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In vitro characterization of UB Forest (Malang, Indonesia) indigenous bacteria as plant growth promoting bacteria (PGPB)

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Abstract. *Aini LQ, Trianti I, Rachmawati SW, Putra AM, Anastasya NA, Setiawan A. 2023. In vitro characterization of UB Forest (Malang, Indonesia) indigenous bacteria as plant growth promoting bacteria (PGPB). Biodiversitas 24: 4558-4565.* UB Forest, an educational forest of Universitas Brawijaya in Malang, East Java, has a mega-biodiversity of microbial germplasm. Previously, we obtained several UB Forest indigenous bacterial isolates and tested them on various plant commodities. However, the comprehensive characterization of the bacteria to produce IAA hormone, to overcome biotic and abiotic stresses as well as their potency as biofertilizers *in vitro* has not been carried out yet. In this study, we conducted *in vitro* assays to elucidate the potency of UB Forest indigenous bacterial isolates as Plant Growth Promoting Bacteria (PGPB). The molecular identification showed that the bacterial strains were dominated by the *Pseudomonads*, namely *Pseudomonas versuta* UB-36, *P. aeruginosa* UB-52, *P. lundensis* UB-53, *P. migulae* UB-54, and *P. koreensis* UB-62. Other strains were *Enterococcus gallinarum* UB-55 and *Lysinibacillus fusiformis* UB-64. Of the 7 bacterial strains, only 3 could inhibit *Xanthomonas campestris*. All bacterial strains were able to produce IAA, whereas five bacteria can solubilize phosphate, six bacteria can fix nitrogen, and four bacteria have both activities. All bacterial strains can grow at pH 5-6, salinity 5-15%, temperature 60°C, and 15% polyethylene glycol (PEG) drought stress media. The results suggested that the UB Forest indigenous bacterial strains have a role as plant growth-promoting bacteria (PGPB) and are expected to support the growth of plants grown under biotic and abiotic stress conditions.

Keywords: Biofertilizer, bioprotectant, biostimulant, biotic and abiotic stress, indigenous bacteria, UB Forest, PGPB

INTRODUCTION

Plants often grow in complex environments that are always changing and interact with biotic and abiotic factors that trigger plant stress (Foyer et al. 2016). Environmental stress is an external condition that affects the growth, development, or productivity of plants. Environmental stress can trigger various plant responses, such as altered gene expression, cell metabolism, changes in growth rates, and yields (Verma et al. 2013). The abiotic stress can be of drought stress, excessive watering, extreme temperatures, high salinity, and mineral toxicity, whereas biotic stress is in the form of injuries caused by plant pests and pathogens (Gull et al. 2019).

Plant Growth Promoting Bacteria (PGPB) are soil or aerial microorganisms that interact with plants and stimulate their growth by influencing plant physiology and development (Souza et al. 2015). PGPB can be found in the phyllosphere, rhizosphere, plant endosphere, and soil (Ibort et al. 2017). PGPB usually stimulates plant growth by producing auxins, gibberellins, cytokinin, and ACC deaminase, as well as providing N fixation, phosphate solubilization, and iron sequestration. PGPB can also protect plants by inhibiting plant pathogenic microorganisms and reducing abiotic stress impacts (Glick 2012). Therefore, PGPB can be utilized as a biostimulant, bioprotectant, or biofertilizer for plants to stimulate growth and alleviate environmental stress as well as nutrient deficiency in the growth medium.

A forest is a natural ecosystem that has the most diverse biodiversity on earth. Forests, especially tropical forests, have high biodiversity because they provide high energy, biomass, and resources, as well as different habitats in the forest structure. The forest environment also provides a cycle of nutrients involving plants, worms, arthropods, insects, fungi, and bacteria that live above and below the ground and are involved in the decomposition process of organic matter derived from dead plant or animal biomass into minerals and organic compounds absorbed by plants to grow in the forest (Viera et al. 2016). The forest environment also has other ecological functions, such as natural control, which involves a very complex biodiversity of biota that includes plants, animals, and microbes that can be used as a source of natural biological control (Brockerhoff et al. 2017). For example, the forest environment can provide a major natural source of rich bacterial biodiversity (Baldrian 2017) that can be utilized in agriculture as an antagonist against plant pathogens or as PGPB. Radhapriya et al. (2018) reported the exploration of indigenous PGPB from Nanmangalam Reserve Forest, India, and examined the effect of the indigenous PGPB on several plant species in the nursery. They found 160

bacterial isolates recovered from nitrogen-free media, and nine of the selected isolates showed plant growth-promoting (PGP) activity. Yasmin et al. (2016) also isolated PGPB from Kacip Fatimah (*Labisia pumila*) grown in Bukit Slim Permanent Forest Reserve, Perak, Malaysia. The PGPB strains showed capability in producing plant-growth hormones, N2 fixation, and P solubilization.

UB Forest is a 554-ha educational forest of Universitas Brawijaya, located in Karangploso, Malang, East Java, Indonesia, at 7.8219 S and 112.5772 E as a southern foothill of Mount Arjuna, with pine and mahogany as the main trees. UB Forest consists of a 42-ha conservation forest area, which is located at an altitude of 1000 to 1200 m above sea level, and a production area, which has several different land uses (Azzahra et al. 2018; Kurniawan et al. 2019). As tropical forests, UB Forests exhibit a high level of microbial biodiversity, including fungi, bacteria, and archaea that inhabit various forest habitats such as plant surfaces, bark surfaces, ground vegetation, roots, rhizosphere, litter, soil, deadwood, rock surfaces, and invertebrates, which have the level of dominance of bacteria or fungi as well as the composition of their communities (Baldrian 2017). This microbial diversity, particularly bacteria recovered from many parts of the forest environment, can be utilized as a germplasm source for beneficial PGPB. However, the utilization of indigenous bacteria from the UB Forest as beneficial PGPB is still limited. Therefore, UB Forest is a potential area to be explored as a source of beneficial bacteria, especially as a PGPB.

