CarakaTani
Journal of Sustainable Agriculture
ISSN 2613-9456 (Print) 2599-2570 (Online)

Caraka Tani: Journal of Sustainable Agriculture, 40(4), 510-526, 2025
URL: https://jurnal.uns.ac.id/carakatani/article/view/107138
DOI: http://dx.doi.org/10.20961/carakatani.v40i4.107138

Halotolerant Rhizobacteria Isolated from Salinity-Impacted Marginal Soils: Characterization and Potential for Plant Growth Promotion

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Abstract

Soil salinity is a significant constraint on crop productivity, particularly in marginal lands, and sustainable biological approaches are needed to mitigate its impact. Plant growth-promoting rhizobacteria (PGPR) with halotolerance represent promising candidates for enhancing plant resilience under saline stress. This study aimed to isolate halotolerant PGPR from saline-impacted soils in Pekalongan, Indonesia, and evaluate their potential to improve plant growth under salinity stress. Five bacterial isolates (WN-01 to WN-05) were successfully obtained. The isolates displayed multiple PGPR traits, including nitrogen fixation, phosphate solubilization, indole-3-acetic acid (IAA) synthesis, siderophore release, cellulase activity, 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, exopolysaccharide production, and tolerance to salinity up to 9% NaCl. Molecular identification confirmed the isolates as *Bacillus subtilis* (WN-01), *Priestia megaterium* (WN-02), *Pseudomonas segetis* (WN-03), *Bacillus pumilus* (WN-04), and *Bacillus cereus* (WN-05). Compatibility analysis indicated their potential to be formulated as a consortium bioinoculant. *In vivo* pot experiments using sweet maize (*Zea mays* saccharata var. Bonanza F1) under saline conditions (4 dS m^{-1}) showed that consortium application, especially at 10^8 CFU ml^{-1}, significantly enhanced plant height, leaf surface area, and chlorophyll content. Moreover, the total microbial population in soil increased proportionally with inoculum density, with the highest values recorded in the 10^8 CFU ml^{-1} treatment. These findings demonstrate that local halotolerant PGPR have strong potential as bioinoculants to support crop growth and soil health in saline-impacted marginal lands.

Keywords: ACC deaminase; bioinoculant; halotolerant rhizobacteria; phosphate solubilization; salinity stress

Cite this as: Putri, S. R., Nugroho, L. H., Sukirno, & Retnaningrum, E. (2025). Halotolerant Rhizobacteria Isolated from Salinity-Impacted Marginal Soils: Characterization and Potential for Plant Growth Promotion. *Caraka Tani: Journal of Sustainable Agriculture*, *40*(4), 510-526. doi: http://dx.doi.org/10.20961/carakatani.v40i4.107138

INTRODUCTION

Soil salinity is one of the primary obstacles in lowland and coastal agricultural systems, threatening the sustainability of food crop production (Mukhopadhyay et al., 2021; Sahab et al., 2021). Land exposed to salt experiences a decrease in fertility due to the disruption of ion balance in the root zone, thereby hindering the accessibility and uptake of vital nutrients and water by plants (Chaudhry et al., 2022; Balasubramaniam et al., 2023). Ions like Na^+ and Cl^+ generate osmotic stress and ionic toxicity, which harm plant cells' structure and functionality

[*] *Received for publication July 30, 2025*
Accepted after corrections August 27, 2025

(Hasanuzzaman et al., 2021). As a result, enzymatic activity, photosynthesis processes, and vegetative growth are disrupted, significantly reducing crop yields (Chaudhry et al., 2022; Balasubramaniam et al., 2023).

Global data shows that more than 20% of agricultural land is currently affected by salinity. This trend is expected to increase due to seawater intrusion, climate change, and environmentally unfriendly agricultural practices such as excessive use of chemical fertilizers (Shahid et al., 2018; Mohanavelu et al., 2021). In Indonesia, this phenomenon is becoming more pronounced in the northern coastal areas of Java, such as Pekalongan and Demak, where the rate of saltwater intrusion reaches more than 1% per year (Karolinoerita and Annisa, 2020; Oelviani et al., 2024). These lands are marginal because they cannot support optimal crop production without adequate technological intervention.

