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RESEARCH ARTICLE
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The Potential of Plant Growth-Promoting Rhizobacteria (PGPR) as Biostimulants and Biodegradation Agents of Chlorpyrifos in Pesticide-Contaminated Shallot Fields

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ABSTRACT

Shallots are a staple commodity in Indonesia, essential both as a culinary ingredient and as traditional medicine, resulting in consistently high demand. However, production is constrained by limited seed and fertilizer availability, while excessive use of synthetic fertilizers can harm the environment. Environmentally friendly alternatives, such as the use of rhizosphere microorganisms, are increasingly considered due to their role in improving soil fertility and promoting plant growth. This study aimed to explore and identify rhizosphere bacterial isolates from shallot plants in Anggeraja Subdistrict, Enrekang Regency, and evaluate their potential as plant growth-promoting rhizobacteria (PGPR), particularly in producing Indole-3-Acetic Acid (IAA) and solubilizing phosphate. Rhizosphere soil samples were collected from four villages Lakawan, Batunoni, Pekalobean, and Tampo—and isolated on nutrient agar. Isolates were characterized morphologically and physiologically, and their ability to produce IAA and solubilize phosphate was assessed using colorimetric and spectrophotometric methods. Among 60 isolates, 20 showed varying IAA production (0.02–3.92ppm) and phosphate solubilization (4.94–11.23ppm). Isolates BN14 and BN25 exhibited the highest IAA production, while BN02 showed the highest phosphate-solubilizing activity. Environmental factors such as soil C/N ratio and organic matter content likely influenced microbial activity. Greenhouse assays showed that several isolates enhanced shallot growth, with TP49 producing the most significant improvements in plant height (9.08cm), leaf number, root length (5.95cm), fresh weight (0.264g), and dry weight (0.0082g) compared to controls. Microscopic analysis confirmed biofilm formation on shallot roots, indicating successful bacterial colonization. Molecular identification using 16S rRNA gene sequencing revealed that selected high-performing isolates were closely related to Bacillus spp. These findings demonstrate the potential of indigenous rhizosphere bacteria as biofertilizers to enhance shallot productivity, support soil fertility, and promote sustainable agriculture in karst highland ecosystems of Enrekang.

Keywords: IAA, PGPR, Phosphate solubilization, Rhizosphere bacteria, Shallot.

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INTRODUCTION

In Indonesia, shallots are almost always needed by households as a complementary ingredient in daily cooking. In addition, shallots are also used as traditional medicine to treat various diseases. Therefore, the availability of shallots is always in demand along with the increasing population (Shahrajabian et al., 2020). One of the obstacles to shallot production is the limited availability of seeds and fertilizers to meet the nutritional needs of the plants, as synthetic chemicals have many negative impacts such as environmental pollution

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(Kolya & Kang, 2024). Awareness of environmental and health issues, along with the development of biotechnology, has driven the use of natural materials in crop cultivation practices, including soil bacteria as microorganisms (Kafrawi et al., 2017).

Microorganisms represent the largest source of biodiversity in the world and are essential for the continuity of ecosystem functions (Delgado-Baquerizo et al., 2016). The presence of microorganisms in soil contributes to the microbial ecosystem, including nutrient cycles, which are indicators of soil fertility and health (Jiao et al., 2021). Soil contains billions of microbes that support plant growth, resulting in symbiotic or non-symbiotic interactions between plants and microorganisms (Yanti et al., 2023).

The narrow rhizosphere area, or the immediate zone around plant roots, strongly influences the microbial biomass surrounding the roots. The rhizosphere is the soil zone affected by plant roots through the release of rhizosphere exudates, mucilage, and detached cells. Root exudates contain various compounds, especially organic acids, sugars, amino acids, fatty acids, vitamins, growth factors, hormones, and antimicrobial compounds (Sun et al., 2021). Each plant root rhizosphere is colonized by a specific group of beneficial bacteria that live symbiotically with plant tissues and cells at various levels of dependence (Oluwafunto et al., 2023). Soil microorganisms (bacteria, fungi) living in the rhizosphere area provide various benefits to plants during their growth and development cycle, creating a mutualistic symbiotic relationship between the two (Raza et al., 2016). These bacteria are capable of producing plant growth-promoting substances such as Indole-3-Acetic Acid (IAA), Abscisic Acid (ABA), Gibberellic Acid (GA), and Cytokinin (CTK), which play important roles in enhancing nutrient uptake and supporting plant growth under suboptimal environmental conditions (Singh et al., 2022).

Bacteria in the soil colonize the root zone (a thin soil layer 1–2mm around the root area) and directly or indirectly stimulate plant growth and development through the production and secretion of various chemicals. These are known as rhizobacteria that promote or stimulate plant growth, referred to as biostimulants or plant growth-promoting rhizobacteria (PGPR) (Ahemad & Kibret, 2014). The presence of rhizosphere bacteria is influenced by environmental conditions, including factors such as soil properties, organic matter content, cultivation techniques, fertilization, and pesticide application. Soil organic carbon content affects the composition of soil microbes, with a stronger effect on bacteria than on fungi (Ren et al., 2021; Zecchin et al., 2023). In this context, one possible approach is to create and enhance sustainable populations of potential bacteria in the endophytic part and root zones of plants (Sturz & Nowak, 2000).

The exploration and identification of these microorganisms were carried out in Anggeraja Subdistrict, Enrekang Regency, which is characterized by karst mountainous terrain separated by flowing rivers (Pekalobean, Lakawan, Tampo, and Batunoni). Most microbial species are influenced by plant biology and their environment (Morris et al., 2011). Environmental factors affect the presence of microbes (Freiberg et al., 1997). Several environmental factors—such as soil type, soil moisture, pH, temperature, as well as plant age and condition—influence rhizosphere effects (Rao, 1995; Reisberg et al., 2013). Currently, exploration and identification of microorganisms in this region are still lacking. Therefore, this study aims to identify the diversity of isolates from shallots that have the potential to produce biostimulants that can be developed as biofertilizers. The findings of this study are expected to contribute to maintaining soil fertility and supporting sustainable agricultural systems in the area.