Previously, we obtained several UB Forest indigenous bacterial isolates, including from soil, plant litter, plant rhizosphere, plant phyllosphere, and plant endosphere, and tested their plant growth-promoting activities on various plant commodities. In the pot and field experiment, the bacteria showed a positive impact on potatoes (Fauzul et al. 2018), shallots (Dinata et al. 2021), chili (Karina et al. 2020), and soybeans (Roekhan et al. 2021). Of 78 UB Forest indigenous bacterial isolates, 7 have been selected based on their higher activity in promoting plant growth. However, comprehensive characterization of the potency of UB Forest indigenous bacteria as PGPB *in vitro* has not been carried out. Therefore, this study aimed to elucidate the role of UB Forest indigenous bacterial isolates as biostimulants, bioprotectants, and biofertilizers *in vitro*.

MATERIAL AND METHODS

Molecular identification of bacterial strains

Total seven bacterial isolates were used in this study that had potency as PGPB selected from previous studies. The test bacterial isolates were provided by the Plant Pathology Laboratory, Faculty of Agriculture, University of Brawijaya, namely UB-36, UB-52, UB-53, UB-54, UB-55, UB-62, and UB-64. These bacteria have previously been characterized both morphologically and biochemically. Bacteria were cultured on Nutrient Broth (NB) medium for 48 hours and harvested by centrifugation at 5000 rpm for 10 minutes. The DNA of bacterial cells was extracted by using the miniprep of bacterial genomic DNA isolation method that involved CTAB precipitation, according to Ausubel et al. (1992). The DNA was then subjected to amplification of the 16S rRNA gene using the PCR method using universal primers, namely 63F (5' CAG GCC TAA CAC ATG CAA GTC 3') and 1387R (5' GGG CGG WGT GTA CAA GGC 3'). The DNA amplification results were validated by agarose gel electrophoresis and visualized with a UV transilluminator. PCR products were sent to PT Indonesian Genetics Science for sequencing. The sequences were analyzed using BIOEDIT software, and homology analysis was performed on the Genbank database using Basic Local Alignment Search Tool (BLAST) on the website www.ncbi.nml.nih.gov. A phylogenetic tree was generated by the neighbor-joining method in the Mega11 software with a bootstrap test of 1000 replicates. The Kimura 2-parameter method was used to calculate the evolutionary distance and the units of the number of base substitutions per site. The pairwise deletion option was used to remove all ambiguous positions for each sequence pair.

Antagonistic assay against *Xanthomonas campestris*

An antagonistic assay was conducted in vitro using the spray method, according to Kawaguchi et al. (2008). Twenty-four hours old bacterial cultures were suspended in 1 mL of sterile distilled water and adjusted to a density of 10^9 CFU/mL. Filter paper discs with a diameter of 5 mm were soaked in the bacterial suspension for 2 minutes, air-dried, embedded on the surface of Nutrient Agar (NA) medium in a Petri dish, and incubated for 24 hours at room temperature. The Petri dish was then inverted, and 1 mL of chloroform was dripped into the lid of the Petri dish and incubated for 1 hour to wipe out the bacterial colonies on the NA medium. The surface of the bacterial culture was atomized with a suspension of the pathogenic bacteria *X. campestris* at a density of 10^9 CFU/mL and then incubated for 3 days at room temperature. The inhibition zone, in the form of a clear area, was then measured by the following formula:

$$I = \frac{(\text{clear zone (cm)} - \text{paper disc diameter (cm)})}{\text{paper disc diameter (cm)}} \quad \ldots\ldots\ldots\ldots (1)$$

Where: I= inhibition zone

The inhibition index was determined using the scale in Table 1.

Table 1. Inhibition index scale

Inhibition Index (IP)	Inhibition zone (cm)
+	<0.50
++	0.51-1.00
+++	1.01-1.50
++++	1.51-2.00
+++++	>2.00

Indole Acetic Acid (IAA) production

Bacteria were cultured in Nutrient Broth (NB) medium with shaking and harvested after 24 h. Totally 1.5 mL of sample culture broth was transferred to an Eppendorf tube and centrifuged at 16,278 × *g* for 5 min using a microcentrifuge. One mL of supernatant was then transferred to a new test tube, mixed with an equal volume (1 mL) of Salkowski reagent, vortexed gently, and incubated at 30°C in a dark condition for 30 min. The presence of IAA was detected by measuring pink or red color development after 30 min. The color intensity was spectrophotometrically measured at 536 nm using a cuvette, and the uninoculated medium was set as the blank. The optical density of the test sample was compared with a standard IAA curve (10-100 g/mL) to calculate the concentration (Gang et al. 2019).

Phosphate solubilization assay

One ose of bacterial culture was suspended in sterile distilled water aseptically. A 5 mm-diameter filter paper disc was dipped into the bacterial suspension for 2 minutes and then air-dried in a laminar air flow cabinet (LAFC). The filter paper was then embedded on the surface of the Pikovskaya medium and incubated for 48 hours at room temperature. Phosphate solubilization activity was positive when a clear zone was formed around the bacteria growing on the filter paper (Kumar et al. 2020).