Various approaches have been developed to address salinity issues, including the engineering of salt-resistant varieties, the use of inorganic ameliorants, and the modification of irrigation systems (Kumawat et al., 2022). However, biological approaches based on soil microorganisms are gaining attention due to their environmentally friendly, economical, and adaptive nature to local conditions (Mokrani et al., 2022). Plant growth-promoting rhizobacteria (PGPR) represent one of the most promising biological agents, consisting of bacterial groups that establish symbiotic associations within the rhizosphere (Kumawat et al., 2022) and have the ability to enhance plant growth through various physiological and biochemical mechanisms (Chandran et al., 2021).

It is well known that the PGPR contributes to the production of growth hormones such as indole acetic acid (IAA), phosphate solubilization, and nitrogen fixation, in addition to generating siderophores and exopolysaccharides (EPS) that enhance plant resilience to abiotic stress (Chandran et al., 2021; Hasan et al., 2024). However, most commercially available PGPR strains originate from normal soil environments, so their effectiveness decreases when applied to land with high salinity (Biswas et al., 2021). Therefore, a more location-specific approach is needed to explore local microbes from saline soils naturally adapted to ionic and osmotic stress.

Bacteria known as halotolerant PGPR can endure and maintain their biological activity in saline environments. Several genera, such as *Bacillus*, *Halomonas*, and *Pseudomonas* have been reported to show promotive activity toward plant growth even in media with high NaCl concentrations (Arora et al., 2020; Kumar et al., 2023). These microorganisms can increase nutrient availability, reduce osmotic pressure, and stimulate the plant's systemic resistance response. The applicative potential of halotolerant PGPR is limited to growth enhancement and includes improving soil physicochemical properties and contributions to fertilization efficiency.

Halotolerant PGPR-based bioinoculants can be developed through single formulations or multi-strain consortia. Although single inoculants are easier to formulate and test, consortia show advantages in terms of functional stability, higher root colonization ability, and potential synergy in facing environmental stress (Santoyo et al., 2021; Ali et al., 2025). Previous studies have shown that combining several compatible PGPR strains can produce more significant additive or synergistic effects than single applications, particularly in improving plant tolerance to abiotic stress and nutrient uptake (Chandran et al., 2021).

However, research on halotolerant PGPR in Indonesia is still very limited, especially those originating from coastal areas with high salinity stress. However, the geographical and edaphic conditions of Indonesia greatly support the unique diversity of rhizosphere microbes that have the potential to be developed as location-specific biological agents. Exploration of local microbes from saline soil in Pekalongan, for example, becomes a strategic step in supporting sustainable agriculture in marginal areas.

In sustainable agriculture, halotolerant PGPR offers a nature-based solution to restore soil health and maintain crop productivity in salt-impacted lands. Unlike chemical inputs, microbial inoculants reduce dependency on synthetic fertilizers, improve nutrient-use efficiency, and enhance soil biological activity, all contributing to long-term ecosystem resilience (Biswas et al., 2021; Mohanavelu et al., 2021). These benefits are particularly relevant for developing countries, where smallholder farmers often face soil degradation, limited access to costly agrochemicals, and high vulnerability to climate change. Bioinoculants can strengthen food security by utilizing locally adapted microbial resources while reducing environmental footprints, aligning with global agendas for sustainable intensification and climate-smart agriculture (Kumar et al., 2023).

Thus, this study aims to isolate and identify halotolerant PGPR from saline soil in the

Pekalongan Region, Central Java, Indonesia. The obtained isolates will be evaluated based on their functional abilities to support plant growth under salinity stress through *in vitro* biochemical property tests and pot bioassays in the greenhouse. This research is expected to produce candidates for bioinoculants based on local microbes that are adaptive to saline conditions and have the potential to be developed into superior biofertilizers for the productive and sustainable utilization of marginal lands.

