol for three minutes. The symptomatic leaf pieces were then rinsed with sterile distilled water for one minute, air dried, and then transferred to a sterile Petri dish. The sample pieces were then placed on a Petri dish containing Nutrient Agar (NA). After 24 hours of incubation, the growing bacteria were purified and further identified (Janse 2006; Abdel-Gaied et al. 2022; De Armas et al. 2022).

Hypersensitive reaction test

A hypersensitivity test (HR) was conducted to determine the identifiable response of plants when infiltrated by bacteria that were potential plant pathogens. The response was characterized by the appearance of necrotic symptoms as a way for plants to inhibit the presence of bacteria, preventing them from spreading to other parts. Pathogenic bacteria were grown on NB media and incubated for 24 hours. One mL of suspended bacterial pathogen were sprayed onto the underside of tobacco leaves, while sterile distilled water was sprayed for positive control (Janse 2006; Amaria et al. 2023). Hypersensitive reactions were observed within 2-7 days after inoculation (HSI).

Pathogenicity test

This test was carried out by inoculating 1 mL bacterial suspension on the test rice plant. For this, rice leaves were wounded using a needle to facilitate the bacterial entry into the tissue. After that, bacterial suspension was sprayed onto the test plants and covered with plastic. Rice leaves were sprayed with sterile distilled water only for the positive control treatment. Observations were made for 7 days to determine the symptoms of blight on rice leaves. After disease symptoms appeared, the affected plant parts were planted on sterile NA media (Abdel-Gaied et al. 2022).

Morphological and physiological test of pathogenic bacteria

Yellow color pigment test on YDCA media

The test bacteria were streaked on sterile yeast dextrose chalk agar media, and then incubated for 24 hours. Positive results were indicated by yellowish-colored bacterial growth on YDCA media (Schaad et al. 2001; Abdel-Gaied et al. 2022).

The growth of Pantoea *Genus on Specific Agar (PGSA) media*

A pure culture of bacteria was taken as much as 1 ose and then streaked on *Pantoea* specific agar media. Observations were made on the color of bacteria that grew on the PGSA media, and positive results were indicated if the bacteria could grow well on the media (Kini et al. 2019).

Gram staining

The procedure started by culturing bacteria for 24 hours and then adding a small quantity of water to a glass object that had been cleaned with 70% alcohol. For this, 24 hours old bacterial culture was used. A small quantity of culture was placed in the center on the glass slide to made a smear and then fixed using a Bunsen burner. Next, liquid crystal violet was added and left for one minute, then rinsed with running water. Next, iodine was added and the mixture was left for one minute, then rinsed with running water. The next step involved decolorization with ethyl alcohol for 30 seconds, then rinsed with running water. Then, a safranin stain was added, and the sample was rinsed again with running water. Finally, the samples were observed via a microscope (Cappucino and Welsh 2020; Sahni and Prasad 2022).

Citrate test

Pure cultures of bacteria were grown on Simon citrate agar media and incubated for 48 hours and positive results were determined by observing a color change from green to blue (Nurjanah et al. 2018).

Indole test

Pure culture of bacteria grown on liquid tryptone media and then incubated for 24 hours. Then, Kovacs reagent was added, and positive results were determined by observing the change to a reddish color (Nurjanah et al. 2018).

Gelatin liquefaction test

Pure cultures of bacteria were grown on bacterial-grade gelatin media and incubated for 24 hours, after which they were refrigerated. Positive results were indicated by gelatin media that remained liquid even though refrigerated (Nurjanah et al. 2018).

Starch hydrolysis test

The tested bacteria were grown on starch media and incubated at 27°C for 48 hours and then added to KI solution. Bacteria that hydrolyze starch form a bright clear zone around the bacterial colony (Schaad et al. 2001; Egorova et al. 2015; Abdel-Gaied et al. 2022).