Nitrogen fixation assay

The assay was conducted using the streak plate method on Burk selective medium. One ose of bacterial suspension in sterile distilled water was streaked on the surface of the medium. The bacteria were then incubated for 48 hours at room temperature. N fixation activity was determined to be positive when bacterial colonies grew on Burk's medium (Chakraborty et al. 2019).

Bacterial tolerance assay against abiotic stress

In the tolerance assay of bacteria to pH stress, NB media with pH 4, 5, and 6 were used. One ose of the bacterial suspension was dipped into NB medium in a sterile test tube and then incubated for 48 hours. Bacteria are determined to be tolerant to an acidic pH when the bacteria grow with the indication that the NB medium turns cloudy. A tolerance assay of bacteria against high temperatures was conducted by growing bacteria on NB medium in sterile tubes, which were grown at 30-60°C for 3 days. Bacteria are determined to be tolerant of extreme temperatures if they can grow at a temperature of 60°C, which is indicated by the turbidity of the medium. A tolerance assay of bacteria to salinity stress was conducted by growing the bacteria on the surface of the NA medium containing NaCl with final concentrations of 5% and 10%. Bacteria are determined to be tolerant to salinity when the bacterial colonies can grow on the NA medium. The tolerance assay of bacteria to drought stress was conducted by growing the bacteria on NA medium containing polyethylene glycol (PEG) with a final concentration of 5%. Bacteria that can grow on NA+PEG 5% medium are determined to be tolerant of drought.

RESULTS AND DISCUSSION

Molecular identification

PCR amplification of the 16S rRNA gene of UB Forest indigenous bacteria using universal primers produced amplicon DNA bands with a length of approximately 1200 bp. After sequencing, the 16S rRNA DNA gene sequence data was subjected to homology analysis with BLAST-N software for identification. The results of molecular identification showed that most of the UB Forest indigenous bacterial strains belonged to the genus *Pseudomonas*. The other genera were *Enterococcus* and *Lysinibacillus* (Table 2).

The results of molecular identification showed that the UB Forest indigenous bacterial strains were dominated by the *Pseudomonads*, namely *P. versuta* UB-36, *P. aeruginosa* UB-52, *P. lundensis* UB-53, *P. migulae* UB-54, and *P. koreensis* UB-62. These results were in line with the previous study carried out by Li et al. (2021) that found 67 PGPB from the rhizosphere were dominated by the genera *Pseudomonas* and *Serratia*. The genus *Pseudomonas*, especially those belonging to the fluorescent group, have been largely known to play a role as PGPs in plants. Several *Pseudomonas* strains are also known to dissolve phosphate by secreting organic acids and protons to dissolve tricalcium phosphate in the medium (Li et al. 2021).

Phylogenetic tree analysis showed that bacterial strain UB-36 was in the same cluster with *P. versuta*, UB-52 was in the same cluster with *P. aeruginosa*, UB-53 was in the same cluster with *P. lundensis*, UB-54 was in the same cluster with *P. migulae*, UB-55 was in the same cluster with *E. gallinarum*, UB-62 was in the same cluster with *P. koreensis*, and UB-64 was in the same cluster with *L. fusiformis* (Figure 1).

Table 2. Similarity index of UB Forest indigenous bacterial strains as a result of BLAST-N analysis

Strain	Nearest Hit Species	Accesion Number	Similarity (%)
UB-36	*Pseudomonas versuta*	MN559449.1	93.69
UB-52	*Pseudomonas aeruginosa*	CP0533587.1	99.61
UB-53	*Pseudomonas lundensis*	MN746219.1	99.44
UB-54	*Pseudomonas migulae*	MT033062.1	99.68
UB-55	*Enterococcus gallinarum*	CP046307.1	99.61
UB-62	*Pseudomonas koreensis*	MN602520.1	99.27
UB-64	*Lysinibacillus fusiformis*	CP010820.1	98.50

In vitro antagonistic activity against *X. campestris*

The results of the antagonistic assay showed that out of the 7 bacterial strains, only 3 had the ability to inhibit *X. campestris* (Table 3). *P. aeruginosa* UB-52, *P. migulae* UB-54, and *E. gallinarum* UB-55 were able to inhibit the growth of *X. campestris* by forming clear zones around the colonies on the NA medium (Figure 2). The clear zone produced by the bacteria was formed by the release of an antibiosis compound that is toxic to the growth of *X. campestris*. Higher inhibition among the three bacteria was shown in *P. migulae* strain UB-54.

Table 3. Inhibition of UB Forest indigenous bacterial strains against *X. campestris*

Bacterial strain	Inhibition Index (I)	Inhibition diameter (mm)
P. aeruginosa UB-52	+	0.43
P. migulae UB-54	+	0.49
E. gallinarum UB-55	+	0.40

Note: +: inhibition diamater <0.50; ++: inhibition diameter 0.51-1.00; +++: inhibition diameter 1.01-1.50; ++++: inhibition diameter 1.51-2.00; +++++: inhibition diameter >2.00

