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Assessment of phosphate-solubilizing microbes isolated from indigenous organic-biofertilizers for enhancing plant growth in acid-stressed

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Abstract. *Kamaluddin NN, Irwandhi, Arum P, Setiawan Y, Khumairah FH, Prihatiningsih N, Simarmata T. 2025. Assessment of phosphate-solubilizing microbes isolated from indigenous organic-biofertilizers for enhancing plant growth in acid-stressed. Biodiversitas 26: 1325-1333.* Indonesia is one of the countries that produces enormous amounts of organic waste every year, but most of this organic waste has not been appropriately managed, thereby worsening climate change. One alternative solution that is environmentally friendly and sustainable is converting organic waste into Indigenous Organic Biofertilizers (IOB). This process involves the controlled decomposition of organic waste, which is then enriched with beneficial microbes, such as Phosphate Solubilizing Microbes (PSM). PSM from IOB can be a candidate for a biofertilizer inoculant that can withstand environmental stress, especially acidity stress caused by climate change. This research aimed to screen, characterize, and assess the biochemical activity of isolates and molecularly identify PSM from IOB that are resistant to acidity stress. In this research, characterization was carried out in the form of macroscopic and microscopic observations, resistance to acidity stress, phosphate dissolution index, production of Indole-3-Acetic Acid (IAA), compatibility test, and identification of the 16S rRNA gene. Bacterial isolates with codes F1ZN1, F2P, and F6P, while fungal isolates F4A, F5B, and F5C were the best PSM isolates. This isolate can produce IAA with a high category of around 57.28-105.94 ppm (bacteria) and a medium category of around 18.34-24.33 ppm (fungi). Apart from that, these isolates are compatible with the isolates to work synergistically. Through molecular identification of 16S rRNA sequencing, F1ZN1 was identified as *Enterobacter cloacae* strain Ec030, F2P was identified as *E. cloacae* strain UT25, and F6P was identified as *E. cloacae* strain ABRL064. These results highlight the potential of PSM isolates from IOB as an effective biofertilizer inoculant candidate for increasing plant growth in environmental stress (acidity) amidst climate change.

Keywords: Acidic soils, *Enterobacter cloacae*, phosphate solubilizing bacteria, phosphate solubilizing fungi, sustainable agriculture

INTRODUCTION

Indonesia is one of the countries that produces large amounts of waste every year, but national waste management is still not optimal. In 2024, the amount of national waste in Indonesia will reach 20.45 million tons per year and around 40.96% (8.38 million tons per year) of these waste piles will be poorly managed (KLH 2025). As much as 70% of Indonesia's waste is organic, most piled up in landfills (Bappenas 2021). This condition will contribute to the formation of greenhouse gas emissions such as methane. The methane formed can trap heat 28 times stronger than carbon dioxide (Waluyo and Kharisma 2023). Poor waste management will encourage air and air pollution, land degradation, and climate change (Abubakar et al. 2022). The importance of efforts to overcome the problem of organic waste is in line with global efforts to implement circular economic principles in the agricultural sector. This principle is a condition where organic waste is reused into valuable products, such as the development of indigenous organic-biofertilizers (Kumar and Gopal 2015).

Indigenous Organic-Biofertilizer (IOB) is a fermented fertilizer made from organic materials that contain many microbes that stimulate plant growth (Roeswitawati et al. 2018). This fertilizer consists of various microbes that can function as decomposers, biopesticides, and biofertilizers. IOB can fertilize the soil and stimulate plant growth (Retnowati and Katili 2021). Currently, there has been a lot of research on the ability of IOB to support the growth of various plants such as lettuce, kale, and broccoli (Roeswitawati et al. 2018; Pane and Marwazi 2020; Yuliana 2021). IOB fertilizer works by increasing the availability of organic matter, nitrogen, phosphorus, and potassium (Irwandhi et al. 2024a). Previous studies have shown that IOB based on organic plant and animal waste contains high macronutrients such as organic carbon (14.80%), nitrogen (4.07%), phosphorus (0.03%), and potassium (0.08%) (Irwandhi et al. 2025). In addition, IOB also contains many beneficial microbes such as *Azospirillum* sp., *Bacillus* sp.,

Aspergillus, and *Sclerotium* which are included in the group of phosphate-solubilizing microbes (Whitelaw 1999; Retnowati and Katili 2021).

Phosphate Solubilizing Microbes (PSM) is a group of bacteria and fungi that can convert unavailable phosphate into available phosphate (Rawat et al. 2020). PSM can produce organic acids and phosphatases that convert insoluble phosphate into soluble phosphate so plants can absorb it (Hii et al. 2020). In addition, PSM can also produce phytohormones such as Indole-3-Acetic Acid (IAA) (Wang et al. 2023). The results of previous studies showed that PSM strains isolated from IOB can produce IAA around 10.59-19.41 ppm (Sodiq et al. 2021). *Bacillus*, *Pseudomonas*, and *Enterobacter* are the most significant Phosphate-Solubilizing Bacteria (PSB) in dissolving phosphate. In contrast, the Phosphate-Solubilizing Fungi (FPS) groups are *Aspergillus* and *Penicillium* (Tariq et al. 2022). PSM inoculant is an alternative technology that can maintain soil health and sustainable plant growth in conditions of phosphorus deficiency due to acidity stress on suboptimal land (Satyaprakash et al. 2017).