Growth test at 37°C

The test bacteria were grown on sterile liquid nutrient media and incubated at 37°C for 3 days. Positive results were indicated by changes in liquid nutrient media from clear and yellow to turbid. The turbidity indicated that bacteria could grow well in media and temperatures of 37°C (Schaad et al. 2001; Egorova et al. 2015; Abdel-Gaied et al. 2022).

Molecular identification of pathogenic bacteria

The bacterial isolates causing bacterial leaf blight of the selected rice were identified molecularly by sequencing the 16S rRNA gene. Molecular identification consists of several stages, namely DNA extraction, Polymerase Chain Reaction (PCR), and DNA sequencing. DNA extraction was performed according to the Quick DNA Fungal/Bacterial Mini preparation Kit (Zymo Research, D6005). The extracted DNA was amplified by PCR technique using universal primers uinF 27F (5'-AGAGTTTGATCATGGGTCAG-3') and uinR 1492R (5'-TACGGCTACCTTGTTACGA-3'). Amplification was performed on a PCR machine using My Taq HS Red Mix (Bioline, BIO-25048) with initial denaturation at 95°C for 1 minute, denaturation at 96°C for 15 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 45 seconds. The denaturation, annealing, and extension step were done at 35 cycles. The PCR results were then visualized using electrophoresis on a 0.8% agarose gel coupled with ethidium bromide and TBE buffer and UV transilluminator. Furthermore, sequencing was performed by the sequencing service provider company PT Genetika Science Indonesia, Jakarta. The sequencing results were used to search for homologous 16S rRNA sequences in the DNA database (GenBank) using the BLAST program from the National Centre for Biotechnology Information (NCBI) (Mondal et al. 2011; Egorova et al. 2015; Kini 2017; Azizi et al. 2019).

Inhibitory effect of rhizobacteria on *Pantoea ananatis* in vitro

For this test, rhizospheric bacterial isolates used were UNS-P1, UNS-P3, UNS-R1, and UNS-R2. The rhizobacteria were collected from the International Center of Agriculture Training, Ketindan, Malang, Indonesia. The methods used were the double-layer, disc diffusion method and spray method. In the double-layer method, pathogen and rhizobacteria were grown on sterile liquid nutrient media and then incubated for 24 hours. In disc diffusion method, pathogenic bacteria were spread on sterile nutrient agar media. A sterile 0.5 cm-diameter filter paper was dipped in 1 mL rhizobacterial suspension, air dried and placed on nutrient agar media then incubated for 24 hours. The formation of a clear zone around the filter paper was observed (Rahma et al. 2019). For the spray method, A sterile 0.5 cm-diameter filter paper was dipped in 1 mL rhizobacterial suspension, air dried and placed on nutrient agar media then incubated for 24 hours. After the bacteria grew around the filter paper, the Petri dish lid was swabbed with chloroform and allowed to stand for 1 hour. Next, the bacterial pathogen was sprayed and incubated for 48 hours. The clear zone formed around the filter paper was then observed (Aini et al. 2023).

RESULTS AND DISCUSSION

Isolation of bacterial and pathogenicity test

The results showed that a milky white to yellowish colony of bacteria was isolated from diseased leaves of rice. The yellowish bacteria were labeled as La. The result of hypersensitivity test revealed that La bacteria caused necrosis of tobacco leaves, which confirmed that La bacteria were pathogenic. Pathogenicity test conducted on new rice leaves showed that La bacteria were pathogenic for rice. The symptoms produced were similar to those of natural symptoms, with green leaves turning yellow to brown, showing symptoms of blight. The Koch's postulate was proved by re-isolating the pathogen from the diseased leaf. The culture was similar to the original bacterial culture (Figure 1).

Physiological test

Morphological observations revealed the that bacteria were rod-shaped bacteria without flagella. Based on the results of the physiological characterization, pathogenic bacteria were Gram-negative, unable to hydrolyze starch, and were positive according to the Levan test. The bacteria formed yellow colonies on yeast chalk dextrose agar at 36°C. Isolate was positive for indole, used citrate as a carbon source, and liquefied gelatin at low temperatures (Figure 2).