MATERIALS & METHODS

Research Location

This study was conducted from May to August 2024 at the Agricultural Biotechnology Laboratory, Department of Plant Pests and Diseases, Faculty of Agriculture, Hasanuddin University, Makassar. Bacterial isolates were collected from several villages in Anggeraja Subdistrict, Enrekang Regency, including Lakawan, Batunoni, Pekalobean, and Tampo. The geographical coordinates of these villages are as follows: Lakawan (-3.4091680, 119.7950860), Batunoni (-3.4384730, 119.8256750), Pekalobean (-3.3770250, 119.7864520), and Tampo (-3.3938130, 119.8150480).

The greenhouse test has not yet been conducted; however, the in vitro (bottle) test under hydroponic conditions was carried out in this study as follows: Bacteria were cultured on NA medium for 24 hours, then three loops of colonies were transferred into 300 mL of sterile distilled water and shaken until homogeneous. The colony density was adjusted to 10^8CFU/mL using a spectrophotometer (OD 0.066). Then, 50mL of the bacterial suspension was placed into a sterile glass bottle. Shallot seeds were surface-sterilized using alcohol (3–5 minutes), followed by 3% chlorox solution (3–5 minutes), and rinsed thoroughly with sterile distilled water. The control seeds were only soaked in sterile distilled water. Seeds were then soaked in the bacterial suspension or sterile distilled water for approximately 24 hours at 22–26°C with 35–50% humidity. After soaking, the seeds were placed on Petri dishes lined with four layers of Whatman No.1 filter paper. After sprouting reached about 2cm (±3 days), the seedlings were transferred into test tubes containing culture media (PA-grade distilled water + inoculum 10^8 CFU/mL, or sterile distilled water for control) in 50 mL culture bottles, supported by perforated sponges.

The cultures were maintained for approximately 14 days before observation was conducted. The experimental design used a Completely Randomized Design (CRD) with 3 replications, consisting of 11 treatments (P0 = control or sterile distilled water medium, P1 = isolate BN01, P2 = isolate BN07, P3 = isolate BN11, P4 = isolate TP47, P5 = isolate TP49, P6 = isolate TP53, P7 = isolate LW31, P8 = isolate PL56, P9 = isolate PL58, and P10 = isolate BN24), resulting in a total of 33 experimental units. Greenhouse testing will be conducted in future research, as well as multi-location

testing of the bacterial isolates obtained in this study.

Sample Collection

Rhizosphere soil samples of shallots were collected from four locations: Pekalobean, Batunoni, Lakawan, and Tampo. Samples were taken randomly by uprooting shallot plants along with their roots, and approximately 100g of soil was collected from each plant. Each sample was placed in a sterile brown envelope, labeled, and stored in a cool box. The samples were then transported to the laboratory for further analysis and testing within 48 hours after collection (Tomlinson et al., 1977).

Molecular Identification

Molecular identification was carried out using the gSYNC DNA Extraction Kit for DNA isolation. PCR amplification of the 16S rRNA gene was performed in a reaction volume of 50µL using primer 63F (5′-CAG GCC TAA CAC ATG CAA GTC-3′) and primer 1387R (5′-GGG CGG WGT GTA CAA GGC-3′). The PCR reaction mixture consisted of 25µL GoTag® Green Master Mix, 2.5µL of 10µM primer 63F, 2.5µL of 10µM primer 1387R, 5µL of template DNA, and 15 µL of nuclease-free water. The PCR program was run for 35 cycles, starting with a pre-denaturation step at 95°C for 2min, followed by denaturation at 95°C for 30s, annealing at 50°C for 30s, and DNA extension at 72°C for 45s. The amplification process was completed with a final post-extension step at 72°C for 5min.

Isolation and Morphological–Physiological Characterization of Bacteria

The soil samples were taken to the laboratory and serially diluted up to 10^{-7}. To prepare the initial dilution, 1g of soil was ground using a mortar and added to 9mL of sterile distilled water in a test tube. The tube was vortexed for several minutes to create the 10^{-1} dilution. Subsequently, 1mL from this suspension was transferred to another tube containing 9mL sterile water to prepare the 10^{-2} dilution. This procedure was repeated until the 10^{-7} dilution was obtained.

Aliquots of 0.1mL from the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were spread on nutrient agar (NA) plates using a sterile spreader and incubated for 24 hours to allow rhizosphere bacteria to grow. Distinct colonies were then purified individually using the streak plate method (Jiang et al., 2016). Pure bacterial isolates were examined macroscopically to observe colony morphology, including size, pigmentation, margin, shape, and elevation. Colony counts were estimated using the total plate count (TPC) method (Mizuochi et al., 2016).

From a total of 60 bacterial isolates, all distinct isolates were collected from rhizosphere soils at four locations: Batunoni (26 isolates), Tampo (16), Pekalobean (10), and Lakawan (8). Among these 60 isolates, 23 isolates (not 20) demonstrated potential for producing IAA, GA_3, phosphate solubilization, and nitrogen fixation. Subsequently, out of these 23 isolates, 10 isolates were selected and tested in vitro under hydroponic conditions, and finally, 6 superior isolates showing strong PGPR (Plant Growth-Promoting Rhizobacteria) potential were further analyzed using PCR assays.

