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Potential of Indigenous Rhizobacteria as Biocontrol Agents of *Xanthomonas* sp.

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Bacterial leaf blight (BLB) disease caused by *Xanthomonas* sp. is one of the main diseases of eucalypt plants. The utilization of rhizobacteria to manage diseases in other agricultural ecosystems has been reported. This study aims to select and characterize rhizobacteria from the rhizospheric zones of *Eucalyptus pellita* with antagonistic nature against *Xanthomonas* sp. and to identify isolates that have the best potential as biocontrol agents in the *Eucalyptus–Xanthomonas* pathosystem. The research was conducted in-vitro. The antagonism test was arranged in a completely randomized design with three replications. Characteristics of antagonistic rhizobacteria were examined based on their ability as plant growth-promoting bacteria (PGPR). Identification of potential rhizobacteria was performed based on 16S rRNA sequences. There were 14 isolates showing antagonistic activities against *Xanthomonas* sp. The results showed that the rhizobacteria had significant antagonistic impacts against the pathogen. Characterization of the 14 isolates indicated that all isolates produced siderophores and indole acetic acid (IAA). As many as 13 isolates produced protease, eight isolates were able to dissolve phosphate, and three isolates synthesized hydrogen cyanide (HCN). The isolate with the highest inhibitory effect was isolate RE081, whose activity was classified as very strong. The RE81 isolate also showed the best properties in the HCN test (+++), IAA production (19,29 mg L^{-1}), and the ability to dissolve phosphates. Molecular analysis of the 16S rRNA gene disclosed that RE81 is *Pseudomonas aeruginosa* (100%).

Keywords: 16S rRNA gene, characteristic, identification, *Pseudomonas aeruginosa*

INTRODUCTION

Bacterial leaf blight (BLB) is one of the main diseases of eucalyptus plants (Ferraz *et al.* 2018). Infected plants will grow with many stems and are stunted (Coutinho *et al.* 2015). BLB was first reported by Truman (1974) in Australia to have been caused by *Xanthomonas deleyii* subsp. *eucalypti* (Truman) (synonym *X. campestris* pv. *eucalypti*). According to Gonçalves *et al.* (2008), *X. axonopodis* is the most widespread species as the cause of BLB of *Eucalyptus* spp. in Brazil. Coutinho *et al.* (2015) reported that *X. vasicola* underwent a host jump from sugar cane to *E. grandis* in South Africa. *X. perforans* was reported to cause leaf blight and dieback of eucalyptus plants in Indonesia (Bophela *et al.* 2019). The reduced leaf cross-sectional area due to lesions and continuous defoliation to carry out photosynthesis is the cause of decreased growth and survival of eucalyptus seedlings. Losses due to late blight by *X. axonopodis* at the nursery

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stage were recorded at USD 8 million from 2003–2008 in Brazil (Alfenas *et al.* 2009).

One of the important components of integrated management of significant eucalyptus diseases is the use of biological control agents. Siregar *et al.* (2022) screened endophytic *Trichoderma* for potential isolates to improve the growth and health of eucalyptus seedlings. Rhizobacteria are also very essential biological agents. The utilization of rhizobacteria, including those thriving in the surrounding areas of plant roots (rhizosphere), to improve plant growth and health in different agricultural ecosystems has been reported. In general, the population of microorganisms in the rhizosphere is generally more numerous and diverse than that in non-rhizosphere soils (Niswati *et al.* 2008). The exudate produced by the roots influences the activity of rhizobacteria. Rhizobacteria can aggressively colonize the rhizosphere, and several types of rhizobacteria have the characteristics of being able to act as biofertilizers, biocontrol, and biostimulants in plants, known as PGPR (Ashrafuzzaman *et al*. 2009; Sutariati and Wahab 2012). According to Ashrafuzzaman *et al*. (2009) and Bhattacharyya and Jha (2012), the application of rhizobacteria has the potential as a substitute for pesticides and, at the same time, improves the efficiency of nutrient uptake by plants. Rhizobacteria can also help plants reduce stress due to drought (Riseh *et al*. 2021a) and high environmental salinity conditions (Riseh *et al*. 2021b). Some rhizobacteria that have been reported to be antagonistic against plant pathogens are from the genera of *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, and *Enterobacter* (Naureen *et al.* 2005).