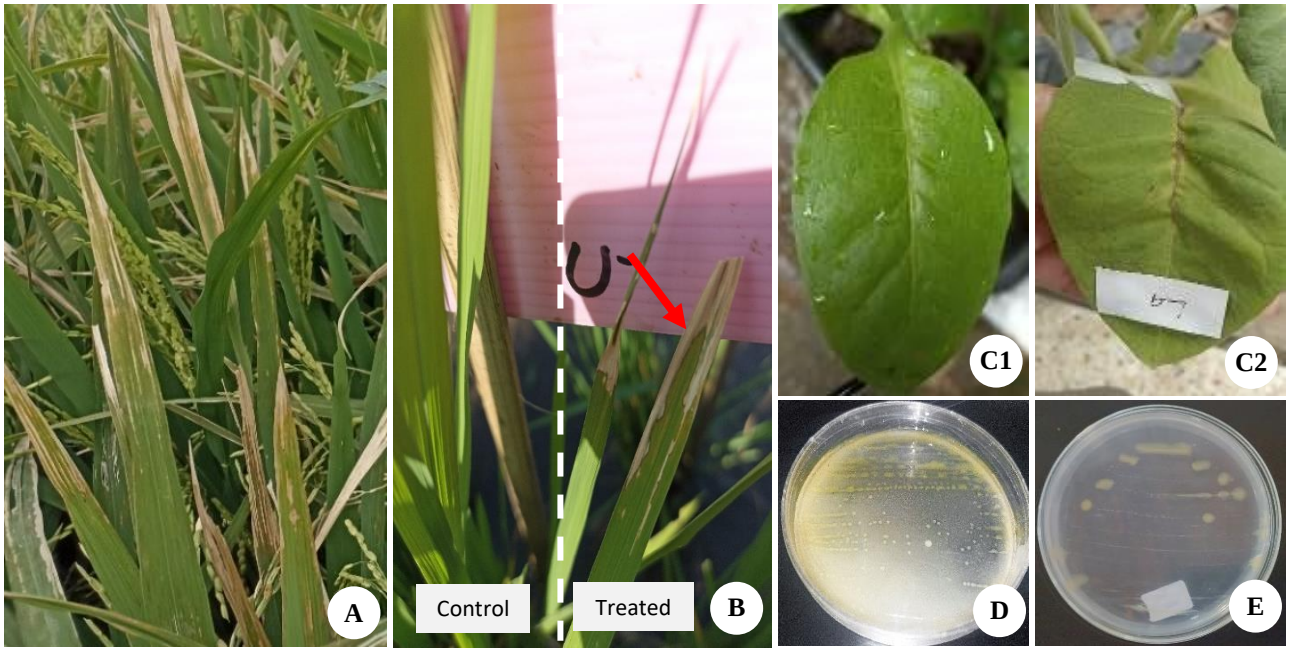

Figure 1. A. Natural leaf blight disease in rice; B. Artificial leaf blight for postulate Koch treatment; C. Hypersensitive reaction test; D. Pathogen on yeast dextrose chalk agar media; E. Bacterial pathogen on *Pantoea* Genus-Specific Agar (PGSA) media