MATERIALS AND METHOD

Experimental time, sites, and sample materials

The research was conducted between March and September 2024. Rhizospheric soil samples were collected from saline-impacted marginal land with an electrical conductivity of approximately 5 dS m^{-1} in the Wonokerto area, Pekalongan Regency, Central Java, Indonesia (109°41'52.0" E, 6°52'01.6" S). Laboratory analyses and experiments were conducted at the Mitra Persada 168 Microbiology Laboratory, Yogyakarta, Indonesia.

Methods

Sample collection and rhizobacteria isolation

Rhizopheric soil samples were obtained from soils adhering to the roots of *Cyperus rotundus*. Soil sampling was carried out on an area of 500 m^2, with root samples collected from 25 plants. The roots were cut and placed in a sterile conical flask containing phosphate-buffered saline (PBS). While being transported to the laboratory, samples were kept at 4 °C. The rhizobacteria isolated by suspending the sample in sterile PBS were vortexed for 5 minutes and serially diluted (Lakshmanan et al., 2017). The 10^{-4} dilution was inoculated onto nutrient agar (NA) (Himedia, India) supplemented with 5% NaCl and incubated for 48 hours at 28 °C. Colonies with distinct morphological features were purified on NA. Pure colonies were stored individually in NA slants (Mishra et al., 2023).

Rhizobacteria plant growth-promoting (PGP) traits screening

Phosphate solubilization assay

Overnight culture (OD_{600} 0.8-1.0) of individual isolates grown in nutrient broth (NB) (Himedia, India) was serially diluted and inoculated onto Pikovskaya's agar (Himedia, India). Plates were incubated at 28±2 °C for 72 hours. The formation of clear zone around the colonies identified phosphate-solubilizing isolates. The phosphate-solubilizing efficiency (PSE) was calculated according to Equation 1.

$$PSE = \frac{Z\text{-}C}{C} \times 100\% \quad (1)$$

Where Z represents the diameter of the clear zone (cm) and C is the colony diameter (cm) (Lihan et al., 2021).

Nitrogen fixation capability assay

The nitrogen fixation ability was tested using a nitrogen-free solid medium by Baldani and Dobereiner (1980) with modifications. The nitrogen-free medium was composed of the following components per liter: 0.1 g NaCl, 4.5 g KOH, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 5 g malic acid, 0.5 g K_2HPO_4, and 15 g agar. The medium is enriched with 2 ml l^{-1} micronutrient solution (including $CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, H_3BO_3, $Na_2MoO_4 \cdot 2H_2O$, and $MnSO_4 \cdot H_2O$), 4 ml l^{-1} 1.64% Fe-EDTA solution, 2 ml l^{-1} bromothymol blue (5 g/0.2 N KOH), along with 1 ml l^{-1} of vitamin mix, prepared by dissolving 10 mg biotin and 20 mg pyridoxal HCl in 100 ml of pre-warmed distilled water. The pure isolate was streaked and incubated at 30 °C for 48 hours—the change in the medium's color to blue shows the ability to fix nitrogen.

Cellulolytic activity assay

Using the pour plate technique, the overnight-grown bacterial culture was serially diluted and inoculated into carboxymethyl cellulose (CMC) media. The CMC medium (per liter) consists of 10 g CMC, 0.2 g $MgSO_4$, 0.75 g K_2HPO_4, 0.02 g $FeSO_4 \cdot 7H_2O$, 0.04 g $CaCl_2$, 2 g yeast extract, 1 g glucose, and 15 g agar. Incubation was carried out at 28 °C for 48 hours. The surface of the agar was then flooded with 1% Congo red solution and left for 15 minutes, followed by sterile 1 M NaCl solution to remove excess dye. Cellulase activity is indicated by forming a clear zone surrounding the colony (Oo et al., 2020).

Salinity tolerance assay

Each isolate was streaked onto NA plates supplemented with NaCl at varying concentrations: 0 to 9% (w/v). The plates underwent a 5-day incubation period at 30±2 °C. Growth at higher salt concentrations was used to evaluate the isolates' capacity for salt tolerance (Oo et al., 2020).