Rhizobacteria have also been utilized to manage *Xanthomonas* sp. on different host plants. However, there has not been any report on the use of rhizobacteria to control *Xanthomonas* on *Eucalyptus pellita*, in particular in Indonesia. Kurniawati *et al.* (2015) reported that the bacteria *Bacillus nealsonii* strain F22, *Chromobacterium* sp. MWU328, *Streptomyces* sp. Antag1, and *Kitasatospora nipponensis* strain H2-4 inhibited the growth of *X. oryzae*. Ernita *et al.* (2016) also found indigenous rhizobacteria – namely, *P. geniculata* strain XJUHX-19 and *B. pumilus* strain TSH22w – which were able to increase the resistance of shallot plants to BLB (*X. axonopodis* pv. *allii*). *B. altitudinis* AP69 was reported to be able to reduce the severity of disease caused by *X. axonopodis* pv. *vesicatoria* on tomato plants by 44.6% (Liu *et al.* 2017). Nguyen *et al.* (2020) reported that root treatment with *P. fluorescens* and *B. subtilis* triggered systemic resistance in *Arabidopsis thaliana* plants to *Botrytis cinerea* and *P. syringae* pv. *tomato* DC3000.

Recent studies have revealed the development of new application technologies, including encapsulation of the rhizobacteria to increase their effectiveness *in vivo* (Fathi *et al*. 2021; Riseh *et al*. 2021c, d; 2022a, b; Pour *et al*. 2021, 2022a, b). Encapsulation technology can reduce plant disease severity by up to 70–95% (Riseh *et al*. 2022b; Riseh and Mojde 2021). Therefore, the contribution of rhizobacteria in the near future in plant disease management is expected to increase rapidly. The present research aims to obtain and characterize effective indigenous rhizobacterial isolates as components of integrated management of BLB on *E. pellita* plants.

MATERIALS AND METHODS

Xanthomonas Inoculum

A *Xanthomonas* sp. isolate of the Sinarmas Forestry PT Arara Abadi R&D Laboratory collection was first tested for its characteristics to ensure its virulence. The bacterium was grown in yeast extract-dextrose-$CaCO_3$ (YDC) (10 mg yeast extract, 20 g dextrose, 20 g $CaCO_3$, 20 g agar, and 1000 mL aquadest) medium and incubated for 48 h at 28–30 °C to determine the colony shape and color. The form of the cell and gram test were observed under a light microscope. The 3 % KOH test was carried out by mixing one loop of isolate and 3% of KOH solution. The formation of threads indicates positive results. The catalase test was carried out by dripping a 3 % H_2O_2 solution. The formation of air bubbles indicates a positive result. The oxidase test was carried out by dripping the p-aminodimethylaniline oxalate solution onto filter paper and then applying the isolate. Positive results are indicated by the formation of blue color on the filter paper (Cappuccino and Sharman 2014). The hypersensitivity test was completed using a method similar to the rhizobacterial hypersensitivity test described in the following section. A virulence test was carried out by spraying a suspension of the inoculum with a density of 10^8 cfu/mL onto plant leaves. Plants were observed up to 21 d after inoculation of the pathogen.

Rhizobacteria Isolates

Soil sampling. Sampling was carried out using the purposive sampling method. Samples were taken from rhizospheres of healthy *E. pellita* surrounded by diseased plants in plots of a relatively high disease incidence with a depth of 5–20 cm. The sample is soil attached to plant roots with a thickness of about 15 mm. Five points were determined randomly in each sample plot, and the samples were composited using sterilized equipment. The soils were stored in plastic clips in a cool box filled with ice cubes and then taken to the laboratory for processing (Saraswati *et al.* 2007).

Rhizobacteria isolation. Isolation of rhizobacteria was carried out using the serial dilution method. Ten grams of the composite soil sample was put into 90 mL of sterile water containing 0.85% NaCl in a 250 ml Erlenmeyer, shaken for 2 h using a rotary shaker, and then five serial dilutions were made. From each dilution, 0.1 mL of suspension was taken and added to nutrient agar (NA) (2.5 g glucose, 5 g peptone, 5 g beef extract, 20 g agar, and 1000 mL aquadest) medium with three replications and incubated for 48 hours at 28–30 °C. Each colony that grew separately was then purified onto a new medium. Rhizobacteria purification was performed using the four-quadrant streak method using the NA medium and incubated for 48 h at 28–30 °C (Khairani and Hesti 2019).