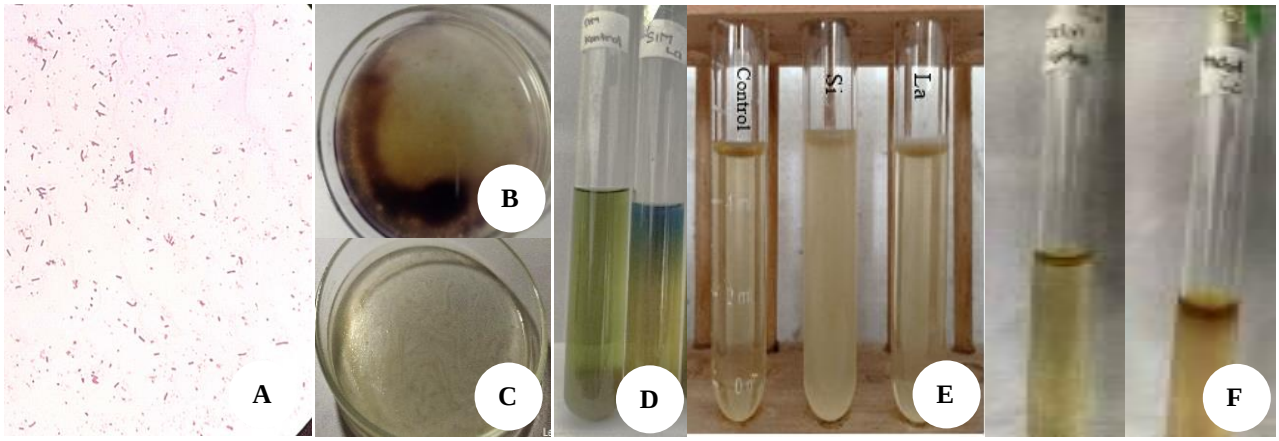

Figure 2. Rod shaped bacteria in: A. Gram staining; B. Starch hydrolysis; C. Levan test; D. Citrate test; E. Growth at 37°C; F. Indole test

Molecular identification

Molecular identification was carried out to determine the identity of the bacteria, from the base sequence to the phylogeny. The results of 0.8% TBE agarose gel electrophoresis revealed that the bacteria within the 16s rRNA range were in a band with a molecular weight of 1404 bp (Figure 3). The electrophoresis results were then sequenced to determine the protein base sequence and its specific identity. The results of the sequence homology analysis, which was conducted via BLAST, revealed that the bacterial pathogen causing leaf blight in rice (isolate code LA) had a similarity of 99.79% with *P. ananatis* strain HJ21L4. The query coverage value of 100% indicates the percentage of the test strain sequence that matches its homologous strain. The results showed that La isolate was similar to *P. ananatis* with 99.79% similarity (Table 1).

The phylogenetic relationship of *P. ananatis* (La) was analyzed with other partial 16S rRNA sequences of related bacterial species available in Gen Bank. The DNA sequences of the 16S rRNA gene fragments of the studied bacteria were aligned and compared using a standard basic local alignment search tool (BLASTn).

The phylogenetic tree between the two isolates showed that *P. ananatis* isolate Lawang (La) was closely related to *Pantoea* sp. and *P. ananatis* strain EM2-53 (Figure 4). The *Pantoea* Lawang isolate had different phylogenetic relationships with plant pathogens such as *X. oryzae* and beneficial bacteria like *Bacillus cereus*, which can be seen in the different branches of the phylogenetic tree with maximum likelihood clustering method.

Potential of rhizosphere bacteria as antagonistic agents of pathogenic bacteria *Pantoea ananatis*

The inhibition potential of rhizobacteria against the bacterial pathogen *P. ananatis* was evaluated with codes UNS-P1, UNS-P3, UNS-R1, and UNS-R2. The results of disc diffusion revealed that rhizobacteria were unable to produce a clear zone as a marker of the ability of bacteria to inhibit pathogen growth when compared to the negative control (only pathogenic bacteria) and positive control (using streptomycin) (Figure 5).

The results of spray method revealed that the bacteria formed a clear zone around the rhizobacteria. This may be because rhizosphere bacteria had an inhibitory mechanism in the form of the formation of antibiotic volatile compounds, instead of the mechanism of competition for space and nutrients. The bacteria were stunned and then the volatile compounds produced by the bacteria were reacted by using chloroform swabs (Figure 6).