Siderophore

Siderophore production was evaluated using Luria Bertani (LB) agar medium enriched with Chrome Azurol S (CAS) solution in a 9:1 ratio

(pH 6.8) (Fuadi et al., 2022). The CAS reagent was made by mixing three sterile solutions: (a) 0.061 g CAS in 50 ml distilled water, (b) 0.027 g $FeCl_3$ in 10 ml 10 mM HCl, and (c) 0.073 g hexadecyltrimethylammonium bromide (HDTMA) in 40 ml distilled water. A total of 50 ml of solution (a), 10 ml of (b), and 20 ml of (c) were combined, then mixed into sterile LB agar. The isolate was streaked on the agar and incubated at 30 °C for 5 days. Siderophore production was indicated by the formation of a coloured halo around the colonies, resulting from iron (Fe) chelation and dye displacement.

1-aminocyclopropane-1-carboxylate (ACC) deaminase production assay

Each isolate was cultured in Dworkin-Foster Minimal Salt medium (pH 7.2), prepared per liter with the following constituents: 6 g Na_2HPO_4, 2 g glucose, 0.2 g $MgSO_4 \cdot 7H_2O$, 10 mg H_3BO_3, 124.6 mg $ZnSO_4 \cdot 7H_2O$, 2 g citric acid, 11.19 mg $MnSO_4 \cdot H_2O$, 4 g KH_2PO_4, 1 mg $FeSO_4 \cdot 7H_2O$, 10 mg MoO_3, 78.22 mg $CuSO_4 \cdot 5H_2O$, 4 ml gluconic acid and 15 g agar. Sterile filtered ACC solution (3 mM) was aseptically added as a nitrogen source (Ariyani et al., 2021). Incubation was conducted at 28 °C for 48 hours. Colony growth showed ACC deaminase activity (Chen et al., 2022).

EPS production assay

Each PGPR isolate was inoculated into 50 ml NB and incubated at 30 °C, 150 rpm for 24 hours. Cultures with $OD_{600} \geq 1.0$ were used for EPS extraction. Ten milliliters of each culture was centrifuged (10,000 rpm, 20 minutes), and the supernatant was mixed with cold ethanol and incubated at 4 °C overnight. Following a second centrifugation, the pellet was dried on filter paper and oven-dried at 60 °C. EPS production was quantified based on dry weight (Patel and Tandel, 2020).

IAA quantification

A modified procedure based on Oo et al. (2020) was used to measure the synthesis of IAA. After transferring 500 µl of the bacterial suspension into 50 ml of NB supplemented with 1% tryptophan, the mixture was continuously shaken at 150 rpm for 72 hours in a dark environment at 30 °C. The culture was centrifuged for 10 minutes at 10,000 rpm following incubation. One milliliter of the resultant supernatant was mixed with 2 ml of Salkowski reagent and allowed to stand for half an hour. The development of a pink hue was seen as a sign that IAA synthesis was occurring. A quantitative analysis was conducted using a spectrophotometer set to 520 nm and a standard calibration curve for IAA.

Rhizobacteria isolates compatibility

The compatibility tests among PGPR isolates were performed using a modified cross-streak technique on NA media (Khan et al., 2022). The petri dishes were incubated at 28 °C for 48 hours. The overlapping growth indicates compatibility, whereas the inhibition zones indicate antagonistic interactions between the isolates.

Rhizobacteria molecular identification

Bacterial genomic DNA was isolated using the Quick-DNA MagBead Plus Kit (Zymo Research). Amplification of the 16S rRNA gene was performed via PCR using MyTaq HS Red Mix 2× (Bioline) and universal primers 27F (5′–AGAGTTTGATCMTGGCTCAG–3′) and 1492R (5′–TACGGYTACCTTGTTACGACTT–3′). The PCR reaction was performed in a total volume of 25 µl, with a final primer concentration of 0.4 µM and a DNA template amount ranging from 5 to 100 µg. PCR's thermal cycle includes a minute initial denaturation at 95 °C, 35 cycles of denaturation at 95 °C for 10 seconds, annealing at 52 °C for 15 seconds, and extension at 72 °C for 15 seconds. On the MinION Mk1B platform, the PCR products were subsequently sequenced (Kai et al., 2019).

