**Exploration of Local Shallot Rhizobacteria with IAA Production and Phosphate Solubilization Ability for Improving Shallot Growth in Saline Soil**

Abstract

Shallot (*Allium ascalonicum* L.) is an important commodity with a growing global demand. However, the expansion of shallot cultivation is limited by the decreasing availability of fertile land and increasing soil salinity. This study investigated the potential of rhizosphere bacteria as plant growth-promoting rhizobacteria (PGPR) to improve shallot growth in salinity-affected soils. This research aims to find bacteria that have the ability to produce indole-3-acetic acid (IAA), dissolve phosphate, and are able to grow at high salinity levels. Research was conducted in Brebes Regency, Indonesia, using a survey method and purposive sampling technique. A total of ten isolates were successfully selected based on their characteristics as PGPR capable of producing IAA and solubilizing phosphate. The results showed that the highest IAA production was observed in isolate KL1K2, while the highest amount of phosphate dissolution was recorded in isolate KL3ASH1. In addition, isolates KL1K2, KL4ASH3, K4K1, K4P1, K4RZ3, K4RZ6, K3RZ1, K3IS4, KL3ASH1, KL1K2, and K4RZ6 showed nitrogen fixation ability. Isolates KL1K2, K3RZ1, K4RZ3, K4P1, and KL4ASH3 can also produce siderophores.

*Keywords*: Shallot, PGPR, IAA, phosphate solubilization, saline soil.

**Introduction**

Shallots (*Allium ascalonicum* L.) are a vital horticultural commodity in many developing countries, including Indonesia. In Indonesia, shallots are also considered a national strategic crop, where they are not only essential for culinary use but also play a significant role in the agro-economy and socio-economy (Saptana et al., 2021).

However, the sustainability of shallot production is increasingly threatened by several problems, including land degradation and the presence of unfit agricultural prospective land nearby the coastal area, which is high in salt concentration. These challenges are further compounded by climate change, which alters precipitation patterns and intensifies drought and seawater intrusion. Salinity stress adversely affects plant physiology, leading to a reduction in water uptake, inhibition of nutrient absorption, and, consequently, a decline in crop productivity. The sensitivity of shallots to these abiotic stressors has been well-documented, often resulting in substantial yield reductions, as evidenced by research conducted by Trisnaningsih et al. (2023). The impact of salt concentration in soils on shallot growth and yield is a salient example of this sensitivity.

Traditionally, farmers have relied on synthetic chemical fertilizers to enhance crop productivity. While these inputs can yield immediate gains, their prolonged utilization frequently results in deleterious effects such as soil acidification, nutrient imbalances, and a decline in soil microbial diversity. The escalating costs of agrochemicals impose financial constraints on small-scale farmers, while environmental concerns surrounding groundwater contamination and greenhouse gas emissions underscore the need for more sustainable agricultural practices.

In this context, plant growth-promoting rhizobacteria (PGPR) have emerged as a promising eco-friendly alternative. PGPR are beneficial soil microorganisms that colonize plant roots and enhance plant growth. These bacteria facilitate plant growth through various mechanisms, including increasing the availability of essential nutrients like nitrogen (N), phosphorus (P), and potassium (K) (Amalia et al., 2025). These microbial traits enhance nutrient availability and help plants better tolerate abiotic stress, such as salinity. This makes PGPR a potential tool for sustainable shallot cultivation under changing climatic conditions.

Previous studies on PGPR have largely focused on isolates obtained from non-saline environments or relied on commercial microbial strains, which may not be well-adapted to saline soils or specific agroecological conditions found in Indonesia (Egamberdieva et al., 2017). There remains a significant knowledge gap regarding the potential of native PGPR strains isolated from saline soils, particularly in key shallot-producing regions such as Brebes, Central Java.

The objective of this study is to explore, investigate and describe local shallot rhizosphere bacterial isolates that possess potential as plant growth-promoting rhizobacteria (PGPR), exhibiting characteristics such as indole acetic acid (IAA) production, phosphate solubilization, nitrogen fixation, and siderophore production for improving shallots’s growth.

**Methods**

Study Site and Sampling Method

This study was conducted from August 2024 to December 2025 in the coastal agricultural area of Brebes Regency, Central Java, Indonesia. The region is known for shallot cultivation under saline soil conditions. Rhizosphere soil samples were collected from the root zone (10–15 cm depth) of healthy shallot plants using purposive sampling. During sampling, in situ measurements of soil parameters were taken, including soil pH, moisture content, and temperature, using portable sensors. Soil electrical conductivity (EC) was measured later in the laboratory using a digital EC meter (modification from Joshi et al., 2021).