Analysis of IAA Production Capability

A total of 10 grams of soil was added to 100mL of NB medium containing L-tryptophan (0.1%), then incubated at 28°C for 24 hours, with three biological replicates for each isolate. The IAA concentration (mg L^{-1}) of each sample was calculated based on a standard curve prepared using pure IAA standard (Kesaulya et al., 2015). The IAA standard curve followed the regression equation Y = 0.0632x + 0.0189 (R^2 = 0.9948), obtained from a serial dilution of the IAA stock solution. The optical density of each solution was measured at a wavelength of 535 nm using a UV-Vis spectrophotometer.

Qualitative and Quantitative Analysis of Phosphate-Solubilizing Activity

Before measuring the phosphate solubilization absorbance, the acidity level (pH) was first measured — initially 10.2 and 6.5 after cultivation. The phosphate solubilization ability test (quantitative test) was carried out using the spectrophotometric method with liquid Pikovskaya medium, following the method of Jastrzębska (2009). After seven days of incubation at 28°C, the culture was centrifuged at 10,000rpm for 20 minutes. The supernatant was mixed with ammonium molybdate, sulfuric acid, and ferrous sulfate to produce a blue color. The absorbance at 693nm wavelength was used to calculate the phosphate concentration using a linear calibration curve with the equation Y = 0.191x + 0.048 (R^2 = 0.957). The blank value on the spectrophotometer was 0.000 Abs (Verma & Srivastav, 2017). Endophytic bacterial isolates were spot-inoculated onto the agar and incubated at 28 °C for three days.

In the phosphate solubilization assay, the negative control (-) used was distilled water. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 693 nm, with the blank value = 0.000 Abs.

Quantitative estimation of soluble phosphate was performed using a modified spectrophotometric method (Jastrzębska, 2009). Each isolate was grown in 30mL of liquid Pikovskaya medium containing: glucose (10g), tricalcium phosphate (5 g), ammonium sulfate (0.5g), potassium chloride (0.2g), yeast extract (0.5g), magnesium sulfate (0.1g), sodium chloride (0.2g), manganese sulfate monohydrate (0.002g), and ferrous sulfate heptahydrate (0.002g) per liter of sterile distilled water. Cultures were shaken at 28°C for seven days.

After incubation, 1.5mL of culture was centrifuged at 10,000rpm for 15min. One milliliter of supernatant was mixed with 3 mL of sterile distilled water and 1 mL of color reagent containing 1.5% (w/v) ammonium molybdate, 5.5% (v/v) sulfuric acid, and 2.7% (w/v) ferrous sulfate. A blue color indicated the presence of soluble phosphate (Dalenberg et al., 2014). The absorbance was measured at 693nm using a UV-Vis spectrophotometer (Genesys 10S UV 840208100). Phosphate concentrations were determined using a standard curve prepared from Titrisol phosphate standards ranging from 0 to 2.5mg L^{-1}, and the

results were expressed in mg L^{-1}.

The phylogenetic analysis in this study was conducted based on 16S rRNA gene sequences to determine the evolutionary relationships between the identified bacterial isolates and bacterial species registered in GenBank. The sequencing process produced amplicons of approximately 1,450 base pairs (bp), covering nearly the entire region of the 16S rRNA gene—from conserved to variable domains. Determining this amplicon length aimed to obtain sequence data that is sufficiently representative to depict the evolutionary relationships among species.

The sequencing was performed with two-directional coverage, using both forward and reverse primers, resulting in overlapping sequence reads. This approach ensures a high level of accuracy, as each base is verified by the complementary read. The resulting sequences were assembled into a consensus contig using sequence analysis software such as BioEdit or MEGA X, minimizing reading errors and generating valid nucleotide sequences for subsequent analysis.

After obtaining the verified sequences, alignment was performed against reference sequences downloaded from the GenBank database. Sequence alignment was conducted using MUSCLE (Multiple Sequence Comparison by Log-Expectation) implemented in MEGA X, as this method provides an optimal balance between speed and accuracy, especially for datasets of medium to large size.

The aligned sequences were then used to construct a phylogenetic tree using the Maximum Likelihood (ML) method, which is known for its robustness in estimating evolutionary relationships based on nucleotide substitution models. The evolutionary model applied was the Tamura–Nei (TN93) model, which accounts for differences in transition and transversion substitution rates and base frequency variation—making it suitable for analyzing the 16S rRNA gene, which is highly conserved yet exhibits variation in specific regions.

The resulting phylogenetic tree illustrates the evolutionary relationships between the isolates and reference bacterial species. According to the tree, TP47, TP49, BN07, and PL53 form a cluster closely related to the Bacillus group, while BN11 is closely related to the Paenibacillus genus, and BN01 is distinctly separated, forming a cluster with Arthrobacter/Pseudarthrobacter. This indicates that the bacterial isolates in this study possess considerable phylogenetic diversity, encompassing several genera within the Firmicutes and Actinobacteria phyla. The relatively short branch lengths in most Bacillus clusters indicate high sequence similarity, whereas the longer branches in the Arthrobacter group suggest a greater evolutionary distance from the Bacillus group.

The reliability of each branching point in the phylogenetic tree was tested using 1,000 bootstrap replications and further supported by the Shimodaira–Hasegawa approximate Likelihood Ratio Test (SH-aLRT). The bootstrap and SH-aLRT values at each node represent the level of confidence in the inferred evolutionary relationships; values above 70% indicate strong branch support. The combination of these two statistical tests provides a stronger justification for the validity of the tree topology.

The phylogenetic tree visualization was performed using MEGA X, displaying the species names and GenBank accession numbers for each branch. Overall, the analysis demonstrated that the bacterial isolates in this study exhibit high genetic similarity with certain reference species, and the 16S rRNA-based phylogenetic approach effectively provided a clear depiction of the evolutionary relationships between the isolates and known reference bacterial species.