Discussion

The disease triangle concept states that the development of plant diseases can be influenced by virulent pathogens, susceptible hosts, favourable environments, and human treatment. This concept is relevant to crop cultivation activities carried out by humans to fulfil food needs because these factors cause plant diseases to be dynamic. Especially with the threat of climate change, the dynamism of plant diseases allows for changes in their status, from secondary diseases to primary diseases, and allows the emergence of new plant diseases (Agrios 2016). In Indonesia, rice is an important commodity, as it is the staple food of most of the population. In other countries, there have been reports of a new cause of disease attacking rice crops namely, the pathogenic bacteria *P. ananatis*. However, there have been no reports of this bacteria attacking paddy rice in Indonesia.

The results of present study showed that new rice blight disease was found in Indonesia. Symptoms of leaf blight include changes in the leaf color to green to yellowish and finally brownish. These symptoms are consistent with the results of previous research, which indicate that rice leaves infected with *P. ananatis* show orange-brown necrotic symptoms along the edges (Kini 2020) and round, reddish-brown lesions on the top of the flag leaf (Aksoy and Boluk 2019). Yellow or brown lesions then dry out on rice leaves, resulting in cell death. The stems of the inoculated seedlings shrink and dry (Yu et al. 2021).

The symptoms of disease found were similar to the symptoms of disease caused by bacterial leaf blight pathogen *X. oryzae* pv. *oryzae*. Therefore, bacterial isolation followed by morphological, physiological and molecular identification were performed to confirm the identity of pathogenic bacteria. The results showed that a milky white to yellowish colony of bacteria was isolated from diseased leaves of rice. The result of hypersensitivity test in this study revealed that *P. ananatis* bacteria caused necrosis of tobacco leaves, which confirmed that *P. ananatis* bacteria were pathogenic.

To determine the pathogenicity of *Pantoea* bacteria, tobacco hypersensitivity and pathogenicity tests were conducted on new rice leaves. This is important because some *Pantoea* may be found as detrimental (pathogenic), and some may be found as beneficial (non-pathogenic) bacteria. Some *Pantoea* have a positive impact on plant growth (Doni et al. 2021), but other cause leaf blight in several plant commodities, such as rice (Mondal et al. 2011; Kini et al. 2017; Aksoy and Boluk 2019; Toh et al. 2019; Yu et al. 2021; Reshma et al. 2022; Luna et al. 2023), garlic (Nurjanah et al. 2018), strawberries (Abdel-Gaied et al. 2022), and corn (Mamede et al. 2018).

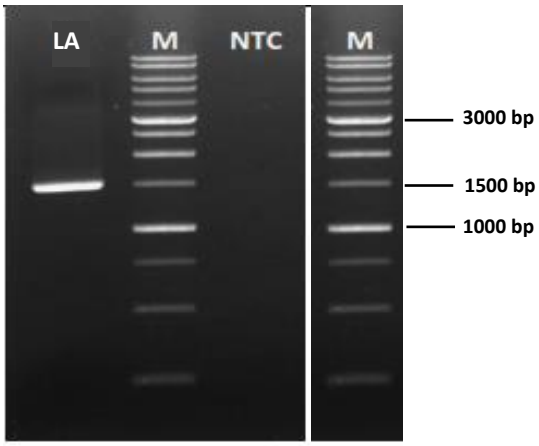

Figure 3. PCR result of bacterial pathogen visualized with UV transilluminator. LA: Bacterial pathogen; M: Marker; NTC: Non Template Control