PGP experiment

This study generated combinations of previously screened isolates in liquid form with cell density series of 10^4, 10^5, 10^6, 10^7, and 10^8 CFU ml^{-1}. The isolate combinations were evaluated on sweet corn plants (*Zea mays saccharata* Sturt.) var Bonanza F1 was grown on soil with a salinity of 4 dS m^{-1} as the model organism. Immersed in distilled water for 24 hours, corn seeds were sown in soil treated with 25 ml of the liquid isolate combination formulation for each pot. The negative control (C-) was applied with distilled water, whereas the positive control (C+) received a commercial liquid biofertilizer treatment. The experiment was performed with five replications. Plant height, leaf surface area, and chlorophyll (Ali et al., 2021) were measured on the 20th day post-planting.

Total count of soil microorganisms

Ten grams of air-dried, sieved soil (2 mm) from each treatment were suspended in 95 ml sterile 0.85% NaCl solution and agitated at 150 rpm for 30 minutes to release soil microorganisms. The suspension underwent serial dilution (10^{-1} to 10^{-8}), and 0.1 ml aliquots from the

dilutions were spread in triplicate on NA plates. Plates were incubated at 30±2 °C for 48 hours, and colonies (CFU) were enumerated manually using a digital counter (Lee et al., 2021).

Data analysis

Data were analyzed using one-way ANOVA and Duncan's test at $p < 0.05$ with SPSS version 27. Normality and homogeneity of variance were checked before analysis. Additional descriptive statistics were processed in Microsoft Excel 2021.

RESULTS AND DISCUSSION

Isolation, characterization, and properties of halotolerant PGPR

Five dominant halotolerant rhizobacteria isolates (WN-01, WN-02, WN-03, WN-04, WN-05) were successfully isolated and purified using NA media enhanced with 5% NaCl (w/v) at a dilution of 10^{-4}. NaCl concentration provides an intense osmotic pressure comparable to 855 mM NaCl for the rhizobacteria in the sample, thereby eliminating non-halotolerant rhizobacteria (Khumairah et al., 2022). The osmotic pressure in the medium will induce the emergence of osmoregulatory mechanisms, which involve the activation of Na^+/H^+ antiport pumps to preserve intracellular ion balance and the buildup of suitable solutes like glycine betaine (Wang et al., 2019; Imhoff et al., 2021). The stable growth of the isolates under those conditions indicates a halotolerant capacity that exceeds the tolerance range of most soil bacteria (1 to 3% NaCl) and provides an ecological advantage due to salinity stress (AbuQamar et al., 2024). The five halotolerant rhizobacteria were then used to evaluate PGP traits.

Of the five halotolerant rhizobacteria isolates, only WN-01 showed phosphate solubilization activity with a solubilization index (PSI) of 6 on the Pikovskaya medium (Table 1). This indicates a unique ability to provide dissolved phosphate in saline environments. In saline soils, phosphate often precipitates into insoluble forms through interactions with calcium (Ca^{2+}) and iron (Fe^{3+}) ions, drastically limiting its availability to plants (Dey et al., 2021). According to Oo et al. (2020), such conditions create a nutrient-deficient environment that hampers plant growth due to restricted phosphorus uptake. Halotolerant rhizobacteria such as WN-01 have been shown to dissolve phosphate through the secretion of organic acids, primarily oxalate, propionate, and gluconate, which lower the local pH and chelate metal ions, thereby releasing bound phosphate (Jiang et al., 2018; Widane et al., 2022). The halotolerant capacity of rhizobacteria to efficiently dissolve phosphate even under high salinity conditions is due to physiological adaptations such as the stability of phosphate-dissolving enzymes in hyperosmotic conditions, the efficiency of organic acid metabolism, and the ion pump system that maintains cellular homeostasis. In the research by Widane et al. (2022), *Burkholderia dolosa* PSR I11 and I12 isolated from arid marginal land solubilize phosphate with a PSI of 3.0 to 3.4. Furthermore, *Achromobacter* spp. and several strains of *Pseudomonas* from saline soil grown in 8 to 15% NaCl stress were able to solubilize up to 86 µg ml^{-1} of phosphate (Kapadia et al., 2022). It indicates that halotolerant phosphate-solubilizing bacteria (PSB) has stable and effective enzymatic and structural mechanisms under ionic and osmotic stress.