Currently, around 70% of the total land in Indonesia is acidic, with a pH of less than 5 (Sumiahadi and Acar 2019). In areas with high rainfall, this soil is susceptible to loss of organic matter and nutrients, resulting in soil degradation (Hindersah et al. 2022). This condition causes limited availability of nutrients such as phosphate (Shi et al. 2017). Phosphorus (P) in acidic soil will be bound by aluminum (Al) and manganese (Mn), making it unavailable to plants (Johan et al. 2021). Limited availability of P for acidic soil plants will inhibit root growth, delay flowering, and reduce yields (Rose et al. 2016; Wei et al. 2017; Hu et al. 2025). Given the potential and role of PSM isolates in maintaining soil health and enhancing plant growth, research on the isolation, characterization, and molecular identification of acid-tolerant PSMs from IOB formulations is very important. Exploring biofertilizer inoculant candidates that can withstand environmental stress, especially acidity stress caused by climate change, is very important. Climate change can increase soil acidity by around 21.43-45.52% (Sun et al. 2023). This study aimed to screen, characterize, and assess the biochemical activities of isolates and molecularly identify PSMs from IOB that are resistant to acidity stress.

MATERIALS AND METHODS

Fermentation of indigenous organic-biofertilizers

Fermentation activities for Indigenous Organic-Biofertilizers (IOB) formulas were carried out as in research by Irwandhi et al. (2025). Six formulas were developed based on organic waste in the form of vegetable, animal, and JAKABA (Eternal Lucky Mushroom) fertilizer. All ingredients used in each formula are put into a fermenter, and water is added to reach 30 L. The ingredients are mixed using a turbo mixer and fermented for 60 days. The fermentation results are then filtered using an 80-100 mesh sieve.

Microbial isolation of indigenous organic-biofertilizers

Microbes from IOB were isolated using serial dilution and the spread plate method. A total of 1 mL of IOB for each formula was dissolved in 9 mL of sterile water and vortexed. An aliquot of 1 mL was transferred using a sterile pipette into 9 mL of sterile water and vortexed for 10 seconds to obtain a 10^{-2} dilution. Serial dilutions were carried out up to 10^{-7} dilution, 1 mL aliquot of 10^{-3} dilution (fungal isolation), and 0.1 mL aliquot of 10^{-7} dilution (bacterial isolation) were inoculated on agar medium using the spread plate method. The agar medium used is nutrient agar (bacteria) and potato dextrose agar (fungus), which is then incubated at a temperature of 27-30°C for 7 days. Single colonies of microbes growing in each growth medium were observed and selected based on colony size. The single colony is then purified until a pure microbial colony is obtained.

Characterization of phosphate solubilizing microbes

After obtaining pure isolates, a characterization of each isolate, both bacteria and fungi, was carried out. Macroscopic observation of bacterial isolates was carried out by observing the colony shape and color. In contrast, the colony shape, colony color, radial lines, and texture were observed for fungal isolates. Apart from that, microscopic observations were also carried out in cell shape, Gram stain (bacteria), and conidia shape (fungus). PSM isolates were characterized by evaluation of the Phosphate Solubilization Index (PSI) and acidity stress (Guardiola-Márquez et al. 2023).

Phosphate solubilizing test

Tests for the ability of microbes to dissolve phosphate were carried through growth in selective Pikovskaya medium (Yeast extract 0.5 g; Dextrose/Glucose 10 g $Ca_3(PO_4)_2$ 5 g; KCl 0.2 g; $MgSO_4.7H_2O$ 0,1 g; Mn $SO_4.7H_2O$ 0.0001 g; Fe $SO_4.7H_2O$ 0.0001 g; $(NH_4)_2SO_4$ 0.5 g; Agar 15 g; topped with distilled water up to 1 L). Inoculation of bacterial and fungal isolates was carried out using an ose needle that was touched to each pure bacterial and fungal isolate. Then the tip of the ose needle was touched to the Pikosyaka medium by making a point (without scratching). Then, it is incubated at room temperature for 2 days (bacteria) and 3 days (fungi). The clear zone formed around the microbial colony shows the ability of the microbes to solubilize phosphate. Each microbial isolate's Phosphate Solubilization Index (PSI) was calculated based on the formula:

$$PSI = \frac{\text{Colony diameter} + \text{Clear zone diameter}}{\text{Colony diameter}}$$ (Pande et al. 2017)

Acidity stress test

Isolates were grown in a liquid medium with various pH in order to test their resistance towards acidity stress. Bacterial isolates were grown in Nutrient Broth (NB) medium, while fungal isolates were grown in Potato Dextrose Broth (PDB) medium, each with a pH of 4, 7, and 9. The bacterial culture was then incubated for 3 × 24 hours, while fungal cultures were incubated for 5 × 24 hours. Test results are interpreted as positive if the medium becomes cloudy and

negative if there is no change in the medium (Amalia et al. 2020).