Hypersensitive reaction. This test aims to determine whether the isolated rhizobacteria are pathogenic or not. The test was applied to 3-mo-old tobacco plants with fully opened leaves (Butar-Butar *et al.* 2018). One to two loops of bacterial colonies were incubated on nutrient broth (NB) (2.5 g glucose, 5 g peptone, 5 g beef extract, and 1000 mL aquadest) medium in a shaker for 24 h at the speed of 100 rpm. The suspension was then infiltrated into tobacco leaves using a 1-mL volume of syringe without a needle and incubated for 48 h. The same thing was completed for control but using sterile water. The presence of necrosis symptoms in the inoculation area indicates that the inoculated bacterium is pathogenic.

Antagonism Tests of the Isolated Rhizobacteria against *Xanthomonas*

The paper disc diffusion method was used with three replications for each treatment. As much as 0.1 mL of the pathogenic bacterial suspension with a density of 10^8 cfu/mL (OD600 = 0.6) was spread using glass beads in a petri dish containing NA medium and allowed to dry for 5–10 min. Three pieces of filter paper with a diameter of 6 mm soaked for ± 10 min in a solution of rhizobacteria were placed on the medium. Antibiosis activity is indicated by the formation of a clear zone around the rhizobacteria (Tridesianti *et al.* 2016). Observations were made by measuring the diameter of the clear zone minus the growth diameter of the tested isolate after being incubated at room temperature for 24–48 h (Ulya 2009). According to Davis and Stout (1971), the inhibition ability of bacteria is divided into four categories based on the diameter of the inhibition zone formed: very strong (> 20 mm), strong (10–20 mm), moderate (5–10 mm), and weak (< 5 mm).

Characterization of the Antagonistic Rhizobacteria

Hydrogen cyanide (HCN) production. Screening of HCN-producing bacteria was carried out using the protocol described by Bakker and Schippers (1987) with slight modifications. Tested isolates were grown on King's B media containing 4.4 g/L glycine. Whatman No.1 filter paper previously soaked in a solution of 2% sodium carbonate in 0.5% picric acid was put on the lid of the Petri dish. The plates were then incubated at 30 °C for 4 d. The color change on the filter paper from yellow to dark yellow or reddish brown indicates the formation of HCN.

Protease enzyme production. The rhizobacterial isolates tested were grown on skim milk agar with a composition of 25 g skim milk and 20 g agar and then incubated at 30 °C for 24–48 h. The clear zone indicates the activity of the extracellular protease enzyme (Hazra and Susanti 2017).

Siderophore production. Siderophore production was tested according to Schwyn and Neilands (1987) using a blue agar medium containing Chrom Azurol-S and hexadecyltrimethylammonium bromide as indicators. The orange halo that forms around the colonies on the blue agar is indicative of the excretion of the siderophore (Louden *et al.* 2011).

Phosphate solubilizing ability. Bacterial isolates were grown on Pikovskaya medium (10 g $C_6H_{12}O_6$, 5 g $Ca_3(PO_4)_2$, 0.5 g $(NH_4)_2SO_4$, 0.2 g KCl, 0.1 g $MgSO_4.7H_2O$, 0.002 g $MnSO_4.7H_2O$, 0.002 g $FeSO_4.7H_2O$, 0.1 g NaCl, 0.5 g yeast extract, and 20 g agar in 1000 mL distilled water). The bacteria were incubated for 7 d at 30 °C. A positive reaction was indicated by the formation of a clear zone (Hazra and Susanti 2017).

Production of indole acetic acid (IAA) hormone. One loop of rhizobacteria aged 48 h was inoculated into 10 mL of NB medium. Following incubation for 24 h, a total of 500 µL of the bacterial culture was inoculated into 50 mL of NB medium containing 0.1% L-tryptophan. The culture was incubated on a shaker for 48 h at 30 °C, 180 rpm in the dark. The bacterial solution was then centrifuged at 10,000 rpm for 10 min at 4 °C. The production of rhizobacterial IAA was analyzed by colorimetric method using Salkowski reagent (150 mL concentrated H_2SO_4; 7.5 mL $FeCl_3.6H_2O$ 0.5 M; 250 mL sterile water). As much as 1 mL of the supernatant was mixed with 4 mL of Salkowski reagent and then incubated for 30 min in the dark. A change in color from pink to dark red indicates that the rhizobacteria produce IAA. The absorbance value was measured using a spectrophotometer at 535nm (Ghevariya and Desai 2014). The resulting IAA concentration was estimated based on the absorbance value using the IAA standard curve (Lebrazi *et al.* 2020).