Isolates WN-01 to WN-04 showed nitrogen fixation activity in nitrogen-free (N_2) media, indicated by the color change of the medium from yellow to blue as an indication of increased pH due to ammonia accumulation (Figure 1). These findings suggest that although nitrogenase is generally very sensitive to salinity stress, the four isolates were still able to maintain their enzymatic activity, indicating the presence of physiological adaptations, such as enzyme structure stabilization through the accumulation of osmoprotectants and modifications to cell membrane structure. Similar results were reported by Khumairah et al. (2022) and Simarmata et al. (2023) who found that *Pseudomonas stutzeri* and *Klebsiella pneumoniae* not only remained active in producing nitrogenase under saline conditions but also produced organic acids that supported nitrogen fixation efficiency. Furthermore, Ji et al. (2024) demonstrate that the introduction of nitrogen-fixing bacteria with halotolerant ability, such as *Bacillus* spp. can increase the abundance of nitrogen-managing microbes in the plant rhizosphere through synergistic interactions with indigenous rhizobacteria. The ability of halotolerant rhizobacteria to maintain nitrogen fixation under salinity stress is closely related to adaptive strategies such as enzyme stabilization, ribosomal structure protection, and the synthesis of osmoprotectant substances (Sagar et al., 2022).

In this study, only the WN-05 isolate showed significant cellulolytic activity on CMC media, with a cellulolytic index of 2.1 (Table 1), indicating its ability to degrade cellulose. This ability can accelerate the decomposition of

Table 1. Identification results and PGP traits of halotolerant rhizobacteria isolates from salinity-impacted marginal soil

Isolate code	PGP traits								Identification result
	PSE	Nitrogen fixation	Cellulolytic	Salinity tolerance (%)	Siderophore	ACC deaminase	EPS production (g ml^{-1})	IAA production (µg ml^{-1})	
WN-01	+ (6)	+	-	7	+	+	0.016	0.170	*Bacillus subtilis*
WN-02	-	+	-	7	+	-	0.023	81.9	*Priesta megaterium*
WN-03	-	+	-	9	+	-	0.257	53.6	*Pseudomonas segetis*
WN-04	-	+	-	9	+	+	0.031	1.046	*Bacillus pumilus*
WN-05	-	-	+ (2.1)	9	+	+	0.009	9.25	*Bacillus cereus*

organic matter by halotolerant rhizobacteria to support the carbon cycle and enhance nitrogen and phosphorus availability through mineralization, especially in saline soils that are generally nutrient-deficient (Dey et al., 2021). It indicates that the decomposition of organic matter by halotolerant rhizobacteria speeds up the restitution of organic compounds that can be utilized by plants and other rhizobacteria (Mishra et al., 2023). Furthermore, cellulolytic capability enhances the health of saline soil microbial ecosystems by providing primary nutrient sources. Ma et al. (2024) investigated the identification of microorganisms that break down soil cellulose, such as *Rhodococcus wratislaviensis* and *Pseudomonas xanthosomatis*, which possess high-performance cellulase enzymes under various conditions, including hypersaline, emphasizing the importance of genetic adaptation and the expression of cellulolytic enzymes under stress conditions. Thus, isolate WN-05 exhibits significant cellulolytic activity and possibly possesses broad adaptive mechanisms such as a stabilized enzymatic system (Zverev et al., 2024).