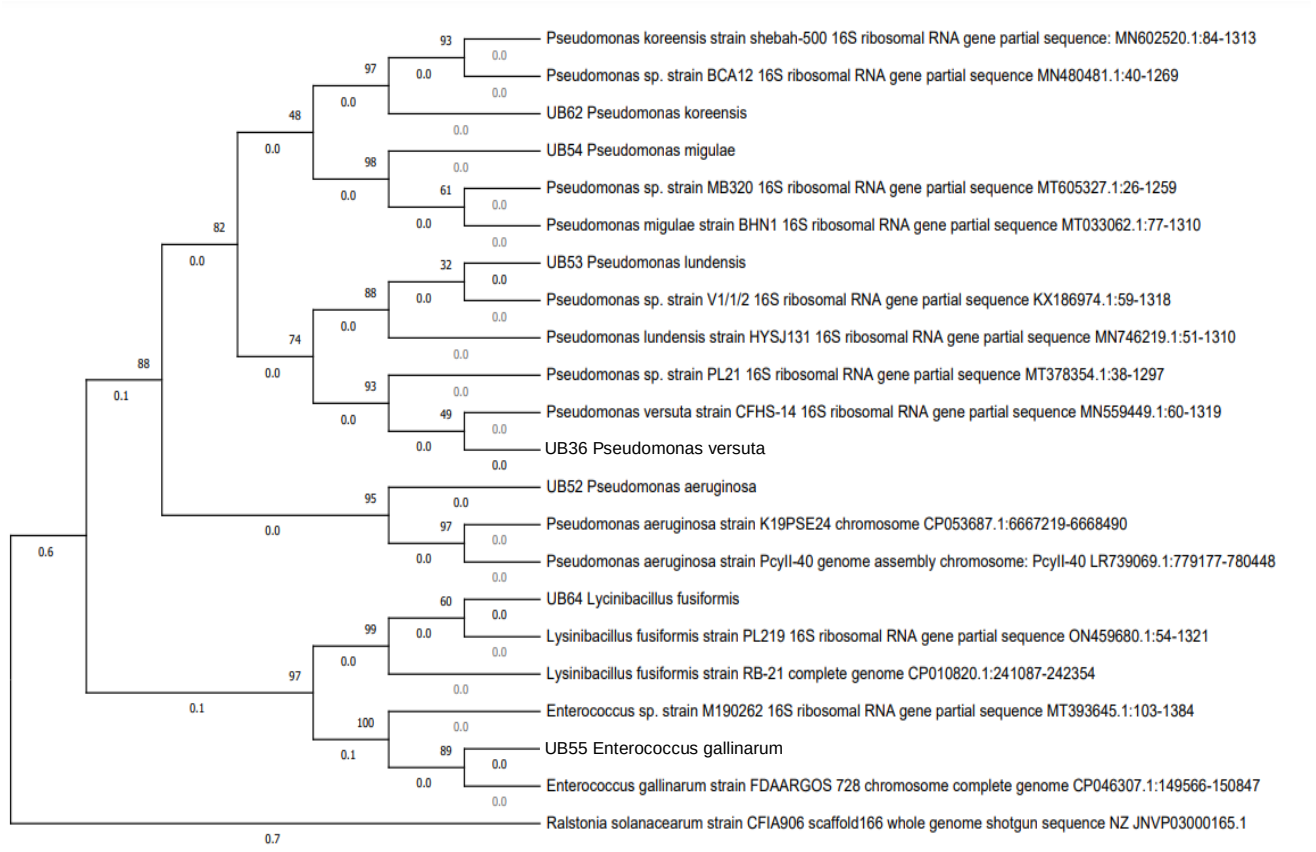
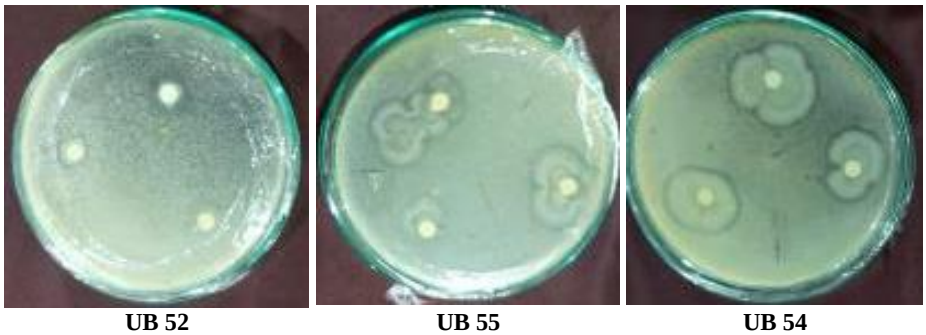


Figure 1. Phylogenetic tree of UB Forest indigenous bacterial strains and reference sequences from GenBank



UB 52 **UB 55** **UB 54**

Figure 2. PGPB inhibition against *X. campestris* in Petri dishes. Bacteria inhibiting the growth of *X. campestris* are indicated by clear zones on surrounding colonies

Pseudomonas spp. has been widely known as a biocontrol agent in plant disease suppression, where one of its mechanisms has the ability to produce antibiosis compounds (Haas and Défago 2005; Raaijmakers et al. 2002; Raaijmakers and Mazzola 2012). One of them is a *P. aeruginosa* subgroup strain that has been known to produce an arsenal of secondary metabolites with broad antimicrobial activity (Höfte 2021). Whereas *P. migulae* strains had previously been found in water or soil samples in mining areas, such as in the oil-contaminated rhizosphere of *Galega orientalis* in Finland (Jussila et al. 2006) and in a selenium mining area in southwest China (Li et al. 2015). *Pseudomonas migulae* has been involved in selenite reduction and has a large number of genes encoding resistance to copper and antibiotics (Li et al. 2015). Also, Beltrán-Acosta et al. (2022) reported that *P. migulae* Pf014 was able to increase the growth of cape gooseberry. In this study, *P. migulae* in UB-54 was able to inhibit the growth of *X. campestris* by releasing antibiosis compounds, although the type of antibiosis compounds is still unknown. These results have shown new insights into the role of *P. migulae* as a biocontrol agent, especially against *X. campestris*. Thus, from this study, it can be suggested that *P. aeruginosa* strains UB-52 and *P. migulae* strains UB-54 have the potential to be PGPBs, which indirectly support plant growth by inhibiting pathogens and preventing negative effects on plant health.