Isolation of Rhizobacteria

Rhizobacteria were isolated using serial dilution and spread plate techniques. One gram of rhizosphere soil was suspended in 9 mL of sterile distilled water and serially diluted up to 10⁻⁶. Each dilution was plated on Nutrient Agar (NA) supplemented with NaCl (5 mS/cm) to simulate saline soil conditions, Yeast Mannitol Agar (YMA) supplemented with Congo red, and Ashby. Plates were incubated at room temperature (~28°C) for 3-7 days. Distinct colonies were purified through repeated streaking and maintained as pure cultures (modification from Joshi et al., 2021).

Salinity Tolerance Test

To evaluate the salinity tolerance of the isolates, cultures were inoculated into nutrient broth media supplemented with NaCl at concentrations of 3%, 7%, and 8% (w/v). The tubes were incubated at room temperature for 72 hours under static conditions. Bacterial growth was monitored visually and measured spectrophotometrically at 600 nm (Manshur et al., 2020).

Phosphate Solubilization Test

Qualitative phosphate solubilization was assessed on Pikovskaya’s agar by observing halo formation after 7 days of incubation. For quantitative analysis, isolates were cultured in 10 mL of Pikovskaya’s broth and incubated at room temperature for 7 days under shaking conditions. After centrifugation, the soluble phosphate content in the supernatant was determined using the molybdenum blue method. Briefly, 1 mL of filtrate was mixed with ammonium molybdate and stannous chloride reagents, and the absorbance was measured at 693 nm. Phosphate concentration was calculated based on a KH₂PO₄ standard curve (Amri et al., 2023; Larasati, 2018).

Indole-3-Acetic Acid (IAA) Production Test

Isolates were grown in nutrient broth supplemented with 0.2% L-tryptophan and incubated at room temperature for 48 hours under shaking conditions. After centrifugation, 1 mL of the supernatant was reacted with 2 mL of Salkowski reagent and incubated in the dark for 30 minutes. Development of a pink coloration was quantified at 530 nm using a spectrophotometer, and IAA concentration was determined from a standard curve (Amalia et al., 2020).

Siderophore Production Test

Siderophore production was assessed qualitatively on Chrome Azurol S (CAS) agar. Cultures were spot-inoculated and incubated at room temperature for 3 days. Orange or yellow halos around the colonies were indicative of siderophore synthesis (Agunbiade et *al*., 2024).

Nitrogen Fixation Ability Test

Isolates were streaked onto Nitrogen-Free Bromthymol Blue (NfB) and incubated for 7 days. Growth on Nfb indicated atmospheric nitrogen fixation potential. Nitrogenase activity was measured using the Acetylene Reduction Assay (ARA). Bacterial isolates were inoculated into tubes containing nitrogen-free liquid medium (NFb broth) and incubated at room temperature for 48 hours. After incubation, 10% of the headspace air in each tube was replaced with acetylene gas (C₂H₂), and the tubes were sealed tightly and incubated for an additional 24 hours. The production of ethylene (C₂H₄), resulting from the reduction of acetylene by the nitrogenase enzyme, was measured using gas chromatography (GC). The results were expressed in units of μmol C₂H₄/mL/h as an indicator of nitrogen fixation activity (Montez-Luz et al., 2022).

**Result and Discussion**

Physicochemical Characteristics

Table 1. Soil temperature, pH, moisture, and electrical conductivity measurements in shallot rhizosphere at Kaliwlingi

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sampling Site | Temperature  (oC) | pH | Moisture (%) | Electrical conductivity/EC (mS/cm) |
| 1 | 25 | 7.9 | 45 | 5 |
| 2 | 34.5 | 6.8 | 50 | 4 |
| 3 | 34.5 | 7.5 | 50 | 4 |
| 4 | 28 | 8 | 49 | 5 |

Note: Sampling site (1, 2, 3, 4) indicating the sampling distance (km) from the shoreline.

The physicochemical properties of shallot rhizosphere soil collected from four sites in Kaliwlingi are presented in Table 1. The soil temperature ranged from 25°C to 34.5°C, reflecting typical microclimatic variation in coastal agricultural environments. Such temperature ranges are within the tolerance limits for mesophilic soil bacteria, including most plant growth-promoting rhizobacteria (PGPR) (Glick, 2012). Meanwhile, sampling site no. 2 is eliminated from this study as it performed neutral pH (not saline soil).

The soil pH ranged from 7.5 to 8.0, indicating slightly to moderately alkaline conditions. Soil pH influences the solubility of nutrients and the composition of microbial communities. Alkaline pH tends to limit the availability of phosphorus and micronutrients, potentially increasing the importance of phosphate-solubilizing bacteria (Nautiyal, 1999). Furthermore, several PGPR strains are adapted to alkaline soils and have been reported to maintain functional activity in such environments (Egamberdieva et al., 2017).

Soil moisture content was relatively stable across sites (45–50%), suggesting suitable water availability for microbial metabolism and root-associated interactions. Adequate soil moisture enhances microbial colonization and nutrient exchange within the rhizosphere (Cappuccino & Sherman, 2014).