RESULTS AND DISCUSSION

IAA Production by Rhizosphere Bacteria

Based on the qualitative test results (Fig. 1), a color change of the suspension to pink was observed during the IAA assay in all rhizosphere bacterial isolates from shallot plants after the application of Salkowski reagent compared to the control. This indicates that rhizosphere bacteria have varying abilities to produce IAA depending on their source locations and isolate types (Randive et al., 2024).

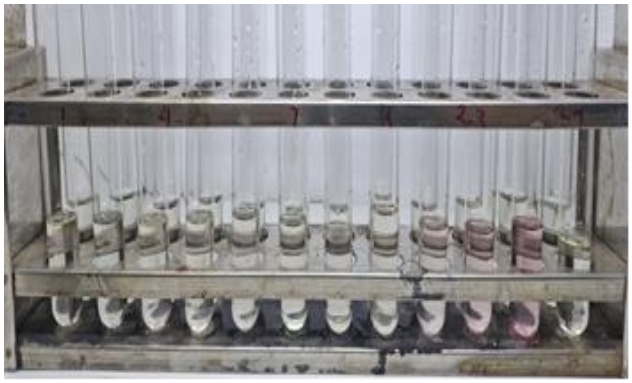

Fig. 1: Qualitative analysis of IAA production by rhizosphere bacteria.

The rhizosphere bacterial isolates coded BN14 and BN25 showed the highest IAA production, reaching 3.92ppm, both originating from Batunoni Village. In contrast, the isolate coded BN07 exhibited the lowest IAA concentration at only 0.02ppm (Fig. 1). Meanwhile, isolates from Lakawan Village (LW27) produced about 1.36ppm, and isolates from Tampo Village (TP48) reached 2.83ppm.

Fig. 2: Qualitative analysis of dissolved phosphate using liquid Pikovskaya medium with the addition of 0.5 mL concentrated ammonium molybdate and potassium antimonyl tartrate blue reagent.

The variation in IAA concentrations is presumably due to differences in the original locations from which the rhizosphere bacterial samples were collected. Environmental and biological factors affect the presence and activity of specific microbial populations, such as soil chemical properties and the availability of precursors like tryptophan, which plays a crucial role in IAA biosynthesis (Lata, 2024a).

This finding is consistent with the results of Lata (2024a), who identified several bacterial genera such as *Pseudomonas*, *Bacillus*, and *Klebsiella*, each exhibiting different IAA production capacities depending on their isolation sites. IAA production by these bacteria is known to play an important role in promoting plant growth.

Furthermore, Lata (2024b) reported that IAA-producing bacteria isolated from the rhizosphere of common beans (*Phaseolus vulgaris*) were able to significantly enhance plant growth variables, indicating that IAA synthesis by these bacteria is an important factor contributing to increased plant growth rates compared to control groups.

In addition, Randive et al. (2024) also reported that IAA production by certain bacteria, particularly Plant Growth-Promoting Rhizobacteria (PGPR) isolated from the rhizosphere of *Pterocarpus marsupium*, has a major influence on plant growth and development. Their study showed that IAA produced by PGPR positively affected wheat plant growth, especially by stimulating better root formation and supporting overall plant development.

Qualitative and Quantitative Analysis of Phosphate Solubilization

The ability of endophytic bacterial isolates to solubilize phosphate quantitatively was measured through the solubilization activity of Ca-phosphate (tricalcium phosphate) in liquid Pikovskaya medium. The test results showed that isolate BN02 had the highest phosphate solubilization concentration at 11.23ppm, while isolate PL55 showed the lowest concentration at 4.94ppm. The measurement of phosphate solubilization concentration was conducted using a UV-VIS spectrophotometer at a wavelength of 693nm (Fig. 2). Factors such as bacterial strain type, microbial growth in culture, phosphate source, and environmental conditions strongly affect the differences in the levels of phosphate solubilization produced by these bacterial isolates.

There was considerable variation in phosphate-solubilizing ability among the bacterial isolates. Several isolates, such as TP46 and PL53, exhibited relatively high phosphate concentrations (around 7–9ppm), while others showed lower values. This indicates that factors such as bacterial strain type, growth conditions, and available phosphate sources influence phosphate-solubilizing ability.

One key factor affecting phosphate solubilization is the bacterial species, as different species have varying capacities to solubilize phosphate, which may be influenced by their ability to produce organic acids or phosphatase enzymes. As reported in several studies, bacteria that produce organic acids such as citric acid tend to be more efficient at solubilizing phosphate (Khuong et al., 2024).

Environmental factors such as soil pH, temperature, and oxygen availability can also influence the activity of rhizosphere microbes in solubilizing phosphate. Some bacteria are more active in acidic or neutral soil conditions, while others are more tolerant of extreme environmental conditions (Rehman et al., 2022).

Bacteria with high phosphate-solubilizing ability, such as isolate BN02 from Batunoni, can be considered as potential biofertilizer candidates to improve phosphate fertilization efficiency in plant growth. Rhizosphere bacterial species such as *Bacillus* and *Burkholderia* are known to produce various organic acids including gluconic, oxalic, citric, tartaric, succinic, formic, and acetic acids. These acids can lower the local pH and help release phosphate bound in mineral forms such as tricalcium phosphate and octacalcium phosphate (Chawngthu et al., 2020; Paul & Sundar, 2021).

Quantitative analysis of the phosphate-solubilizing ability of rhizosphere bacterial isolates was carried out using a UV-VIS spectrophotometer at 693nm. The results of the quantitative analysis (Fig. 2 and 3) showed that, among the total of 60 rhizosphere bacterial isolates found in the rhizosphere area of shallots, 20 isolates exhibited varying abilities to solubilize phosphate. The highest IAA concentration was observed in rhizosphere bacterial isolates BN14 and BN15, while the highest phosphate-solubilizing activity was shown by isolates BN02 and TP35.