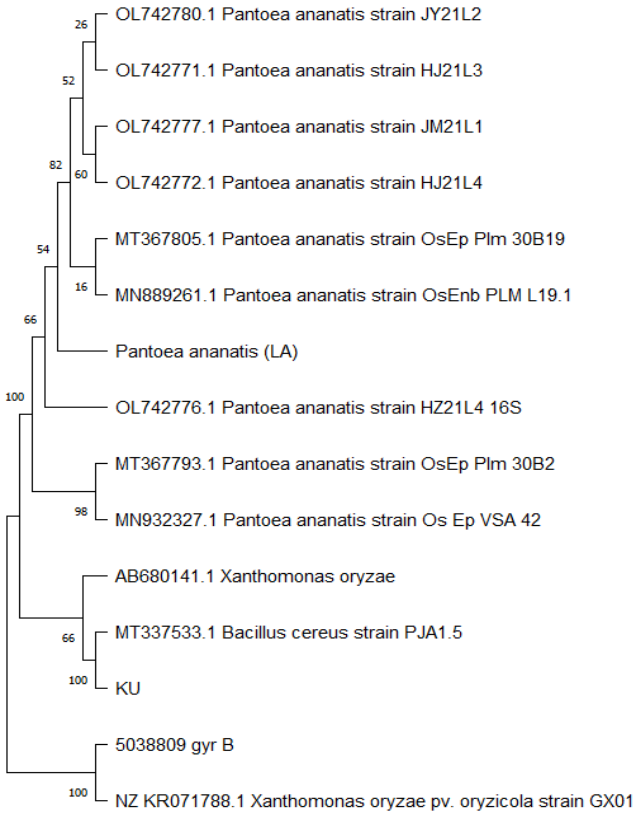

Figure 4. The maximum likelihood phylogenetic tree of bacterial pathogens created using MEGA 11 software

Table 1. Results of homology tested via BLAST

Isolate	BLAST result	Percent identity	Accession no.
LA	*Pantoea ananatis* strain HJ21L4 16 S ribosomal RNA gene	99.79%	OL742772.1
	Pantoea ananatis strain HJ21L3 16S ribosomal RNA gene, partial sequence	99.79%	OL742771.1
	Pantoea ananatis strain JY21L2 16S ribosomal RNA gene, partial sequence	99. 86%	OL742780.1
	Pantoea ananatis strain HZ21L4 16S ribosomal RNA gene, partial sequence	99.72 %	OL742776.1
	Pantoea ananatis strain OsEp_Plm_30B19 16S ribosomal RNA gene, partial sequence	99.72 %	MT367805.1
	Pantoea ananatis strain OsEp_Plm_30B2 16S ribosomal RNA gene, partial sequence	99.72 %	MT367793.1
	Pantoea ananatis strain OsEnb_PLM_L19.1 16S ribosomal RNA gene, partial sequence	99.72 %	MN889261.1
	Pantoea ananatis strain Os_Ep_VSA_42 16S ribosomal RNA gene, partial sequence	99.72 %	MN932327.1
	Pantoea ananatis strain JM21L1 16S ribosomal RNA gene, partial sequence	99.72 %	OL742777.1

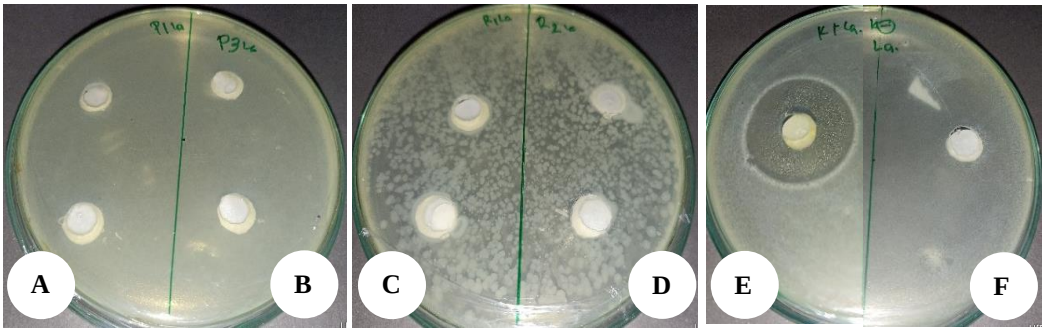

Figure 5. Double layer/disc diffusion method antagonistic assay of rhizobacteria isolates against pathogenic bacteria *Pantoea ananatis* (La). A. UNS-P1; B. UNS-P3; C. UNS-R1; D. UNS-R2; E. Positive control; F. Negative control