Isolates WN-01 to WN-05 showed high salinity tolerance, with WN-01 and WN-02 surviving up to 7% NaCl and WN-03, WN-04, and WN-05 surpassing 9% NaCl (Table 1). The usual PGPR salinity tolerance threshold (~5 to 7% NaCl) is exceeded by this level, according to Latif et al. (2024) and Kapadia et al. (2022). This tolerance places the isolates in the intermediate to high halotolerant category, allowing them to function in soils with salinity approaching 10 dS m^{-1}. This capacity is crucial because the effectiveness of PGP traits depends on the bacteria's ability to survive and remain active in supporting plant growth under saline conditions (Li et al., 2025). The review by Oliva et al. (2023) also emphasizes that bacteria such as *Halomonas* spp. and *Bacillus* spp. are often resistant to 8 to 12% NaCl and capable of promoting plant development in harsh conditions through a combination of mechanisms such as EPS production, osmoprotectant accumulation, and adaptive enzymes.

The formation of colored zones surrounding colonies on CAS media (Figure 1) was the primary indicator that all isolates in this investigation could produce siderophores. The colored zones that appeared were caused by the separation of Fe from the blue chromophore complex (CAS–Fe^{3+}) by the siderophores produced by the microbes, resulting in a color

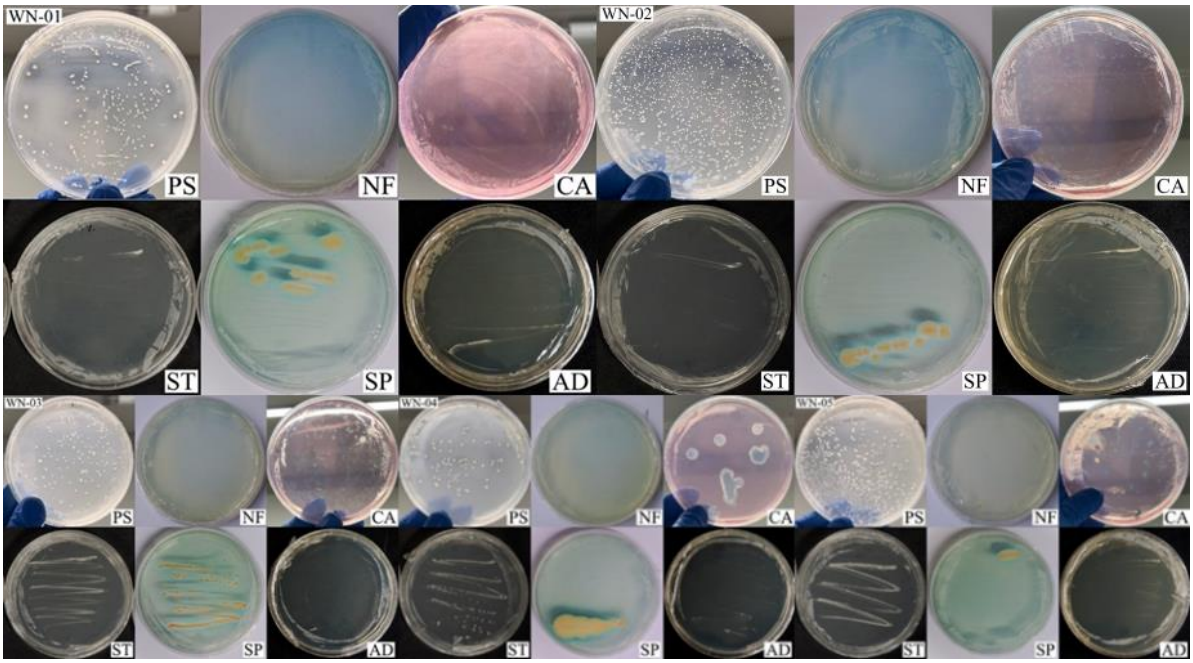

Figure 1. Five rhizobacteria were isolated from salinity-impacted soil (WN-01 to WN-05)
Note: All isolates show multiple PGP traits (Table 1). PS = Phosphate solubilization; NF = Nitrogen fixation; CA = Cellulolytic ability; ST = Salinity tolerance; SP = Siderophore; AD = ACC-deaminase activity