The soil analysis results (Table 1) showed that the soil from the Batunoni location had the highest phosphate content (14.15ppm) and a C/N ratio of 14%. This indicates that rhizosphere bacteria play an important role in enhancing the availability of phosphorus (P) in the soil by solubilizing bound phosphate. Research has shown that different C/N ratios can affect the structure and biomass of microbial communities, which in turn can influence phosphate-solubilizing function (Sun et al., 2017). A higher C/N ratio can increase microbial activity, including phosphate-solubilizing bacteria, which contribute to improved phosphate solubilization (Zhu et al., 2021).

Table 1: IAA, GA_3, Siderophore, Phosphate, and Nitrogen Fixation Data

Isolat Code	IAA	GA3	Siderofor	Fosfat	N Fiksation
BN01	1.30	9.256	1.04	6.00	688.77
BN03	0.81	7.25	1.13	4.80	322.50
BN04	0.34	7.57	2.73	3.84	423.43
BN05	0.22	7.63	3.65	4.50	336.78
BN06	1.00	7.33	2.21	5.24	344.76
BN07	0.02	7.13	3.94	6.83	576.19
BN08	0.91	6.87	2.07	6.34	356.19
BN09	0.88	7.66	2.17	4.74	345.76
BN10	0.45	7.54	4.61	5.31	440.75
BN11	1.27	7.27	4.12	6.59	649.90
BN12	0.50	8.10	2.43	4.88	402.23
BN13	1.20	8.68	1.51	6.36	307.95
BN16	1.14	8.06	2.12	5.97	428.50
BN18	0.21	7.35	2.02	3.91	526.54
BN24	0.42	8.81	1.17	7.82	724.82
LW31	0.80	9.75	2.86	6.66	652.96
TP45	0.42	7.93	2.55	3.33	550.75
TP47	1.27	8.12	1.81	7.15	601.39
TP49	1.28	9.52	0.97	4.95	647.09
TP50	1.28	8.07	0.92	5.94	527.87
PL53	1.35	7.95	1.18	6.82	623.82
PL56	0.97	7.99	1.00	2.98	645.16
PL58	0.23	8.29	0.25	6.20	623.10

Fig. 3: Quantitative Analysis of Phosphate-Solubilizing Ability of Rhizosphere Bacterial Isolates.

Phosphate is a major nutrient essential for plant growth and development. Although phosphate is abundant in soil in both organic and inorganic forms, most of it (about 95–99%) is insoluble and therefore not readily available for plant uptake (Akplo et al., 2025). Thus, the use of rhizosphere bacteria capable of solubilizing phosphate is highly beneficial to help provide available phosphate for plants. These bacteria can also produce plant growth hormones such as indole acetic acid (IAA) (Barea et al., 1976; Mahdi et al., 2020), which stimulate root and shoot growth (Luo et al., 2024).

The quantitative analysis of IAA production by rhizosphere bacteria showed concentrations ranging from 0.02 to 3.92ppm (Fig. 3). This variation is presumably due to differences in sampling site conditions. It is also suspected that the availability of precursors such as tryptophan in the medium influences IAA production. In addition to functioning as mediators for adaptation to different environmental conditions, auxins modify the plant root system in response to biotic and abiotic signals from the root rhizosphere (Kazan, 2013; Mroue et al., 2018). Auxins also interact with other plant hormones such as cytokinins and gibberellins, either synergistically or antagonistically, to trigger a series of morphogenetic processes and root development in plants (Saini et al., 2013; Gao et al., 2024)

Plant Height (cm), Number of Leaves (Leaves), Root Length (cm), Fresh Weight (g) and Dry Weight (g)

The results of the analysis of variance (ANOVA) showed significant differences in all observed variables (Table 2). The mean plant height of shallot shoots at 14 days after planting (DAP) (Fig. 4 and Fig. 5) indicated that treatment with isolate TP49 produced the highest average height of 9.08 cm, which was significantly different from the control but not significantly different from treatments TP53, LW31, PL56, PL58, BN07, BN11, TP47 and BN24.

Treatment with isolate TP49 also showed the highest mean number of leaves, with an average increase of 2.50 leaves, which was significantly different from the control and BN07 treatments, but not significantly different from the other treatments. The control treatment produced the lowest average number of leaves, with only 1.00 leaf.

The mean results of the HSD 5% post-hoc test showed that, for the root length variable, the longest root length was observed in the TP49 treatment with a value of 5.95 cm, which was significantly different from the control treatment with a value of 1.70cm. For the fresh weight variable, the TP49 treatment showed the highest mean fresh weight of 0.264g, which was significantly different from the control (0.053 g), BN07 (0.103g), and other treatments. For the dry weight variable, the highest value was also recorded in the TP49 treatment with an average of 0.0082 g, while the lowest was found in the control treatment at 0.0027g.

Table 2: Mean plant height (cm), number of leaves (leaves), root length (cm), fresh weight (g), and dry weight (g) of shallot shoots under different PGPR (Plant Growth-Promoting Rhizobacteria) isolates