Electrical conductivity (EC) values ranged from 4 to 5 mS/cm, classifying the soil as moderately saline according to USDA salinity standards. Soils with EC >4 mS/cm can hinder plant growth and affect microbial viability. However, salinity-tolerant PGPR can thrive under such stress and even contribute to alleviating salt-induced damage in host plants (Upadhyay et al., 2011). Therefore, the rhizosphere environment in Kaliwlingi provides an appropriate ecological context for isolating halotolerant PGPR with potential application in salinity-stressed agricultural systems.

Salinity Tolerance Test

Table 2.  Number and codes of selected isolates

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Isolate code | Sampling site | NaCl tolerance level | | |
| 3% | 5% | 8% |
| KL1K2 | 1 | + | - | - |
| KL3ASH1 | 3 | + | + | + |
| K3IS4 | 3 | + | + | + |
| K3RZ1 | 3 | + | + | + |
| K4RZ6 | 4 | + | + | + |
| K4K6 | 4 | + | + | + |
| K4RZ3 | 4 | + | + | + |
| K4P1 | 4 | + | + | + |
| K4K1 | 4 | + | + | + |
| KL4ASH3 | 4 | + | + | + |

Salt tolerance testing was conducted to assess the ability of bacterial isolates to grow under high salinity conditions, specifically at NaCl concentrations of 3%, 7%, and 8%. All isolates were able to grow at 3% NaCl, indicating a basic tolerance to low salinity. However, only one isolate, KL1K2, failed to grow at 7% and 8% NaCl, while the remaining nine isolates continued to grow even at higher salt concentrations. This result suggests that most of the isolates exhibit strong salinity tolerance and have potential for application in soils with moderate to high salinity levels.

High salinity creates osmotic stress that inhibits the growth of non-halotolerant microbes. However, certain PGPR are physiologically adapted through mechanisms such as exopolysaccharide production, osmolyte regulation (e.g., proline or glycine betaine), and stabilization of enzyme function under hyperosmotic conditions (Nguyen et al., 2019; Szymańska et al., 2020). These adaptations allow the bacteria to remain active and continue supporting plant growth under salt stress. The ability of the isolates to survive up to 8% NaCl indicates that most bacteria isolated from shallot rhizosphere in coastal areas are already adapted to extreme abiotic stress, including salinity. This tolerance is a key criterion for selecting PGPR candidates suitable for use in marginal lands, such as coastal and tidal zones that are increasingly affected by climate change (Mansour et al., 2021). The failure of KL1K2 to grow at 7–8% NaCl suggests a limited salinity tolerance, possibly due to differences in cell membrane structure or an inability to efficiently synthesize osmoprotective compounds (Ali et al., 2020).

Phosphate Solubilization

Fig. 1. Phosphate solubilization capacity in selected isolate

All isolates exhibited the ability to solubilize inorganic phosphate, with values ranging from 4.49 to 16.05 ppm. Isolate KL3ASH1 recorded the highest solubilization activity (16.05 ppm), followed by K4K6 (11.67 ppm), while several others fell within the moderate range of 6–8 ppm. Phosphate solubilization is one of the key mechanisms by which PGPR enhance phosphorus availability for plants. In many soils, phosphate exists in insoluble forms that cannot be directly absorbed by plant roots. Phosphate-solubilizing bacteria (PSB) produce organic acids such as gluconic and citric acid, which reduce local pH and release phosphate ions from their bound forms (Sharma et al., 2013; Zaidi et al., 2016).

The high solubilization value observed in KL3ASH1 indicates strong potential for improving phosphate fertilization efficiency in saline soils, which typically have lower available phosphorus due to precipitation with calcium and magnesium ions (Chen et al., 2021). Nonetheless, isolates with moderate solubilizing activity remain functionally relevant, as phosphate availability in the rhizosphere is also influenced by environmental interactions and microbial dynamics (Alori et al., 2017). The combination of phosphate-solubilizing ability and salt tolerance is a critical factor in selecting promising PGPR strains. While some isolates may not exhibit the highest solubilization levels, their ability to grow under high salinity and maintain functional activity still makes them worthy of further evaluation in plant-based assays.

IAA Production Assay

Fig. 2. IAA production of selected isolate

All isolates demonstrated the ability to produce IAA in the range of 1.25 to 2.01 mg/L. The highest production was recorded in isolate KL1K2 (2.01 mg/L), followed by others that generally ranged between 1.56 and 1.90 mg/L. IAA is a key phytohormone from the auxin group that plays an important role in promoting root elongation, cell division, and vascular tissue development in plants (Spaepen et al., 2014). IAA production by PGPR is known to enhance nutrient and water uptake, especially under abiotic stress such as salinity (Arora & Ramawat, 2017).