Identification of the Selected Rhizobacterium Based on the 16S rRNA Gene

A selected rhizobacterial isolate with the highest inhibition activity was identified molecularly based on the 16S rRNA gene. Total rhizobacterial DNA was extracted using the Geneaid Genomic DNA extraction kit according to the

manufacturer's instructions. The isolated DNA was amplified by PCR (Nanbei Double Block Gradient Thermal Cycler) using universal bacterial primers with a forward 27 F primer (5'-TACGGYTACCTTGTTACGACTT-3') and a reverse 1492 R primer (5'-AGAGTTTGATCCTGGCTCAG-3') (Jiang *et al.* 2006). Each reaction mixture (25 µL) contained 5 µL of PCR buffer, 0.5 µL of dNTP, 2.5 µL of $MgCl_2$, 0.25 µL of Taq DNA polymerase (Green GoTaq, Promega, US), 0.1 µL of primers, 4 µL of DNA template/sample, and 12.55 µL of H_2O. The cycle conditions used were as follows: denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 92 °C for 30 s, primer binding at 55 °C for 30 s, extension at 72 °C for 1 min, and final elongation at 72 °C for 2 min. The PCR products were visualized by agarose gel electrophoresis at 1% using MUPID EX-U of 100 V for 20 min. Subsequently, sequencing was performed at the 1st Base (Selangor, Malaysia). Sequence analysis was carried out through the edit sequence stage using the BioEdit 7.2 version for Windows program, followed by comparison of the sequence with the GenBank DNA database *via* the National Center for Biotechnology Information (NCBI), National Institute for Health, USA, using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.org). The phylogenetic tree construction was completed using the Mega 11 program.

RESULTS

Characterization of *Xanthomonas*

Xanthomonas sp. is a Gram-negative bacterium with bright yellow and mucoid shapes. Bacterial cells are rod-shaped and show positive results in the 3% KOH, catalase, and oxidase tests. In the hypersensitive test on tobacco leaves, the bacterium also caused necrosis. In the pathogenicity test on *E. pellita* plants, *Xanthomonas* caused symptoms of soaked spots consistent with BLB, which started to appear on the seventh day after inoculation. Figure 1 shows symptom development on the 21st day after inoculation.

Figure 1. Bacterial leaf blight symptoms caused by *Xanthomonas* on the 21st day after pathogen inoculation.

Rhizobacteria Isolates and Hypersensitive Reaction

A total of 123 rhizobacterial colonies were successfully isolated. The results of the hypersensitive test (Figure 2) showed that one isolate, *i.e.* RE008, caused necrosis symptoms on the tobacco leaves after infiltration for 48 h. This indicates that the RE008 isolate is a plant pathogen, whereas the other 122 isolates are not pathogenic.

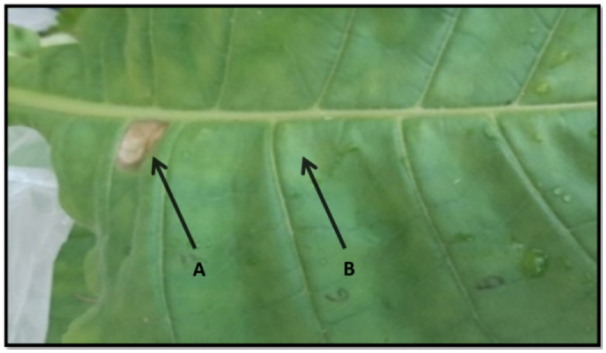

Figure 2. Hypersensitive reaction test results: [A] rhizobacterial isolate RE008 forms necrosis; [B] a rhizobacterial isolate that does not form necrosis.