Treatment	Variable				
	Plant height	Number of leaves	Akarroot length	Fresh weight	Dry weight
control	5.97^{c}	1.00^{d}	1.70^{f}	0.053^{g}	0.0027^{d}
BN01	7.40^{abc}	2.00^{bc}	2.57^{cdef}	0.43^{bcdef}	0.0044^{bc}
BN07	8.30^{abc}	1.87^{c}	2.02^{def}	0.103^{fg}	0.0038^{c}
BN11	8.33^{abc}	2.00^{bc}	2.45^{cdef}	0.173^{bc}	$0,0037^{c}$
TP47	6.33^{bc}	2.00^{bc}	3.50^{bcde}	0.130^{cdefg}	0.0056^{b}
TP49	9.08^{a}	2.50^{a}	5.95^{a}	0.264^{a}	0.0082^{a}
TP53	8.97^{ab}	2.00^{bc}	3.16^{cdef}	0.177^{b}	0.0043^{bc}
LW31	8.03^{abc}	2.00^{bc}	4.62^{ab}	0.111^{efg}	0.0046^{bc}
PL56	8.57^{abc}	2.00^{bc}	3.64^{bcde}	0.173^{bcd}	0.0048^{bc}
PL58	8.18^{abc}	2.00^{bc}	3.78^{bc}	0.147^{bcde}	0.0046^{bc}
BN24	7.53^{abc}	2.00^{bc}	2.00^{ef}	0.116^{defg}	0.0042^{bc}
NP BNJ	2.09	0.49	1.41	0.036	0.0011

Numbers followed by the same letter in the same column (a, b, c, d, e, f, g) indicate no significant difference according to the Honestly Significant Difference (HSD) test at $\alpha = 0.05$.

The performance results shown in Fig. 4 indicate that the treatment with isolate TP49 exhibited the most prominent average plant height and root weight. This suggests that the rhizosphere bacterial isolate treatment can stimulate plant height growth and directly enhance plant fresh weight (Vasava et al., 2025). Furthermore, Fig. 5 illustrates the successful colonization activity of PGPR bacteria on shallot roots. In the TP49 treatment, the formation of biofilms (arrows 1, 2, 3, and 4) serves as visual evidence that the bacteria are not only present around the root surface but also form stable colonies, which are crucial for ensuring that PGPR effects (such as growth promotion and increased plant resistance) can be sustained over time.

With the presence of biofilms, the bacteria can work synergistically to produce beneficial metabolites that support root growth, as reflected by the healthy root development shown in Fig. 5.

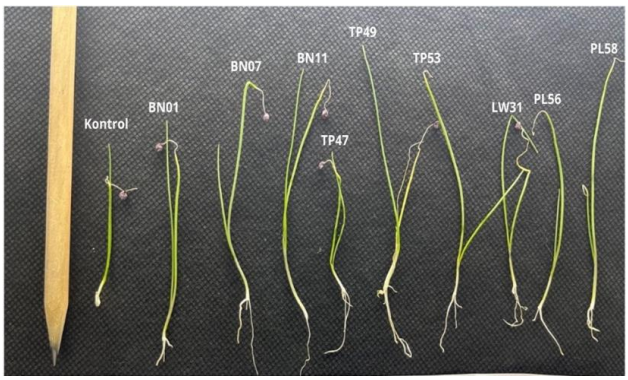

Fig. 4: Average performance of shoot growth of shallot plants at 14 days after planting under control and various PGPR isolate treatments.

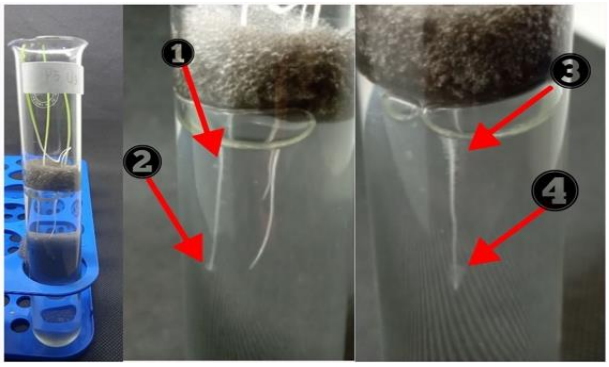

Fig. 5: Performance of bacterial biofilm activity indicated by arrows showing PGPR isolates on the roots of shallot plants treated with PGPR isolate (BN49 = P5).

Molecular Identification Based on 16S rRNA Gene Primers

16S ribosomal RNA (rRNA) gene sequencing analysis is currently the most widely used and highly accurate method for bacterial identification. This sequencing analysis aims to obtain information about the identity of PGPR bacterial isolates based on data from the NCBI (National Center for Biotechnology Information) database through the BLAST-N program. The BLAST analysis provides information on the bacterial isolates and the percentage similarity of their DNA nucleotide sequences compared to those in the GenBank database. (Janda & Abbott, 2007; Johnson et al., 2019).

Six superior bacterial isolates were selected, namely BN11, BN01, BN07, TP49, TP47, and PL53, based on the results of screening from Phase I to Phase III research after in vitro testing. These six isolates were compared with DNA sequences available in the NCBI database through sequence alignment. (Kim et al., 2014; Yarza et al., 2014). Based on alignment results using the NCBI BLAST-N database (Table 2), the bacterial isolate from Batunoni (BN11) showed a maximum identity of 83.45% with *Bacillus subtilis* subsp., 82.71% with *Bacillus manausensis*, 82.24% with *Bacillus chungangensis*, 81.78% with *Bacillus rhizoplanae*, 81.86% with *Bacillus thuringiensis*, and 81.99% with *Bacillus pseudomycoides*. (Fritze, 2004; Logan & De Vos, 2015; Agusta et al., 2022; Akkale, 2023).

Isolate BN01 showed similarity with *Arthrobacter* sp. at 80.67% and the lowest similarity was with *Pseudarthrobacter enclensis* at 78.71%. Meanwhile, isolate BN07 showed high maximum identity with *Bacillus albus* (92.77%), *Bacillus pseudomycoides* (92.69%), *Bacillus mycoides* (92.77%), *Bacillus hominis* (92.69%), *Bacillus thuringiensis* (92.44%), and *Bacillus rhizoplanae* (92.19%). (Rooney et al., 2009; Busse, 2016; Liu et al., 2017) The isolate from Tampo (TP49) showed maximum similarity with *Bacillus flexus*, *Priestia flexa*, and *Bacillus* sp., all at around 97.91%. Isolate TP47 had maximum identity with *Bacillus aerius* (97.75%), *Bacillus stratosphericus* (97.46%), *Bacillus altitudinis* (97.38%), *Bacillus aerophilus* (97.38%), *Bacillus xiamenensis* (97.31%), and *Bacillus pumilus* (96.32%). (Shivaji et al., 2006; Dunlap et al., 2016; Liu et al., 2020; Mol, 2025).