Selection of phosphate-solubilizing microbial isolates

The results of observations of PSI and acidity stress for each PSM isolate were given a score. The ability of the isolate to dissolve P was given 1-9 points, with the isolate having the highest IPF getting 9 points. Stress resistance score was given based on changes in the color of the growth medium, such as cloudy (2 points), slightly cloudy (1 point), and clear/constant (0). After that, the points for all parameters were added, and the 3 best bacterial and fungal isolates with the highest points were selected, respectively (Ambarita et al. 2024).

Indole acetic acid production test

Each bacterial isolate was inoculated in 30 mL NB medium supplemented with sterile L-tryptophan (800 µg mL^{-1}). L-tryptophan was dissolved in distilled water and sterilized using a 0.22 µm membrane filter. Bacterial cultures were incubated using a shaker at 180 rpm and 30°C for 72 hours and then centrifuged at 5,000 rpm for 20 minutes. 150 µL of supernatant was mixed with 150 µL of Salkowski reagent and then incubated in dark conditions at 25°C for 30 minutes. The absorbance value at 530 nm was measured to detect the pink color and was repeated three times for each isolate. The fungal isolate was inoculated into 30 mL of PDB medium supplemented with sterile L-tryptophan (2 mg mL^{-1}) and incubated using a shaker at 130 rpm for 7 days. The rest of the procedure followed the same steps as performed for bacterial cultures (Guardiola-Márquez et al. 2023).

Compatibility test

The synergy ability between PSM isolates was seen by scratching each pure isolate on an NA medium in a petri dish so that the isolates met each other. Then, it was incubated, and observations were made at 3, 5, and 7 Days After Inoculation (DAI) through the presence of a clear, inhibitory zone around or in the intersection of the isolates. Isolates are assumed to be synergistic if no inhibition zone is observed in the area where the two isolates intersect, and they are said to be antagonistic if an inhibition zone is present in the area where the two isolates intersect (Khumairah et al. 2018).

Identification of bacteria based on 16S rRNA gene

A total of three selected PSM isolates were sent to PT. Genetic Science Indonesia (Bogor, West Java, Indonesia) for molecular identification. DNA extraction was done using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082). DNA amplification of bacterial isolates using the MyTaq HS Red Mix kit, 2X (Bioline, BIO-25048), and electrophoresis of the 16S Gene amplification product. DNA sequencing of isolates was carried out in two directions using the Sanger DNA Sequencing method using Capillary Electrophoresis. The bidirectional sequencing data were collected with BioEdit 7.2 software. Next, to identify the species, the nucleotide sequence of each isolate was analyzed using BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic tree construction of bacterial isolates was carried out by MEGA-11 software (Irwandhi et al. 2024b).

Data analysis

The characteristics of microbial isolates, including microbial morphology, resistance to acidity testing, phosphate solvent ability for IAA production, and microbial compatibility, were analyzed based on descriptive analysis. This data was used to determine the potential of microbes to support plant growth under humidity checks. DNA-sequencing characterization of PSM isolates was compared to data available in NCBI GenBank.

RESULTS AND DISCUSSION

Characteristics of phosphate solubilizing microbial isolates

The characterization of Phosphate Solubilizing Bacterial isolates (PSB) in Table 1 shows that each bacterial isolate has different characteristics. Macroscopic observations (Figure 1.A) showed that six bacterial isolates had circular colony shapes, while the other three had irregular colony shapes with predominantly white colony colors. Microscopic observation (Figure 1.B) showed that seven isolates had bacilli cell forms, while the other two had coccus forms. All bacterial isolates belonged to the Gram-negative bacteria group, which was indicated by red Gram staining results. Tripathi and Sapra (2021) stated that the Gram staining results showed that Gram-positive bacteria had purple bacterial cells, while Gram-negative bacteria had red bacterial cells. This difference occurs due to variations in the composition of bacterial cell walls. Gram-positive bacteria have a thick peptidoglycan layer, while Gram-negative bacteria have a thick lipid layer.

The characteristics of Phosphate-Solubilizing Fungal isolates (PSF) in Table 2 show that each has different characteristics. Macroscopic observation (Figure 1.C) shows that each isolate has a different color, such as white (5 isolates), gray (2 isolates), black (1 isolate), and gray-green (1 isolate), with four isolates having radial lines. Microscopic observation (Figure 1.D) showed that seven isolates had round conidia, oval conidia, and rod-shaped conidia.