Rhizobacterial Antagonism Test against *Xanthomonas*

The *in vitro* antagonism test, from 122 non-pathogenic rhizobacteria, produced 14 rhizobacterial isolates that formed clear zones, indicating inhibition (inhibitory zones). Control did not generate clear zones. The results of the analysis of variance showed that the rhizobacterial isolates produced significantly different clear zone diameters (Table 1). RE081 had the highest inhibitory effect and was significantly different from the other isolates. The lowest inhibitory zone of the rhizobacterial treatments was exhibited by isolate RE065. Meanwhile, in control (without rhizobacterial treatment), no inhibitory zone was formed. Thus, the inhibitory effects of the isolates tested in the current study ranged from weak (4 isolates), moderate (3 isolates), strong (6 isolates), to very strong (1 isolate).

Characterization of the Antagonistic Rhizobacteria

As shown in Table 2, three isolates are capable of producing HCN. A strong reaction (+++) was observed in isolate RE081, a moderate reaction (++) in isolate RE086, and a weak reaction (+) in isolate RE085. Qualitative analysis results showed that 13 isolates produced protease (Figure 3C), whereas isolate RE065 did not.

All the rhizobacteria antagonistic to *Xanthomonas* sp. were able to produce siderophores and IAA (Table 2, Figures 3B and E). Eight rhizobacterial isolates generated clear zones in the selective Pikovskaya medium, indicating their ability to dissolve phosphates. These rhizobacteria are RE020, RE034, RE066, RE081, RE083, RE085, RE086, and RE114 with different diameters.

Table 1. Diameter of inhibitory zone of antagonistic rhizobacteria against *Xanthomonas* sp.

Isolate code	Diameter of inhibition of (mm)[a]	Inhibitory activity
Control	0.00 [a]	–
RE007	3.00 [bc]	Weak
RE013	13.33 [g]	Strong
RE020	6.67 [e]	Moderate
RE034	7.67 [e]	Moderate
RE065	1.67 [ab]	Weak
RE066	6.00 [de]	Moderate
RE072	4.67 [cd]	Weak
RE073	11.33 [f]	Strong
RE081	26.00 [i]	Very strong
RE083	14.33 [g]	Strong
RE084	12.75 [fg]	Strong
RE085	17.00 [h]	Strong
RE086	18.00 [h]	Strong
RE114	3.00 [bc]	Weak

The numbers followed by the same letter(s) are not significantly different according to the DMRT test at $\alpha = 5\%$.

Molecular Identification of the Selected Rhizobacterium

From the molecular analysis of the 16S rRNA gene of isolate RE081 (the isolate with the highest inhibitory zone in the antagonism test), a 1500 bp PCR product was obtained (Figure 4). Comparison of the obtained sequence with the GenBank DNA database using BLAST indicated that isolate RE081 has a 100% similarity to five strains of *Pseudomonas aeruginosa*. They are *Pseudomonas aeruginosa* strain JCM 5962, *Pseudomonas aeruginosa* strain DSM 50071T, *Pseudomonas aeruginosa* strain DSM 50071, *Pseudomonas aeruginosa* strain NBRC 12689, and *Pseudomonas aeruginosa* strain ATCC 10145. *Pseudomonas aeruginosa* strain DSM 50071 was isolated before 1946 from an unknown source by researchers from Merck Sharp & Dohme, which later changed their names to DSM 50071T and ATCC 10145 (Kavanagh 1947). Kim *et al.* (2020) reported that *Pseudomonas aeruginosa* strain DSM 50071 was a polystyrene-degrading bacterium.

Phylogenetic analysis was made by taking some sequences from the NCBI GenBank database and comparing them with isolate RE081. The phylogenetic tree analysis using the maximum-likelihood method showed that isolate RE081 Place belonged to the group of *P. aeruginosa* and it was most similar to *P. aeruginosa* strain MAJPIA03 (Figure 5).

DISCUSSION

Xanthomonas collection of the Sinarmas Forestry PT Arara Abadi R&D Laboratory is a Gram-negative, rod-shaped bacterium. It shows positive results in the 3% KOH, catalase, and oxidase tests. On the YDC medium, *Xanthomonas* colonies are bright yellow and have a mucous shape. This pathogen also causes necrosis in the hypersensitive test, indicating the pathogenicity of the collection. The pathogenicity of *Xanthomonas* on *E. pellita* is characterized by soaked lines to necrotic reactions. This

Table 2. Characterization of rhizobacterial isolates that are antagonistic to *Xanthomonas* sp.