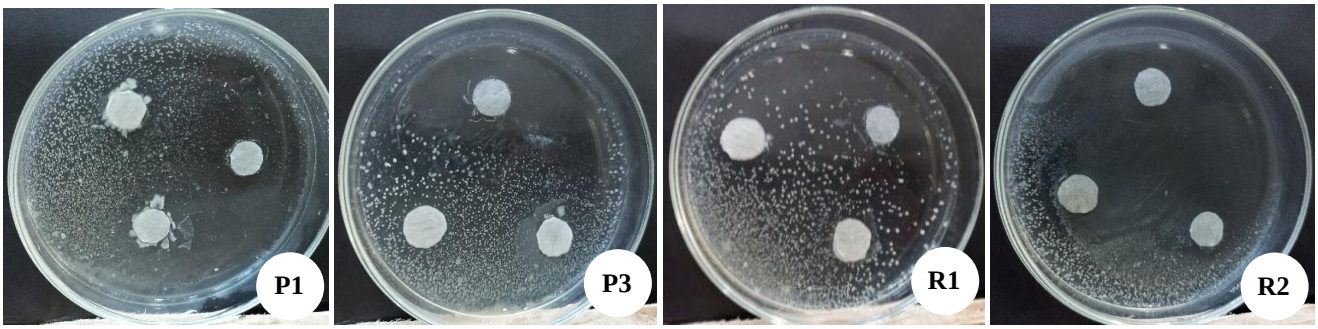

Figure 6. Spray method antagonistic assay of rhizobacteria isolates against *Pantoea ananatis* (La)

The Koch's postulate test shows that pathogenic bacterial isolates retransmitted on rice leaves showed the symptoms produced were similar to those of natural symptoms, with green leaves turning yellow to brown, showing symptoms of blight. The result is consistent with the Koch's postulate that pathogenic bacteria isolated from symptomatic leaves must show the same symptoms after being isolated on artificial media, re-inoculated on new plants, and then re-isolated on new artificial media again (Hou et al. 2023).

The results of physiological characterization exhibited that pathogenic bacteria were Gram-negative, unable to hydrolyze starch, and were positive according to the Levan test. The bacteria formed yellow colonies on yeast chalk dextrose agar at 36°C. Isolate was positive for indole, used citrate as a carbon source, and liquefied gelatin at low temperatures The semi-selective media utilized in isolating *P. ananatis* included Yeast Dextrose Chalk Agar (YDCA) and a genus-specific agar for the Pantoea family of bacteria (PGSA). PGSA was used to filter out *X. oryzae* bacteria, which have similar symptoms of attack in the form of brownish leaves and drying, especially on the flag leaf, and to isolate these pathogenic bacteria with a yellowish color on semi-selective YDCA media. This finding aligns with previous studies, which show that bacteria belonging to the Pantoea family can grow well on PGSA media (Kini et al. 2019).

The results of sequence homology analysis revealed that bacterial pathogen causing leaf blight in rice (isolate code LA) had a similarity with *P. ananatis.* The construction of phylogenetic tree based on the genetic sequences of the two isolates revealed that *P. ananatis* isolate Lawang (La) exhibited a close relationship with *Pantoea* sp. and *P. ananatis*. The *Pantoea* Lawang isolate exhibited distinct phylogenetic relationships with plant pathogens such as *X.*

oryzae and beneficial bacteria like *B. cereus*, as observed in the different branches of the phylogenetic tree using the maximum likelihood clustering method. Results of present findings are consistent with those from previous studies, which also utilized 16SrRNA primers to molecularly identify the pathogenic bacteria *P. ananatis* (Mondal et al. 2011; Egorova et al. 2015; Kini 2017; Azizi et al. 2019; Abdel-Gaied et al. 2022).