Based on the molecular analysis of the bacterial isolate from Pekalobean (PL53), this isolate showed significant similarity (identity) with several species within the genus *Paenibacillus*. The highest identity was recorded at 91.09% with *Paenibacillus* sp. Enrichment, followed by *Paenibacillus ripae* (90.41%), *Paenibacillus yanchengensis* (90.86%), *Paenibacillus sputi* (90.29%), *Paenibacillus turicensis* (89.60%), and *Paenibacillus spongiae* (89.09%) (Grady et al., 2016; Roux & Raoult, 2021). This high level of identity indicates that the PL53 isolate is genetically very similar to these species, suggesting it likely belongs to the *Paenibacillus* group or shares a close phylogenetic relationship with it. In addition, the query cover values reaching 100% for each comparison indicate that nearly the entire sequence of the PL53 sample matched the reference sequences in the database. The higher the query cover value, the more of the sample sequence aligns with existing database sequences, which shows a more complete match between the sample and the reference species. (Chun et al., 2018; Stackebrandt & Goebel, 1994). These findings support the conclusion that the PL53 isolate has significant similarity with species within the genus *Paenibacillus*. Overall, the molecular identification results provide a clear picture of the microbial composition in Batunoni, Tampo, and Pekalobean, and highlight the potential of these isolates for biotechnological applications such as bioremediation or biological control, considering the important ecological and functional roles played by *Bacillus*, *Paenibacillus*, and *Arthrobacter* species. (Lugtenberg & Kamilova, 2009; Hayat et al., 2010; Bhattacharyya & Jha, 2012; Hamburger et al., 2025).

Phylogenetic Tree Identification

Phylogeny is the study of evolutionary relationships among organisms, which places them based on their characteristics on a relationship map represented as a dendrogram or branching diagram. The phylogenetic results of the six isolates (Fig. 6) show a dendrogram that represents the outcome of phylogenetic analysis based on the genetic sequence data of these six bacterial samples. This phylogenetic tree was constructed based on the genetic sequence similarities of each sample compared to registered reference sequences, providing an overview of the evolutionary relationships between these samples and other documented bacterial species. Based on the clustering patterns, some samples appear to be more closely related to each other, suggesting that they share similar genetic characteristics. For example, isolates PL53, BN07, and TP49 appear more similar to one another, while other isolates show closer relationships to more distantly related bacterial species, such as *Bacillus* and *Pseudomonas*.

The PL53 isolate, which is located on a higher branch, shows high similarity to *Bacillus aerius* (accession number: KX941840.1) and other *Bacillus* species. This suggests that this isolate may possess genetic characteristics associated with the *Bacillus* group, which are well known for their abilities in bioremediation or the production of specific enzymes (Logan & De Vos, 2015; Alnaimat et al., 2025). The BN07 and TP49 isolates also show phylogenetic proximity to *Bacillus* species as well as more specific groups of *Pseudomonas* (Peix et al., 2018). Meanwhile, the

BN01 isolate, which is located on a more separate branch, shows a relationship to more general bacterial taxa such as *Arthrobacter* and *Pseudodethrobacter*. This indicates that the BN01 isolate is more phylogenetically isolated from the other samples, which may reflect differences in its environmental origin or genetic traits (Busse, 2016).

In this analysis, the reference sequences used for comparison were genetic sequences published in various Scopus-indexed journals, including *Paenibacillus* spp., *Bacillus* spp., and other species known for their roles in organic matter degradation or the production of bioactive compounds (Grady et al., 2016; Yadav et al., 2017; Yao et al., 2023). Overall, this dendrogram provides a clear picture of the genetic relatedness among the isolates and other identified bacterial species in this study. These results are important for further understanding the potential applications of each isolated bacterium, either in the context of microbial ecosystems or industrial applications such as bioremediation, enzyme production, or the development of microbe-based biotechnological products (Singh et al., 2021).

The correlation analysis (Table 3) showed that as the incubation time increased, the number of bacterial colonies also tended to increase, as reflected by the overall *r*-value reaching 1, indicating a strong positive trend. This pattern is consistent with recent studies showing that bacterial populations commonly undergo exponential growth during the initial stages of incubation under favorable environmental conditions (Shade et al., 2022; Liu et al., 2023; Uthami & Irdawati, 2024).

However, when comparing the values of individual isolates, most bacterial isolates exhibited weak and non-significant relationships between colony number and incubation time. Nonetheless, several significant relationships were detected. Isolate BN11 showed a significant positive correlation with isolate TP49 (r = 0.65*), indicating that both tended to increase in colony numbers concurrently during incubation. Similarly, isolate BN07 showed a very strong positive correlation with LW31 (r = 0.89**), suggesting that their growth patterns were highly synchronized. These results are in line with recent findings that some bacterial strains display cooperative or facilitative growth interactions when sharing the same ecological niche (Venturelli et al., 2018; Madsen et al., 2023). In contrast, some significant negative correlations indicated competitive interactions, such as between TP47 and LW58 (r = −0.60*) and between TP49 and LW58 (r = −0.60*), where the growth of one isolate was accompanied by a decline in the other. Such negative correlations suggest antagonistic or competitive relationships, which are well-documented in microbial interaction studies (Rivett et al., 2021; Ghoul & Mitri, 2023). Meanwhile, other isolates such as PL53, PL56, and BN24 did not show significant correlations with any other isolates, suggesting that their colony growth patterns were relatively independent and less influenced by the presence of other isolates (Miguel Trabajo et al., 2024). Overall, these findings indicate the presence of groups of isolates that tend to grow synergistically, as well as other groups that appear to compete under the same incubation conditions, reflecting the complex ecological dynamics within bacterial communities (Shade et al., 2022; Madsen et al., 2023).