The growth medium's turbidity level changes indicated the resistance of Phosphate-Solubilizing Microbial isolates (PSM) to acidity stress. All bacterial (Table 1 and Figure 2) and fungal isolates (Table 2 and Figure 3) demonstrated resistance to acidity stress. Notably, specific bacterial isolates (F1ZN1, F2P, F4Zn3, and F6P) showed higher resistance levels, highlighting the diversity of resistance levels among the isolates and surviving acidity stress (pH 4).

Microbes respond to acidity stress by increasing intracellular levels of glutamic acid, proline, cysteine, and alanine to maintain intracellular pH homeostasis through decarboxylation and alkali neutralization (Feng et al. 2021). Previous research showed that *Acidithiobacillus ferrivorans* isolated from acid mine drainage contain various transporter proteins that are used to adapt to low pH environments (Peng et al. 2017). This PSM capability significantly increases plant productivity on suboptimal acidic land (Rashid et al. 2016).

Table 1. Traits of phosphate-solubilizing bacterial isolates from indigenous organic-biofertilizers

Variable	F1ZN1	F2P	F2AAK	F3AAK	F4P	F4AAK	F4Zn3	F5AAK	F6P
Colony shape	Circular	Circular	Circular	Irregular	Circular	Circular	Irregular	Irregular	Circular
Colony color	White-yellow	White	White-yellow	Clear white	Cream	Cream	Orange	Clear white	Cream
Cell shape	Bacilli	Bacilli	Bacilli	Coccus	Bacilli	Coccus	Bacilli	Bacilli	Bacilli
Gram stain	-	-	-	-	-	-	-	-	-
pH tolerance									
4	++	++	+	+	+	+	++	+	++
7	++	++	++	++	++	++	++	++	++
9	++	++	+	++	+	+	++	++	++
Phosphate solubilizing index	4.00	4.67	1.00	2.20	3.67	3.33	3.00	2.67	4.67

Notes: The positive sign (+) in the pH tolerance test indicates microbial resistance to acidity stress, categorized as resistant (++) and somewhat resistant (+)