Plant Growth Promoting (PGP) activities

The results showed that all UB Forest indigenous bacteria were able to produce IAA, and the concentration of IAA varied in each bacterium (Table 4). The highest concentration of IAA was found in *Lysinibacillus fusiformis* UB-64 at 9.92 ppm. IAA production by bacteria varies due to environmental factors, growth rate, availability of amino acids, and sources of N. IAA is the main auxin in plants involved in cell enlargement and division, tissue differentiation, and physiological processes. IAA produced by PGPB could increase plant growth and development, such as cell elongation, cell expansion, and cell division (Cao et al. 2023). IAA-producing bacteria can influence the growth process, starting with the amount of IAA produced and tissue sensitivity to changes in IAA concentration. Examples of endophytic bacteria capable of producing significant IAA hormones are *Bacillus cereus* and *Pseudomonas putida* (Herlina et al. 2017).

Table 4. PGP activities of UB Forest bacteria

Bacterial strains	IAA production (ppm)	Phosphate solubilization	Nitrogen fixation
P. versuta UB-36	1.42	-	+
P. aeruginosa UB-52	2.87	+	+
P. lundensis UB-53	1.34	-	+
P. migulae UB-54	2.45	+	+
E. gallinarum UB-55	6.59	+	+
P. koreensis UB-62	5.75	+	-
L. fusiformis UB-64	9.92	+	+

Note: (+) indicates bacteria can solubilize phosphate or fixing nitrogen; (-) indicates bacteria are unable to solubilize phosphate and fixing nitrogen

Two PGP traits of UB Forest indigenous bacteria as a biofertilizer were tested through their activity in fixing nitrogen and solubilizing phosphate. The capacity of the bacteria to solubilize phosphate was determined by the clear zone around the colonies formed on the Pikovskaya medium. While the traits of the bacteria in N fixation were observed from their ability to grow on Burk's medium, The results of the PGP assay showed that 5 bacteria were able to solubilize phosphate, 6 bacteria were able to fix nitrogen, and 4 bacteria had both activities (Table 4). These results indicate that the UB Forest indigenous bacterial strains used in this study could increase plant growth by providing P nutrients and/or fixing N from the air. Therefore, inoculation of plants with PGPB is expected to increase nitrogen and phosphate uptake, resulting in higher plant growth and productivity. The results of this study also showed that four bacterial strains, i.e., *P. aeruginosa* UB-52, *P. migulae* UB-54, *E. gallinarum* UB-55, and *L. fusiformis* UB-64, were able to both solubilize phosphate and fix nitrogen. This is in accordance with a study by Li et al. (2021), which found that PGPB with the dual traits of fixing nitrogen and solubilizing phosphate showed significant growth-promoting effects on cucumber, corn, alfalfa, and oat.

Bacteria can fix nitrogen because they possess nitrogenase, a conserved enzyme of the two metalloproteins FeMO-protein and Fe-protein, which captures N2 from the air and converts it into ammonium, which can be utilized by plants (Souza et al. 2015). Whereas, phosphate solubilizing bacteria dissolve inorganic phosphates such as Ca2(PO4)2, FePO4, and AlPO4 through the production of organic acids, siderophores, and hydroxyl ions. The clear zone surrounding the colonies shows the ability of bacteria to dissolve phosphate in the Pikovskaya selective medium containing insoluble phosphate. The capacity of bacteria to dissolve phosphate differs depending on their ability to produce organic acids (Rossi et al. 2021). Solubilization of phosphate from minerals occurs through the secretion of organic acids, the release of protons, or the production of chelating agents. Whereas, mineralization of organic phosphorus occurs through the synthesis of phosphomono-esterase, phosphodiesterase, and phosphotriesterase, which can catalyze the hydrolysis of phosphorus esters (Ajijah et al. 2023).

Tolerance of UB Forest indigenous bacteria against abiotic stress

The tolerance of UB Forest indigenous bacterial strains to abiotic stress can be observed through their ability to grow in culture medium adjusted to a range of pH, salinity, and drought. The results showed that all bacteria can grow at pH 5-6, salinity 5-15%, and 15% PEG drought stress medium (Table 5). These results indicated that all selected UB Forest indigenous bacterial strains used in this study can survive in abiotic stress conditions such as low pH, high salinity, and drought. Thus, the application of these bacterial strains on plants has the potential to support plant growth and tolerance to abiotic stress conditions in the form of changes in pH, salinity, and drought.

Table 5. Tolerance of UB Forest indigenous bacteria against abiotic stress

Bacterial strains	pH			Salinity (%)			Drought	Temperature
	4	5	6	5	10	15	15%	60°C
P. versuta UB-36	-	+	+	+	+	+	+	+
P. aeruginosa UB-52	-	+	+	+	+	+	+	+
P. lundensis UB-53	-	+	+	+	+	+	+	+
P. migulae UB-54	-	+	+	+	+	+	+	+
E. gallinarum UB-55	-	+	+	+	+	+	+	+
P. koreensis UB-62	-	+	+	+	+	+	+	+
L. fusiformis UB-64	-	+	+	+	+	+	+	+

Note: (+) indicates bacteria are tolerant to pH, salinity, drought, and temperature in a modified medium; (-) indicates bacteria are not tolerant to pH, salinity, drought, and temperature in a modified medium

Abiotic stresses such as pH, salinity, drought, and temperature inhibit plant growth and result in yield loss. Inoculation of abiotic stress-tolerant bacterial strains in plants has been widely known to increase the growth and yield of plants grown in soil or media with abiotic stress conditions by increasing plant tolerance to abiotic stresses (Ajijah et al. 2023). For example, Patel et al. (2017) found that of 67 bacterial isolates from the rhizosphere of *Commiphora wightii*, 29 isolates (43.0%) were able to grow on 15 gram NaCl/mL medium, 40 isolates (58.20%) were tolerant to temperatures up to 70°C, and 32 isolates (47.8%) were tolerant to PEG 13 gram/100 mL. Those abiotic stress-tolerant PGPBs were then observed to have plant growth-promoting characteristics by producing IAA, fixing atmospheric nitrogen, and dissolving phosphate and potassium in a saline medium (Patel et al. 2017).