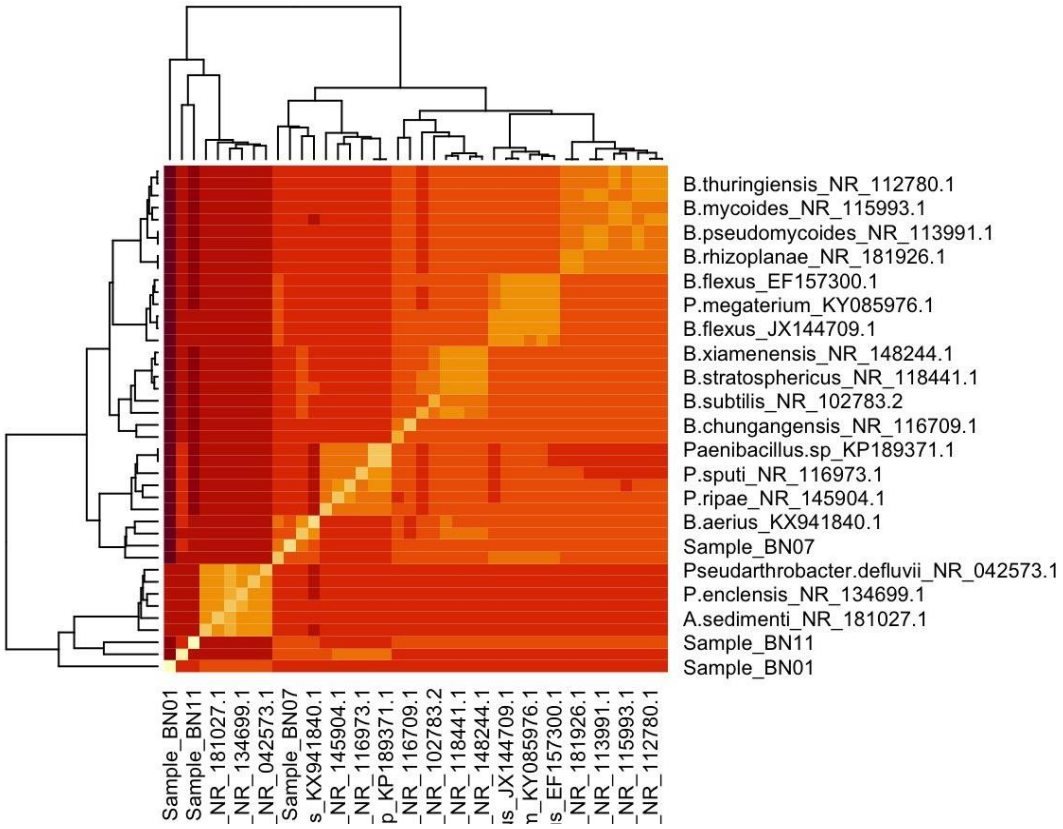

Fig. 6: Dendrogram of phylogenetic tree analysis of six bacterial isolates based on 16S ribosomal RNA sequences.

Table 3: Correlation Analysis of Colony Number with Incubation Time

Isolat	BN01	BN11	BN07	TP47	TP49	PL53	PL56	LW31	BN24	LW58
BN01	1.00**	-0.06tn	-0.39tn	-0.05tn	0.11tn	-0.11tn	0.11tn	-0.42tn	-0.20tn	-0.11tn
BN11		1.00**	-0.16tn	0.14tn	0.65*	-0.17tn	0.16tn	-0.19tn	0.34tn	-0.44tn
BN07			1.00**	-0.26tn	-0.27tn	0.02tn	0.27tn	0.89**	-0.14tn	0.52tn
TP47				1.00**	0.13tn	-0.12tn	0.18tn	-0.12tn	0.22tn	-0.60*
TP49					1.00**	-0.42tn	0.60*	-0.31tn	-0.23tn	-0.60*
PL53						1.00**	-0.40tn	0.32tn	0.03tn	0.36tn
PL56							1.00**	0.32tn	-0.56tn	-0.36tn
LW31								1.00**	-0.21tn	0.52tn
BN24									1.00**	-0.15tn
LW58										1.00**

Conclusion

Rhizosphere bacterial isolates from shallot plants show potential as Plant Growth-Promoting Rhizobacteria (PGPR) through their ability to produce IAA, solubilize phosphate, and form biofilms. Among them, isolate TP49 was the most effective in enhancing plant growth. Molecular analysis revealed high genetic diversity and close relationships to beneficial genera such as *Bacillus*, *Paenibacillus*, and *Arthrobacter*. The diverse interactions among isolates (synergistic, competitive, and independent) highlight their strong potential for application in agricultural biotechnology.

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Conflict of Interest: The authors declare no conflict of interest

Data Availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request. All primary data analyzed in this study are included in this article.

Ethics Statement: This study did not involve human or animal subjects. Therefore, no ethical approval or ethics code from an ethics committee was required.

Author's Contribution: Suharman: Conceptualization, data collection, drafting the manuscript, and final revision. Fachira Ulfa: Conceptualization, data collection and tabulation, drafting the manuscript, and final revision; Baharuddin: Conceptualization, drafting the manuscript, and final revision Katriani Matnja: Conceptualization, data collection and tabulation, and final revision.

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