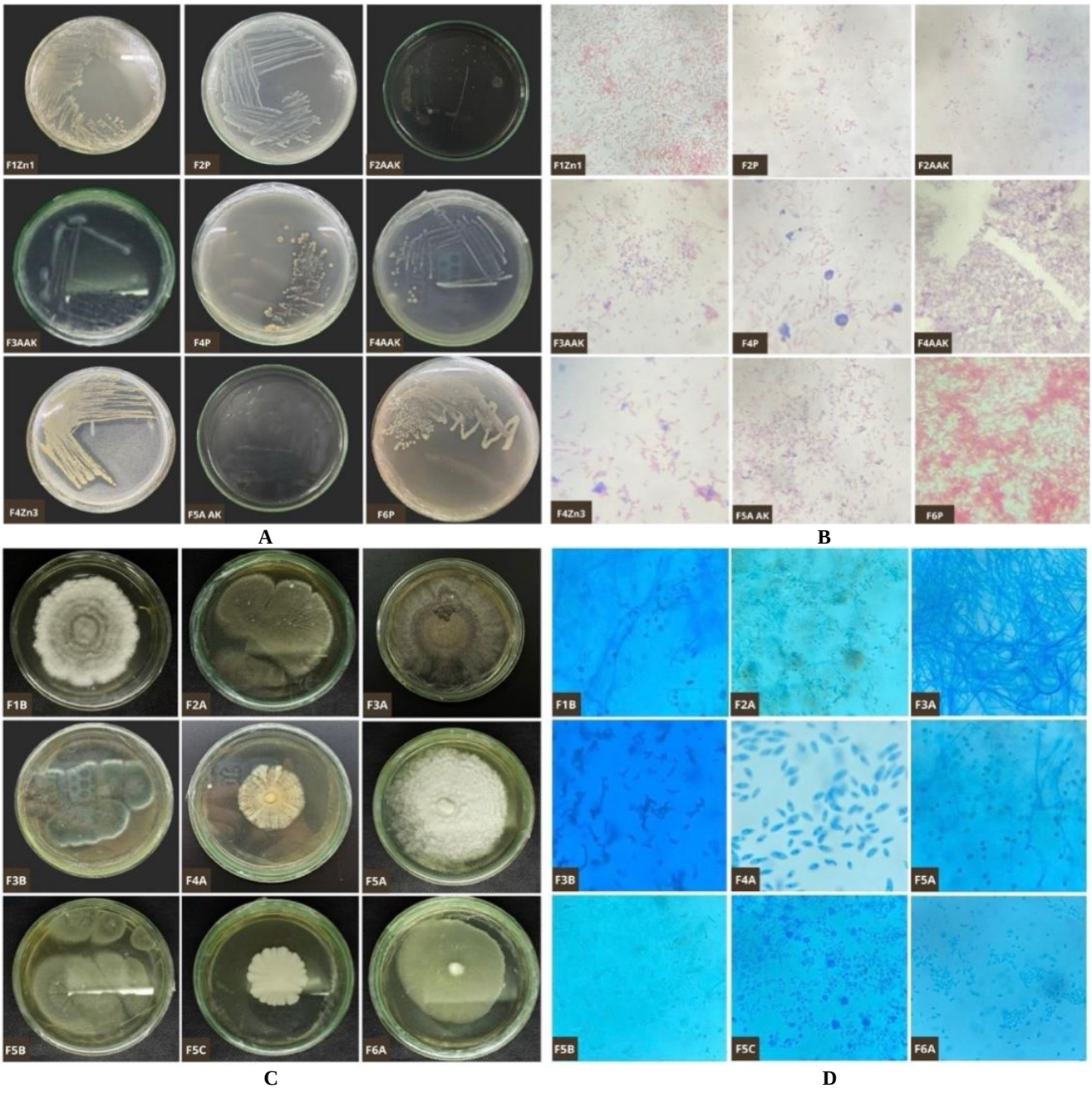

Figure 1. Macroscopic morphology of: A. Bacteria; and C. Fungi. Microscopic morphology of: B. Bacteria; and D. Fungi isolated from indigenous organic biofertilizers

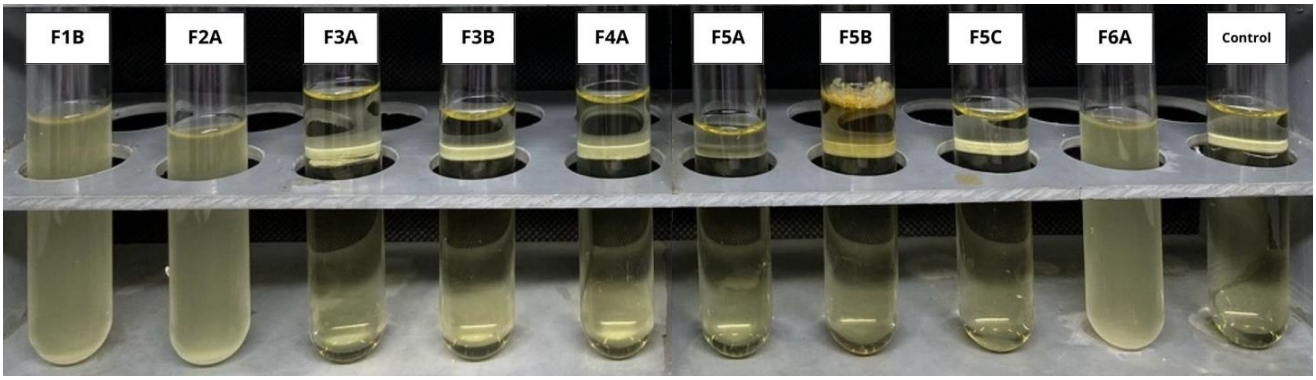

Figure 2. Acid stress tolerance of phosphate-solubilizing bacterial isolates at pH 4, assessed through changes in growth medium turbidity relative to the control

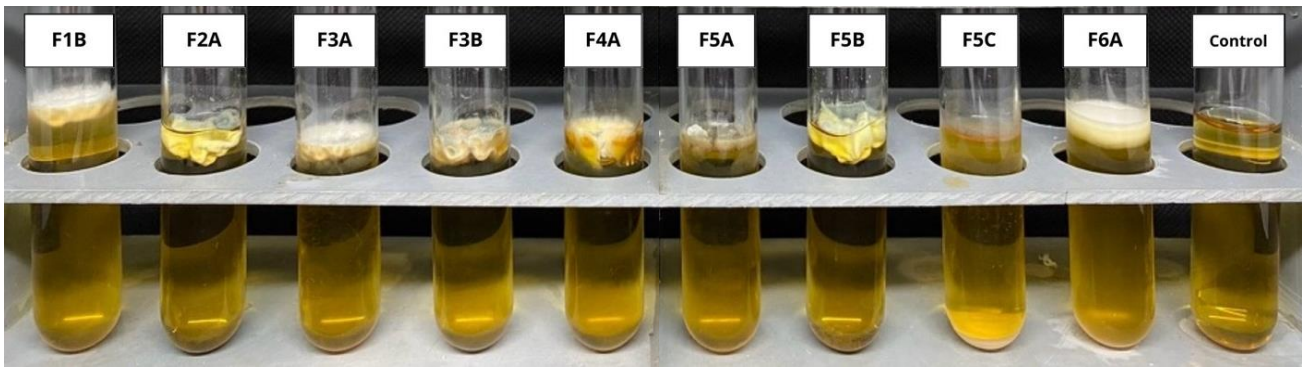

Figure 3. The resistance of phosphate-solubilizing fungal isolates to acidity stress (pH 4) is shown by the growth of fungal isolates in an acidic medium

Table 2. Traits of phosphate-solubilizing fungi isolate from indigenous organic-biofertilizers