Although KL1K2 produced the highest level of IAA, it did not tolerate high salinity (7–8% NaCl). This indicates that high IAA production does not necessarily correlate with stress tolerance. In fact, excessive IAA levels may be counterproductive, as too much auxin can inhibit root growth and lead to hormonal imbalance in plants (Vacheron et al., 2013; Remans et al., 2014). Therefore, isolates that produce moderate but stable levels of IAA under stress conditions are more suitable as potential PGPR candidates.

In this study, most isolates produced IAA at concentrations considered optimal for promoting plant growth without inducing adverse effects. This confirms that the isolates from shallot rhizosphere in saline soils of Kaliwlingi are not only physiologically active but also hold strong potential for application in sustainable agriculture systems.

Nitrogen Fixation Ability and Siderophore Production

Table 3. Nitrogen fixation ability and siderophore production

|  |  |  |
| --- | --- | --- |
| Isolates Code | Nitrogen Fixation | Siderophore Ability |
| KL1K2 | + | + |
| KL3ASH1 | + | + |
| K3IS4 | - | + |
| K3RZ1 | + | + |
| K4RZ6 | + | - |
| K4K6 | - | - |
| K4RZ3 | + | + |
| K4P1 | + | + |
| K4K1 | + | + |
| KL4ASH3 | + | + |

Qualitative nitrogen fixation was evaluated using nitrogen-free bromothymol blue (Nfb) medium, where eight out of ten isolates demonstrated visible growth. The isolates showing positive results on Nfb were KL1K2, KL3ASH1, K3IS4, K3RZ1, K4RZ6, K4RZ3, K4P1, and KL4ASH3. In contrast, isolates K4K1 and K4K6 did not grow, indicating the absence of biological nitrogen fixation ability. Nitrogen fixation is a key trait of PGPR, particularly relevant for improving soil fertility in the absence of synthetic nitrogen inputs. Nitrogen-fixing bacteria convert atmospheric nitrogen into ammonia, a form readily taken up by plants. This trait is especially valuable in marginal environments such as saline soils, where fertilizer efficiency is often reduced and input costs are high (Rilling et al., 2020).

Siderophore production was assessed using Chrome Azurol S (CAS) medium. Most isolates produced siderophores, except for K4K1, K4K6, K4RZ3, and KL4ASH3. Siderophores enhance iron availability in the rhizosphere, particularly under Fe-deficient conditions such as alkaline and saline soils. In addition, they contribute to biological control by limiting iron access to soilborne pathogens through competitive mechanisms (Ahmed & Holmström, 2014; Sharma et al., 2021).

Two isolates, KL3ASH1 and K3IS4, demonstrated outstanding performance, meeting all the observed PGPR criteria—including nitrogen fixation, phosphate solubilization, IAA production, high salinity tolerance, and siderophore synthesis. These isolates were therefore selected for further evaluation using the Acetylene Reduction Assay (ARA) to quantify their nitrogen-fixing capacity more accurately (Jha & Subramanian, 2014).

Fig. 3. Nitrogenase activity of selected isolate

Nitrogenase activity was assessed using the Acetylene Reduction Assay (ARA) on two selected isolates, KL3ASH1 and K3IS4, as both fulfilled all major PGPR criteria. The results indicated that KL3ASH1 exhibited the highest nitrogenase activity at 0.069 μmol C₂H₄/mL/h, while K3IS4 recorded an activity of 0.044 μmol C₂H₄/mL/h.

ARA is a widely used indirect quantitative method for measuring biological nitrogen fixation, based on the enzymatic reduction of acetylene (C₂H₂) to ethylene (C₂H₄) by nitrogenase (Hardy et al., 1968; Jha & Subramanian, 2014). Higher ARA values indicate greater potential for atmospheric nitrogen fixation. The activities observed in both isolates are considered moderate but stable, suggesting their suitability for field application, particularly in saline soils, which are often nitrogen-deficient.

Moreover, the presence of complementary traits such as phosphate solubilization, IAA production, and salinity tolerance further supports the multifunctional potential of these isolates as effective PGPR (Bhattacharyya et al., 2020).

**Conclusion**

Ten bacterial isolates from shallot rhizosphere in saline soil were successfully characterized. Most isolates were tolerant to up to 8% NaCl and exhibited various PGPR traits, including IAA production (1.26–2.01 mg/L), phosphate solubilization (4.49–16.05 ppm), nitrogen fixation, and siderophore production. KL3ASH1 and K3IS4 were identified as the most promising strains, meeting all selection criteria and demonstrating stable nitrogenase activity. These isolates are potential candidates for bio-inoculant development, particularly for saline soil applications.

**Recommendation**

Further studies should include molecular identification through 16S rRNA sequencing, greenhouse trials to evaluate plant growth effects, and bio-inoculant formulation testing under variable environmental conditions prior to field application.

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