PGPB has several mechanisms to mitigate salt stress, one of which is being able to produce osmolytes to maintain cell turgidity and metabolism under unfavorable conditions. In addition, some PGPBs can maintain the Na^+/K^+ ratio through the secretion of exopolysaccharide (EPS) extracellular polymeric substances. Under salinity stress, EPS can chelate various cations, including NA^+, which reduces the toxicity of saline in the soil. It is already known that plants in a saline environment have a higher EPS-producing population in the rhizosphere zone to reduce NA^+ concentrations, thereby reducing the effect of salt stress (Shultana et al. 2020). Another study found that sunflower inoculated with salt-tolerant and biofilm-forming strains of *Pseudomonas plecoglossicida* could resist salt stress better than non-inoculated sunflower and increase in dry mass, photosynthetic pigment, gas exchange activity, and nutrient uptake (Ajijah et al. 2023). PGPB-inoculated plants can also decrease membrane damage through an antioxidant response that can counteract the excess production of reactive oxygen species (ROS) due to salinity. It is known that ROS cause depolarization of cell membranes, destabilize membranes, and cause cell death due to increased cytoplasmic C^{2+} concentration (Rossi et al. 2021). Several studies have also shown that plant inoculation with halotolerant bacterial strains is an alternative to improve the health of salt-sensitive plant cultivars (Aini et al. 2022; Aini et al. 2021, 2023).

Other studies have shown that PGPR can tolerate a pH range of 5-9 and increase the rate of seed germination. Bacteria with a broad pH tolerance can be used to increase plant growth and yield. These bacteria stimulate plant growth through various mechanisms at varying soil pHs (Kumar et al. 2019). In addition, it is known that biofilms produced by bacteria can provide protection, increase the potential for the accumulation of nutrients, increase tolerance to antimicrobial agents, and survive in adverse environments. PGPB can survive in a biofilm matrix resistant to microbes, drought, UV light, environmental stress, and osmotic stress (Ajijah et al. 2023).

Previously, we proposed the hypothesis that tropical forests such as the UB Forest have microbial germplasm, especially bacteria, that can reduce abiotic stress since tropical forests are mostly exposed to abiotic stress throughout their lives (Harfouche et al. 2014). The ability of trees in tropical forests to survive against abiotic stress is thought to be supported by stress-tolerant bacteria associated with plants. In this study, we have provided evidence that several species of bacteria from UB Forest are tolerant to abiotic stress and can produce IAA hormones and provide nutrients for plants. Although we have not tested the ability of these bacteria to protect plants from abiotic stress in vivo, at least their ability to survive under stress conditions of low pH, high temperature, and high salinity indicates that these bacteria have potential as bioprotectants against abiotic stress. However, the ability of bacteria to protect plants against abiotic stress needs to be tested further in vivo in greenhouses or the field.

This study also showed that various bacterial species from the genera *Pseudomonas*, *Enterococcus*, and *Lysinibacillus* have been found to provide insight into the richness of biodiversity found in UB Forest. Taken together, our results showed that the UB Forest indigenous bacterial strains were tolerant to pH, salinity, drought, and temperature stress; hence, they are expected as PGPB to support the growth of plants under abiotic stress conditions that occur in the field, in pots, or hydroponic greenhouses.

In conclusion, the results of molecular identification showed that the UB Forest indigenous bacterial strains were dominated by the *Pseudomonads*, namely *Pseudomonas versuta* UB-36, *P. aeruginosa* UB-52, *P. lundensis* UB-53, *P. migulae* UB-54, and *P. koreensis* UB-62. Other strains were *Enterococcus gallinarum* UB-55 and *Lysinibacillus fusiformis* UB-64. Of the 7 bacterial strains, only 3 had the ability to inhibit *X. campestris.* All bacteria

were able to produce IAA, whereas 5 bacteria showed the ability to solubilize phosphate, 6 bacteria to fix nitrogen, while 4 bacteria had both the activities. All bacterial strains can grow at pH 5-6, salinity 5-15%, and 15% PEG drought stress media. Taken together, our results showed that the UB Forest indigenous bacterial strains had traits as plant growth-promoting bacteria (PGPB) and are expected to support the growth of plants grown under biotic and abiotic stress conditions that occur in the field, in pots, or hydroponic greenhouses.

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REFERENCES

Aini LQ, Aini N, Yamika WSD, Setiawan A. 2022. Screening of plant growth-promoting halotolerant bacteria isolated from weeds rhizosphere grown in saline soil. Agrivita J Agric Sci 44: 322-331. DOI: 10.17503/agrivita.v44i2.3756.

Aini N, Yamika WSD, Aini LQ, Kurniawan AP. 2021. Application of saline tolerant bacteria and soil ameliorants improved growth, yield and nutrient uptake of tomato in saline land. Aust J Crop Sci 15: 827-34. DOI: 10.21475/ajcs.21.15.06.p2903.