Variable	F1B	F2A	F3A	F3B	F4A	F5A	F5B	F5C	F6A
Colony color	White	Gray	Gray	Black	White	White	Green-Grey	White	White
Radial lines	+	-	+	-	+	+	-	-	-
Texture	Cottony	Velvety	Filamentous	Powdery	Downy	Cottony	Velvety	Waxy	Downy
Conidia shape	Round	Round	Round	Round	Oval	Round	Round	Round	Rod
TP									
4	++	++	++	++	++	++	++	++	++
7	++	++	++	++	++	++	++	++	++
9	++	++	+	++	+	+	++	++	++
Phosphate solubilizing index	1.00	2.50	1.00	2.43	3.00	1.00	2.70	3.17	2.12

Notes: The positive sign (+) in the pH tolerance test indicates microbial resistance to acidity stress, categorized as resistant (++) and somewhat resistant (+)

PSM isolate can dissolve P with a Phosphate Solubilizing Index (PSI) value of 1.00-4.67 (bacteria) and 1.00-3.17 (fungi). The bacterial isolate F2AAK could not dissolve P, but other bacterial isolates showed the ability to dissolve P with the highest PSI for isolates F2P and F6P of 4.67. In fungal isolates, F1B, F3A, and F5A could not dissolve P, while other fungal isolates could solubilize P with the highest PSI in isolate F5C of 3.17. These results align with previous research, which showed that bacteria can dissolve P with a PSI of up to 2.87 (Fitriatin et al. 2024). This ability is related to organic acids produced by microbes, such as gluconic acid, citric acid, and oxalic acid. Low molecular organic acid plays an important role in the solubilization of phosphate bound to soil minerals (Sharma et al. 2013).

Selected phosphate solubilizing microbial isolates

The selection of PSM isolates as a candidate biofertilizer inoculant was based on the ability to solubilize P and the isolate's resistance to acidity stress at various pH levels (4, 7, and 9). This ranking aims to identify isolates with the most potential to increase phosphorus availability for plants

on suboptimal land. Based on the ranking results shown in Table 3, the three bacterial isolates with the highest scores are F6P, F2P, and F1Zn1. These three isolates show the best ability to dissolve P. They can survive in environments with different acidity levels, so they have a high potential for application in various soil conditions.

Based on the ranking results of fungal isolates, as in Table 4, the three isolates with the highest total scores are F5C, F4A, and F5B. This fungal isolate was chosen because it has a high capacity to solubilize P and shows tolerance to pH variations. Fungi can dissolve P because they can produce organic acids and convert unavailable P into a form that plants can absorb (Sembiring and Sabrina 2022). In addition, the presence of PSF in microbial consortia can increase the stability of microbial communities and strengthen synergistic interactions with PSB in the soil (Chaudhary et al. 2023).

Indole acetic acid production

The results of the Indole Acetic Acid (IAA) production test on selected PSM isolates in Table 5 show that each isolate can produce IAA with different concentrations. The IAA concentration produced by the selected PSM isolates was around 18.34 to 105.94 ppm. All PSB isolates could produce IAA in the high category (57.28-105.94 ppm), while PSF isolates were in the medium category (18.34-24.33 ppm). Isolate F2P was the PSB isolate that produced the highest IAA (105.94 ppm), while F5C was the PSF isolate with the highest IAA (24.33 ppm).

The ability to produce IAA is categorized into three levels, namely low, medium, and high. This level can be seen from the concentration of IAA produced, respectively 0-10, 11-25, and more than 26 ppm (Guardiola-Márquez et al. 2023). The differences in microbes in producing IAA were influenced by its ability to convert tryptophan in the environment into IAA (Fitriatin et al. 2024). The results of IAA synthesis by high PSM can stimulate plant growth through cell division, differentiation, and elongation of plant cells (Etesami et al. 2015). The research results of Rafique et al. (2017) show that inoculation of PSB in the form of *Lysinibacillus fusiformis*, which produces an IAA of 32.1 ppm, can increase the growth of corn plants.

Compatibility between isolates

The results of the compatibility test analysis between isolates (Figure 4) brought about a significant finding. The PSM isolates were found to be synergistic, with no antagonistic symptoms observed. This absence of an inhibitory zone at the meeting point between isolates provides reassurance about the safety of these microbial isolates. They can work together without inhibiting each other, enhancing the effectiveness of the microbial consortium as a biofertilizer. The PSM consortium has the potential to increase P availability for plants, surpassing the benefits of single inoculant inoculation (Gupta et al. 2012). For instance, inoculation of PSB in the form of *L. fusiformis* on corn plants can boost N, P, and K uptake by 29.9%, 169.6%, and 20.9%, respectively (Rafique et al. 2017).

Biomolecular identification of selected isolates

Figure 5 visualizes the gel results from amplification of the 16S rRNA sequences of three PSB isolates with the codes F1Zn1, F2P, and F6P. The amplification results show that the three PSB isolates have PCR reaction products ±1500 bp in length. This indicates that the three isolates have intact 16S rRNA genes and can be analyzed further.