Isolate code	HCN	Protease (mm)	Siderophore	Phosphate solubilizing	IAA (mgL^{-1})
RE007	–	6.1	+	–	4.49
RE013	–	5.3	+	–	17.65
RE020	–	5.9	+	+	2.55
RE034	–	5.2	+	+	13.45
RE065	–	0.0	+	–	6.59
RE066	–	6.4	+	+	3.35
RE072	–	5.8	+	–	5.00
RE073	–	7.1	+	–	2.33
RE081	+++	3.2	+	+	19.29
RE083	–	4.1	+	+	5.25
RE084	–	5.4	+	–	4.35
RE085	+	4.6	+	+	13.25
RE086	++	2.6	+	+	7.78
RE114	–	5.8	+	+	5.41

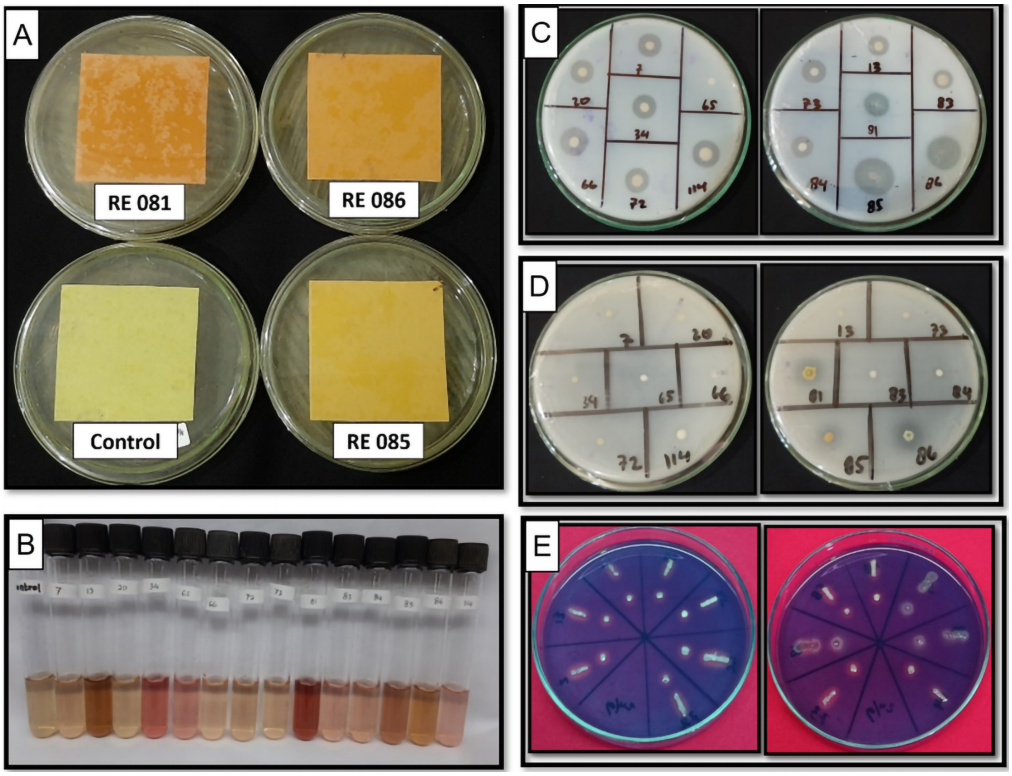

Figure 3. The results of the characterization tests of the isolates: [A] HCN test; [B] IAA test; [C] protease test; [D] phosphate solubilizing test; [E] siderophore test.

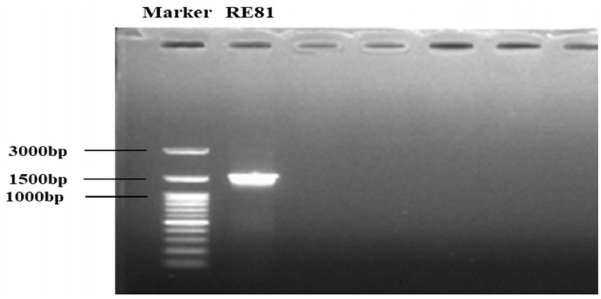

Figure 4. Visualization of the electrophoresis result of the PCR product of the the 16S rRNA gene of *Pseudomonas aeruginosa* isolate RE081 on 1% agarose gel, producing a fragment of 1500 bp.