Aini N, Yamika WSD, Kurniasari N, Nugroho A, Aini LQ. 2023. The effect of halotolerant bacteria isolated from saline soil on growth and yield of maize in saline soil. J Degraded Mining Lands Manag 10: 4341-4347. DOI: 10.15243/jdmlm.2023.103.4341.

Ajijah N, Fiodor A, Pandey AK, Rana A. 2023. Plant growth-promoting bacteria (PGPB) with biofilm-forming ability: A multifaceted agent for sustainable agriculture. Diversity 15: 1-21. DOI: 10.3390/d15010112.

Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K. 1992. Current protocols in molecular biology. Greene Publishing Association, Wiley-Interscience, New York.

Azzahra AK, Yusuf M, Arisoesilaningsih E. 2018. Characteristic of epiphytes host trees in protected area of UB forest. In: Siswanto D, Mastuti R, Zaman F, Treesubsuntom C (eds.) The 9th International Conference on Global Resource Conservation (ICGRC) and AJI from Ritsumeikan University. Brawijaya University, Malang, 7-8 March 2018. DOI: 10.1063/1.5061888. [Indonesian]

Baldrian P. 2017. Forest microbiome: diversity, complexity and dynamics. Fed Eur Microbiol Soc-Microbiol Rev 41 (2): 109-130. DOI: 10.1093/femsre/fuw040.

Beltrán-Acosta CR, Zapata-Narváez YA, Millán-Montaño DA, Díaz-García A. 2022. Efecto de *Bacillus amyloliquefaciens* y *Pseudomonas migulae* sobre el crecimiento de plántulas de uchuva (*Physalis peruviana* L.) en semillero. Agron Mesoamericana 34: 50669. DOI: 10.15517/am.v34i1.50669.

Brockerhoff EG, Barbaro L, Castagneyrol B, Forrester DI, Gardiner B, Olabarria JRG, Lyver POB, Oxbrough NMA, Taki, H, Thompson ID, Plas FV, Jactel H. 2017. Forest biodiversity, ecosytem functioning and the provision of ecosystem services. Biodivers Conserv 26: 3005-3035. DOI: 10.1007/s10531-017-1453-2.

Cao M, Narayanan M, Shi X, Chen X, Li Z, Ma Y. 2023. Optimistic contributions of plant growth-promoting bacteria for sustainable agriculture and climate stress alleviation. Environ Res 217: 114924. DOI: 10.1016/j.envres.2022.114924.

Chakraborty P, Sarker RK, Roy R, Ghosh A, Maiti D, Tribedi P. 2019. Bioaugmentation of soil with Enterobacter cloacae AKS7 enhances soil nitrogen content and boosts soil microbial functional-diversity. 3 Biotech 9: 1-10. DOI: 10.1007/s13205-019-1791-8.

Dinata GF, Aini LQ, Abadi AL. 2021. Pengaruh pemberian plant growth-promoting bacteria indigenous terhadap pertumbuhan tanaman bawang merah (*Allium ascalonicum*). In Agropross: National Conference Proceedings of Agriculture. DOI: 10.25047/agropross.2021.231. [Indonesian]

Fauzul IJ, Qurata AL, Rizkyta KR. 2018. Pemanfaatan rhizobakteri dari gulma di UB forest sebagai agen antagonis penyakit layu bakteri pada kentang. Biotropika J Trop Biol 6 (2): 54-63. DOI: 10.21776/ub.biotropika.2018.006.02.03. [Indonesian]

Foyer CH, Lam H-M, Nguyen HT, Siddique KHM, Varshney RK, Colmer TD, and Cowling W, Bramley H, MOri TA, Hodgson JM, Cooper JW, Miller AJ, Kunert K, Vorster J, Cullis C, Ozga JA, Wahlqvist ML, Liang Y, Shou H, Shi K, Yu J, Fodor N, Kaiser BN, Wong F-L, Valliyodan B, Considine MJ. 2016. Neglecting legumes has compromised human health and sustainable food production. Nat Plants 2: 16112. DOI: 10.1038/nplants.2016.112.

Gang S, Sharma S, Saraf M, Buck M, Schumacher J. 2019. Analysis of indole-3-acetic acid (IAA) production in *Klebsiella* by LC-MS/MS and the salkowski method. Biol Protocol 9 (9): e3030. DOI: 10.21769/BioProtoc.3230.

Glick BR. 2012. Plant growth-promoting bacteria: Mechanisms and applications. Scientifica (Cairo) 2012: 963401. DOI: 10.6064/2012/963401.

Gull A, Lone AA, Wani NUI. 2019. Biotic and abiotic stresses in plants. Edited by Alexandre Bosco de Oliveira. Rijeka: IntechOpen. DOI: 10.5772/intechopen.85832.

Haas D, Défago G. 2005. Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3: 307-319. DOI: 10.1038/nrmicro1129.

Harfouche A, Meilan R, Altman A. 2014. Molecular and physiological responses to abiotic stress in forest trees and their relevance to tree improvement. Tree Physiol 34 (11): 1181-1198. DOI: 10.1093/treephys/tpu012.

Herlina L, Pukan KK, Mustikaningtyas D. 2017. The endophytic bacteria producing IAA (indole acetic acid) in *Arachis hypogaea*. Cell Biol Dev 1 (1): 31-35. DOI: 10.13057/cellbioldev/v010106.

Höfte M. 2021. The use of *Pseudomonas* spp. as bacterial biocontrol agents to control plant disease. In Microbial bioprotectants for plant disease management. Burleigh Dodds. DOI: 10.19103/AS.2021.0093.11.