The species identification results using BLASTn analysis (Table 6) show that the three isolates belong to the *Enterobacter* genus. Isolate F1Zn1 was identified with accession number PP702267.1 as *Enterobacter cloacae* strain Ec030, F2P was identified with accession number MW479759.1 as *E. cloacae* strain UT25, while F6P was identified with accession number MZ597842.1 as *E. cloacae* strain ABRL064.

The results of the phylogenetic analysis carried out by alignment (MUSCLE) and constructed using the maximum likelihood statistical method were obtained, as shown in Figure 6. This figure shows the genetic closeness between the three selected isolates, which are part of the genus *Enterobacter*. *Enterobacter* is a growth-promoting bacteria that can fix N, dissolve P, and produce phytohormones. This strengthens the potential of the three isolates as candidates for biofertilizer inoculants.

Table 3. Ranking of phosphate-solubilizing bacterial isolates derived from indigenous organic biofertilizers

Bacterial isolate	Phosphate solubilizing index	Acidity stress			Total points	Ranking
		pH 4	pH 7	pH 9		
F1ZN1	7	2	2	2	13	3
F2P	8	2	2	2	14	2
F2A AK	1	1	2	1	5	9
F3A AK	2	1	2	2	7	8
F4P	6	1	2	1	10	4
F4A AK	5	1	2	1	9	6
F4Zn 3	4	2	2	2	10	4
F5A AK	3	1	2	2	8	7
F6P	9	2	2	2	15	1

Table 4. Ranking of phosphate-solubilizing fungal isolates derived from indigenous organic-organic-biofertilizers

Bacterial isolate	Phosphate solubilizing index	Acidity stress			Total points	Ranking
		pH 4	pH 7	pH 9		
F1B	3	2	2	2	9	7
F2A	6	2	2	2	12	4
F3A	3	2	2	2	9	7
F3B	5	2	2	2	11	5
F4A	8	2	2	2	14	2
F5A	3	2	2	2	9	7
F5B	7	2	2	2	13	3
F5C	9	2	2	2	15	1
F6A	4	2	2	2	10	6

Table 5. Indole acetic acid (IAA) production of selected phosphate-solubilizing microbial isolate

Selected microbial isolates	Indole acetic acid concentration (ppm)	Category
F1ZN1	64.52	High
F2P	105.94	High
F6P	57.28	High
F4A	20.97	Medium
F5B	18.34	Medium
F5C	24.33	Medium

Khalifa et al. (2016) stated that *E. cloacae* is known as a microbe that can stimulate plant growth. It can fix N, dissolve P, produce phytohormones (IAA), and resist abiotic stresses in acidity and salinity. *Enterobacter cloacae* isolates that can fix N can increase N availability for plants (Liu et al. 2017). These bacteria can dissolve inorganic P-bound in the soil by producing organic acids and phosphatase (Zuluaga et al. 2023).

Previous research revealed that *E. cloacae* can produce IAA around 23.35-105.78 ppm (Kumar and Prasad 2025). The presence of these hormones is used by plants to regulate plant growth and development processes such as cell division and root elongation (Fu et al. 2015). Apart from that, this bacterium also has potential as a candidate for biological control of plant diseases.

Table 6. Determining isolated bacterial species through analysis of 16S rRNA sequences

Code	Description	Per. ident	Accession
F1Zn1	*Enterobacter cloacae* strain Ec030 16S ribosomal RNA, *partial sequence*	100%	PP702267.1
F2P	*Enterobacter cloacae* strain UT25 16S ribosomal RNA, *partial sequence*	99.93%	MW4797591
F6P	*Enterobacter cloacae* strain ABRL064 16S ribosomal RNA, *partial sequence*	100%	MZ597842.1

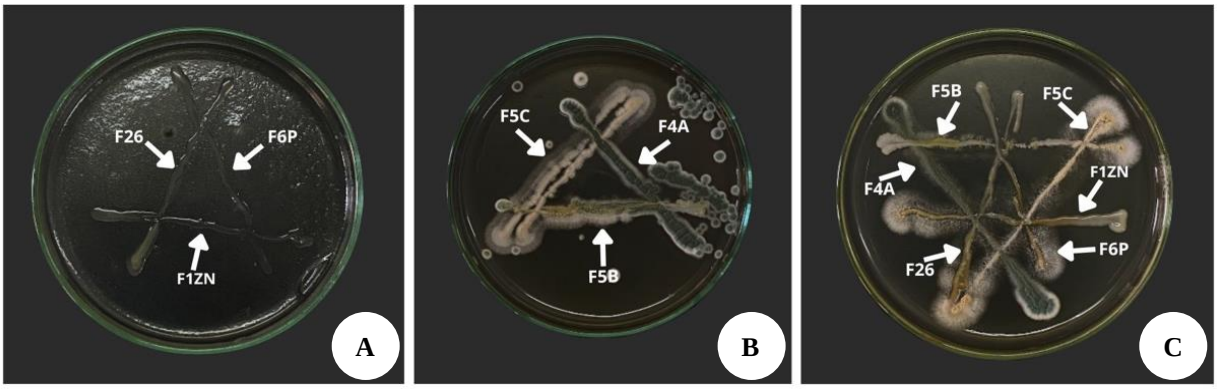

Figure 4. Compatibility analysis of phosphate-solubilizing microbial isolates from indigenous organic biofertilizers: A. Bacterial isolates; B. Fungal isolates; and C. Combined selected isolates