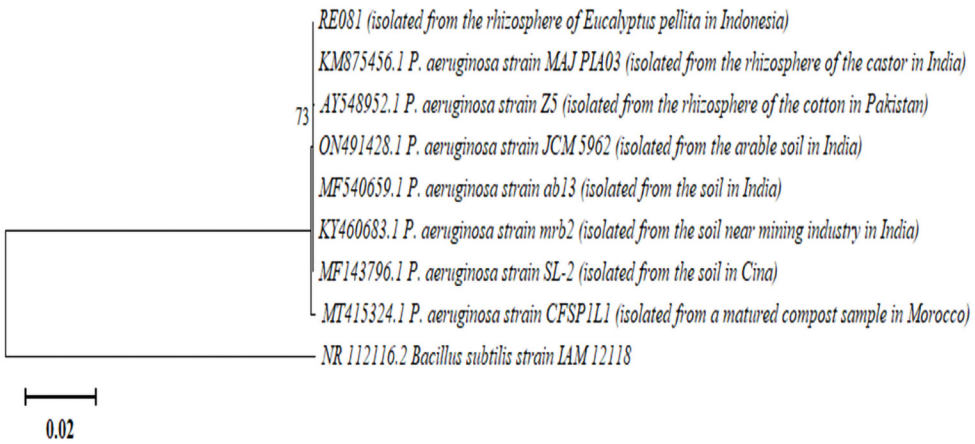

Figure 5. Phylogenetic tree based on the 16S rRNA sequence showing the position of *Pseudomonas aeruginosa* isolate RE081 (using the maximum-likelihood method).

is in line with the symptoms of BLB on eucalyptus plants reported by Gonçalves *et al.* (2008), which are generally characterized by watery lesions forming corners parallel to the leaf veins and amphigenous, which develop into necrotic lesions with a brown color, spread throughout the leaf or only on half of the leaf sheet.

Results of the ANOVA of the antagonism *in vitro* test against *Xanthomonas* showed that the rhizobacteria isolates generated clear zones of significantly different diameters. The formation of a clear zone in the antagonism test indicates the presence of competition, parasitism, or antibiotic interactions. This follows the characterization tests that have been carried out, which show that these rhizobacteria have several compounds that can inhibit the growth of pathogens – namely, HCN, protease, siderophores, and phosphates solubilizers (Figure 3). Agustiansyah *et al.* (2013) reported that rhizobacteria from rice rhizosphere – namely *P. diminuta* A6, *P. aeruginosa* A54, *B. subtilis* 11/C, and *B. subtilis* 5/B – are antagonistically effective against *X. oryzae* pv. *oryzae in vitro*. This is related to the ability of these four bacteria to produce siderophores. *P. diminuta* has also been indicated to produce HCN.

Three isolates that produce the highest inhibition zones are RE081, RE086, and RE085, respectively – all of which can produce HCN (Figure 3A), a secondary metabolite synthesized by many rhizobacteria. It has a strong effect on many organisms, and it can inhibit electron transport and disrupt the energy supply to cells, causing death. Many bacterial genera can produce HCN – including *Alcaligenes*, *Aeromonas*, *Bacillus*, *Pseudomonas*, and *Rhizobium* (Alemu 2016). In addition, the three isolates have all the inhibitory factors tested, *i.e.* proteases, siderophores, and phosphate solubilizers. These results indicate that the rhizobacteria that can produce these inhibitory factors have the potential as biocontrol agents against *Xanthomonas* sp.

The RE065 isolate has the lowest inhibitory diameter (weak type) (Table 1). It was revealed that this isolate only produced siderophore (Table 2). Siderophore is an iron chelator produced by rhizobacteria, acting as an iron solubilizing agent. The siderophore will form a complex with Fe3+ on the bacterial membrane, then reduce it to Fe^{2+}, making it available for itself and the plant in iron-limited conditions (Indiragandhi *et al*. 2008; Srivastava *et al*. 2022). Compared to the RE073 isolate, the latter has a stronger-type inhibition, with the characteristic of producing protease and siderophore. Protease is a hydrolytic enzyme produced by biocontrol agents. It hydrolyzes polymer compounds in the form of protein, one of the components of the bacterial cell wall, thus suppressing the growth of pathogens or killing them. Khalil *et al.* (2022) reported that *B. megaterium*, *B. subtilis*, and *P. fluorescens* are protease producers. The isolate RE065, with a weak inhibition type and most minor inhibition zone, was the only rhizobacteria that did not produce protease. Rhizobacteria that produce siderophore and protease tend to have a higher potential to inhibit the growth of pathogens than those that only produce siderophore (RE065). The presence of HCN also has a strong effect in inhibiting the growth of pathogenic bacteria. This indicates that the inhibitory zone formed is highly related to the type of compounds produced by each isolate.