Ibort P, Molina S, Nunez R, Zamarreno AM, Mina JMG, Lozano JMR, Mosqueda MDCO, Click BR, Aroca R. 2017. Tomato ethylene sensitivity determines interaction with plant growth-promoting bacteria. Ann Bot 120 (1): 101-122. DOI: 10.1093/aob/mcx052.

Jussila MM, Jurgens G, Lindström K, Suominen L. 2006. Genetic diversity of culturable bacteria in oil-contaminated rhizosphere of Galega orientalis. Environ Pollut 139 (2): 244-57. DOI: 10.1016/j.envpol.2005.05.013.

Karina N, Roekhan A, Diah AFC, Qurata AL. 2020. The potency of UB forest bacteria as chitinolytic bacteria to inhibit anthracnose disease on cayenne pepper. Res J Life Sci 7 (1): 41-51. DOI: 10.21776/ub.rjls.2020.007.01.5.

Kawaguchi A, Inoue K, Ichinose Y. 2008. Biological control of crown gall of grapevine, rose, and tomato by nonpathogenic *Agrobacterium vitis* strain var03-1. Phytopathology 98 (11): 1218-1225. DOI: 10.1094/PHYTO-98-11-1218.

Kumar A, Kumari M, Swarupa P, Shireen. 2019. Characterization of pH dependent growth response of agriculturally important microbes for development of plant growth promoting bacterial consortium. J Pure Appl Microbiol 13 (2): 1053-1061. DOI: 10.22207/JPAM.13.2.43.

Kumar P, Aeron A, Shaw N, Singh A, Bajpai VK, Pant S, Dubey RC. 2020. Seed bio-priming with tri-species consortia of phosphate solubilizing rhizobacteria (PSR) and its effect on plant growth promotion. Heliyon 6 (12): e05701. DOI: 10.1016/j.heliyon.2020.e05701.

Kurniawan S, Utami SR, Mukharomah M, Navarette IA, Prasetya B. 2019. Land use systems, soil texture, control carbon and nitrogen storages in the forest soil of UB Forest, Indonesia. Agrivita J Agric Sci 41 (3): 416-427. DOI: 10.17503/agrivita.v41i3.2236.

Li M, Wang J, Yao T, Wang Z, Zhang H, Li C. 2021. Isolation and characterization of cold-adapted pgpb and their effect on plant growth promotion. J Microbiol Biotechnol 31 (9): 1218-30. DOI: 10.4014/jmb.2105.05012.

Li X, Kot W, Wang D, Zheng S, Wang G, Hansen LH, Rensing C. 2015. Draft genome sequence of Se(IV)-reducing bacterium *Pseudomonas migulae* ES3-33. Genome Announcements 3 (3): 10-1128. DOI: 10.1128/genomea.00406-15.

Patel S, Jinal HN, Amaresan N. 2017. Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. Biocatalysis Agric Biotechnol 12: 85-89. DOI: 10.1016/j.bcab.2017.09.002.

Raaijmakers JM, Mazzola M. 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. Ann Rev Phytopathol 50: 403-424. DOI: 10.1146/annurev-phyto-081211-172908.

Raaijmakers JM, Vlami M, Souza JT. 2002. Antibiotic production by bacterial biocontrol agents. Antonie Van Leeuwenhoek 81 (1-4): 537-47. DOI: 10.1023/A:1020501420831.

Radhapriya P, Ramachandran A, Palani P. 2018. In Indigenous plant growth-promoting bacteria enhance plant growth, biomass, and nutrient uptake in degraded forest plants. 3 Biotech 8: 154. DOI: 10.1007/s13205-018-1179-1.

Roekhan A, Dayanti AI, Oktaviani R, Shinta F, Anastasia NA, Aini LQ. 2021. The potency of UB forest pchitinolytic bacteria to Promote plant growth and inhibit damping off disease on soybean. Res J Life Sci 8 (1): 25-33. DOI: 10.21776/ub.rjls.2021.008.01.4.

Rossi M, Borromeo I, Capo C, Glick BR, Gallo MD, Pietrini F, Forni C. 2021. PGPB improve photosynthetic activity and tolerance to oxidative stress in *Brassica napus* grown on salinized soils. Appl Sci 11 (23): 11442. DOI: 10.3390/app112311442.

Shultana R, Kee Zuan AT, Yusop MR, Saud HM. 2020. Characterization of salt-tolerant plant growth-promoting rhizobacteria and the effect on growth and yield of saline-affected rice. Plos One 15: e0238537. DOI: 10.1371/journal.pone.0238537.

Souza RD, Ambrosini A, Passaglia LM. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. Genet Mol Biol 38 (4): 401-419. DOI: 10.1590/S1415-475738420150053.

Verma S, Nizam S, Verma PK. 2013. Biotic and abiotic stress signaling in plants. Stress Signaling in Plants: Genomics and Proteomics Perspective, Vol. 1: 25-49. DOI: 10.1007/978-1-4614-6372-6_2.

Viera M, Caldeira MVW, Rovani FF M, Castro KC. 2016. Ecological and environmental aspects of nutrient cycling in the atlantic forest Brazil. InTech, Croatia. DOI: 10.5772/64188.

Yasmin S, Zaka A, Imran A, Zahid MA, Yousaf S, Rasul G, Arif M, Mirza MS. 2016. Plant growth promotion and suppresion of bacteria blight in rice by inoculated bacteria. Plos One 11 (8): e0160688. DOI: 10.1371/journal.pone.0160688.