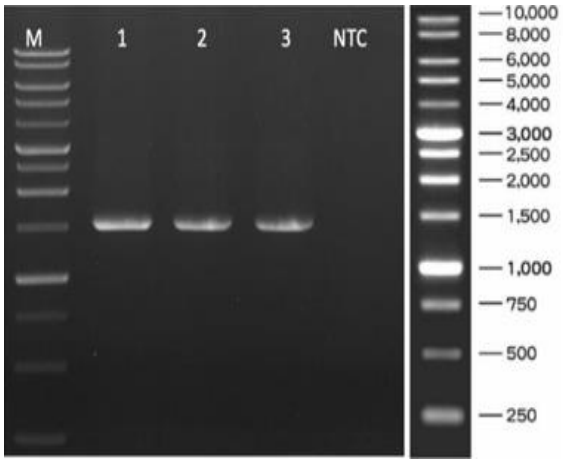

Figure 5. Visualization of the 16S rRNA sequence from isolates F1ZN1, F2P, and F6P showing PCR reaction products approximately ±1500 bp long. Note: 1: F1ZN1; 2: F2P; 3: F6P; and M: 1 Kbp DNA Ladder

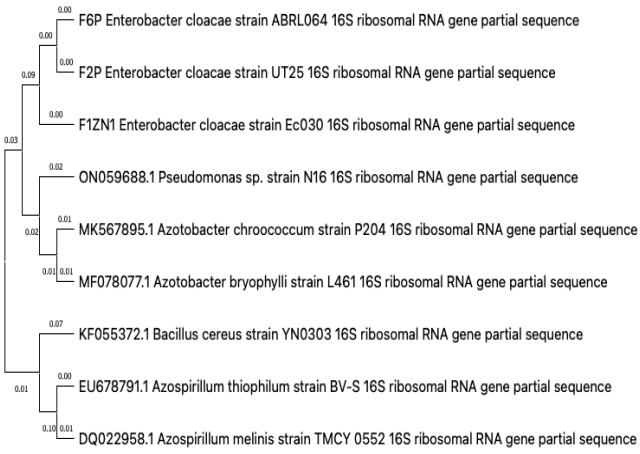

Figure 6. Phylogenetic tree obtained from RNA gene sequences of isolates F1ZN1 (*Enterobacter cloacae* strain Ec030), F2P (*E. cloacae* strain UT25), and F6P (*Enterobacter cloacae* strain ABRL064)

Enterobacter cloacae isolates can induce plant-induced resistance by producing acetoin as 3-hydroxy-2-butanone (Khalifa et al. 2016). Acetoin is an elicitor that mediates plant resistance to pathogens (Rudrappa et al. 2010). Acetoin produced by *Enterobacter* increases the resistance of corn plants to *Setosphaeria turcica*, which causes corn leaf blight disease (D'Alessandro et al. 2014). Previous research results also show that *E. cloacae* can produce around 43.78-65.41% siderophores (Kumar and Prasad 2025). The siderophores produced can suppress pathogen growth and increase P availability by chelating Fe from mineral complexes (Ahmed and Holmström 2014).

In conclusion**,** bacterial isolates with codes F1ZN1, F2P, and F6P, while fungal isolates F4A, F5B, and F5C were the best phosphate-solubilizing isolates. This isolate can produce indole-3-acetic acid with a high category of around 57.28-105.94 ppm (bacteria) and a medium category of around 18.34-24.33 ppm (fungi). These isolates can work synergistically and be formulated into a microbial consortium for plant nutrient supply. Through molecular identification of 16S rRNA sequencing, F1ZN1 was identified as *E. cloacae* strain Ec030, F2P was identified as *E. cloacae* strain UT25, and F6P was identified as *E. cloacae* strain ABRL064. These results show that the IOB formula based on organic waste from vegetable, animals, and JAKABA (Eternal Lucky Mushroom) contains microbial diversity that can provide additional benefits through microbial interactions, increasing phosphate solubilization and plant resistance in the face of acid stress. These results highlight the potential of PSM isolates from IOB as an effective biofertilizer inoculant candidate for increasing plant growth in environmental stress (acidity) amidst climate change. Further studies are needed to assess the effectiveness of these isolates as biofertilizer inoculants in field conditions.

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