All rhizobacterial isolates can produce growth factors – namely, the IAA hormone. IAA can stimulate growth, such as cell elongation, cell division, and differentiation (Hassan *et al.* 2015). The RE081 isolate produced the highest IAA (19 mg L^{-1}). Sandilya *et al.* (2017) reported that *P. aeruginosa* strain MAJPIA03 also produces IAA with high concentration (27.84 mg L^{-1}). In a similar study, Ratnaningsih *et al.* (2023) reported that some rhizobacteria isolated from the rhizosphere of pineapple plants in Lampung also produce IAA. The rhizobacteria are able to increase soybean growth.

In addition to the defense mechanism, the ability of rhizobacteria to dissolve phosphates is also very beneficial for plant phosphate availability, also referred to as a biofertilizer agent. Eight isolates can dissolve phosphates with different capabilities (Figure 3D). Visual observations showed that RE081 had a clear zone that was larger than the other rhizobacteria. Some studies have also reported that rhizobacteria can produce IAA and dissolve phosphates (Sutariati and Wahab 2012; Paiter *et al.* 2019).

From the six types of tests performed (antagonism, HCN test, protease, phosphate solubilization, IAA, and siderophore) in the current study, isolate RE081 has four superior characters compared to other rhizobacteria isolates. These include the largest inhibition zone in the antagonism test, as well as better results in HCN, IAA, and phosphate solubilization tests. Thus, RE081 isolate is a strong biocontrol agent candidate as a potential component of integrated management of BLB on *E. pellita*, pending *in vivo* screenings.

Molecular analysis of its 16S rRNA gene disclosed that the RE081 isolate produced a 1500 bp PCR fragment (Figure 4). This is consistent with the report by Janda and Abbott (2007), which state that the 16S rRNA gene has a length of 1500 bp. This gene is commonly used in bacterial identification because it is present in all bacteria, and its function remains unchanged over time. RE081 has a 100% similarity to *P. aeruginosa*, indicating that the RE081 isolate belongs to the same species of *P. aeruginosa*.

Many studies have suggested that *P. aeruginosa* has the ability to function as a biological agent. Phylogenetic analysis shows that RE081 is closely related to *P. aeruginosa* strain MAJPIA03 (Figure 5). According to Sandilya *et al.* (2017), *P. aeruginosa* strain MAJPIA03 produces NH3, HCN, siderophores, ACC deaminase, IAA, and gibberellins. This bacterium can improve the

growth of *Ricinus communis* and inhibit the growth of five different plant pathogens (*Fusarium oxysporum ciceri*, *F. monoliformes*, *F. oxysporum*, *F. glycopersicum*, and *Rhizoctonia solani*). The RE081 isolate is also related to *P. aeruginosa* strain CFSP1L1, a bacterium that can produce bioactive substances as a biocontrol of phytopathogenic fungi (Amir *et al.* 2021). Ghadamgahi *et al.* (2022) reported that *P. aeruginosa* strain FG106 – isolated from the rhizosphere of tomato plants – inhibited the growth of pathogenic *Phytophthora infestans*, *Alternaria alternata*, *P. colocasiae*, *B. cinerea*, *R. solani*, *Clavibacter michiganensis* subsp. *michiganensis*, and *X. euvesicatoria* pv. *perforans*.

CONCLUSION

This study provides novel information that *P. aeruginosa* isolate RE081, a rhizobacterium isolated from the rhizosphere of eucalyptus plants, is able to inhibit the growth of *Xanthomonas in vitro* by producing inhibitory factors such as HCN, protease, siderophores, and phosphatase. In addition, the bacterium also produces plant growth promoting factors such as IAA hormone. Thus, *P. aeruginosa* isolate RE081 has the potential to be developed as biocontrol agent of *Xanthomonas* sp., the cause of BLB disease on *E. pellita* plants. This research is significant because information on the isolation and application of effective rhizobacteria to manage forestry diseases has so far been very limited.

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STATEMENT ON CONFLICT OF INTEREST

The authors have no conflict of interest.

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