The control of *P. ananatis* must be environmentally friendly, in accordance with the concept of integrated disease control, which focuses on healthy plant cultivation, regular observation, farmers as integrated disease control experts, and the utilization of natural enemies. Certain rhizosphere bacteria can control plant diseases. The mechanism of rhizobacteria as biocontrol of plant diseases includes space competition, producing antibiotic compounds, producing siderophores, producing lytic enzymes, producing HCN compounds to suppress pathogenic fungi, detoxifying and degrading toxins produced by pathogens, modifying host plant cell walls, inducing plant resistance, and regulating plant ethylene levels through the enzyme ACC deaminase (Reddy 2014; Alawiye and Babalola 2019). Based on the results of spray treatment, rhizobacteria UNS-P1, UNS-P3, UNS-R1 and UNS-R2 were showed clear zone around the bacteria. This suggests that bacteria can inhibit the growth of pathogenic bacteria, likely due to the presence of volatile antibiotic compounds. This assumption is reinforced by the results of the previous treatment with the double layer or disc diffusion method, which did not show a clear zone, suggesting that the bacteria cannot successfully compete with pathogens for living space. This finding is consistent with the previous research showing that the pathogen that causes corn leaf blight, *Pantoea* sp., may be effectively controlled by antagonistic bacteria, specifically *Pseudomonas* sp. and *Bacillus* sp. Antagonistic assays of these strains on agar plates and subsequent leaf blight assays in plants led to the conclusion that both *Bacillus* sp. and *Pseudomonas* sp. have the potential to control the disease by inhibiting the growth of *Pantoea* sp. The observed mechanisms were bactericidal and bacteriostatic. The *Pseudomonas* sp. and *Bacillus* sp. strains were demonstrated to be capable of surviving on corn leaves and reducing the severity of corn leaf blight caused by *Pantoea* sp. (Javandira et al. 2013). Another study reported that *P. ananatis* can be controlled by *Bacillus* strain *licheniformis* HN-5 bacteria through the release of an antibiotic compound called bacitracin. Bacitracin A causes cell leakage and changes in the membrane. The application of bacitracin has been demonstrated to cause cell lysis and alter the cell permeability of the pathogen *P. ananatis*. Consequently, this antibiotic is an optimal choice as a biocontrol agent for the pathogen *P. ananatis* (Jin et al. 2021). The ability of *Bacillus* genus bacteria to control *P. ananatis* was also shown in a study on the inhibitory ability of *Bacillus velezensis* B-27 bacteria against *P. ananatis* on leatherleaf fern compared to cooper hydroxide and zinc thiazol bactericides (Tsaniyah et al. 2024). The potential of rhizosphere bacteria as growth inhibitors of the disease-causing *P. ananatis* can be used as a basic reference to mitigate the attack of new disease-causing *P. ananatis* in Indonesia.

In this study, *Pantoea ananatis* was identified and characterized as the causal agent of leaf blight disease in Indonesian rice crops. Based on the results of physiological characterization, pathogenic bacteria were Gram-negative, unable to hydrolyze starch, and positive according to the Levan test. The bacteria produce yellow colonies on yeast chalk dextrose agar, can grow at 36°C, were positive for indole, can utilize citrate as a carbon source, and can liquefied gelatin at low temperatures. The results of molecular identification revealed that the bacterial pathogen causing leaf blight in rice (isolate code LA) has a similarity of 99.79% with *P. ananatis* strain HJ21L4. The inhibition test of pathogenic bacteria by antagonistic bacteria showed that rhizobacteria can inhibit pathogenic bacteria through the production of antibiotic compounds. This is the first report on the identification and characterization of *P. ananatis* in rice in Indonesia and the biocontrol potential using rhizobacteria. These findings can be used as new knowledge about the types of leaf blight pathogens caused by *P. ananatis* and their control alternatives to determine tactical steps in integrated crop management towards sustainable agriculture.

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