change as an indicator of chelating activity. This ability is very important because, under saline soil conditions, Fe availability tends to be very low due to complex ionic interactions (Sultana et al., 2021; Timofeeva et al., 2022). The siderophores produced by halotolerant rhizobacteria function as strong chelators to bind Fe, enhancing plant absorption efficiency and supporting microbial and plant growth by reducing Fe competition from pathogens (Oliva et al., 2023). *Bacillus aryabhattai* MS3 continued to produce siderophores up to 43% under 200 mM NaCl concentration, accompanied by the expression of the *entD* gene, which is involved in siderophore biosynthesis (Sultana et al., 2021). Another study showed that *Acinetobacter johnsonii*, *Pseudomonas* spp., and *Bacillus* spp. from saline soils were able to consistently produce siderophores at high salinity while also suppressing pathogens such as *Fusarium* and *Erwinia* through Fe competition and enhancing the growth of tomatoes and corn (Shabaan et al., 2022; Yan et al., 2024). Overall, the siderophore capabilities strengthen the function of the isolates as multifunctional halotolerant bioinoculant agents—enhancing nutrient absorption, supporting plant growth, and providing biocontrol effects in saline soil.

Isolates WN-01, WN-04, and WN-05 show ACC deaminase activity, as shown by their growth on Dworkin–Foster media with ACC as the only nitrogen source (Table 1 and Figure 1). Amid high salinity stress, plants tend to produce ethylene in excess through the ACC precursor pathway, which can potentially inhibit root expansion and the absorption of water and nutrients (Orozco-Mosqueda et al., 2020). This enzyme breaks down ACC, a direct precursor of the stress hormone ethylene, into ammonia and α-ketobutyrate, which lowers ethylene levels, especially under high salinity conditions. ACC deaminase is one of the main mechanisms of halotolerant PGPR to reduce ethylene stress and help recover plant growth under abiotic stress such as salt (Egamberdieva et al., 2019; Shahid et al., 2023). Azadikhah et al. (2019) showed that inoculation with ACC deaminase-producing *Pseudomonas fluorescens* on barley plants grown under 150 mM NaCl increased root length by 30 to 45% and shoot weight by 25 to 40% compared to the non-inoculated control. Additionally, the strain *B. subtilis* with high ACC deaminase activity is capable of suppressing ethylene stress and increasing biomass as well as root length in peas (*Pisum sativum*) by 35 to 50% under 3% NaCl salinity conditions (Gupta et al., 2022).

All halotolerant rhizobacteria isolates in this study demonstrated the ability to produce EPS, with the highest production recorded in isolate

WN-03 at 0.257 g ml^{-1} (Table 1). EPS production is an essential adaptive mechanism to enhance the survival and function of microbial PGP in high-salinity environments. EPS is a high molecular weight polymer secreted into the extracellular matrix and has various protective and regulatory roles in the rhizosphere zone. One of its primary functions is as an osmotic protector that maintains cell turgor pressure and prevents water loss due to high osmotic pressure. Furthermore, EPS contributes to the absorption of ions like Na^+ and Cl^+, which lessens the ionic toxicity frequently present in salty soils (Sunita et al., 2020). Hydroxyl, carboxylate, and sulfate are functional groups in EPS with a high cation exchange capacity and the capability to chelate toxic ions. By lowering the concentration of free ions in the root zone, this interaction produces a more favorable microenvironment for root growth—microbial colonization and nutrient availability (Orhan et al., 2023). EPS also contributes to forming soil physical structure by enhancing aggregate stability and water retention, which is crucial for maintaining plant-microbe interactions under drought or salinity stress (Bhagat et al., 2021; Dragojević et al., 2023). Bhagat et al. (2021) and Naseem et al. (2018) reported that EPS produced by microbes enhances soil structure and acts as a biochemical buffer that strengthens plant tolerance to abiotic stress by modulating hormonal signals and nutrient flow. These results are in line with several earlier investigations, including Sultana et al. (2021) and Liu et al. (2022), which show that EPS from *Bacillus* and *Pseudomonas* spp. are capable of reducing salt toxicity and enhancing the growth of crops like rice and wheat under saline irrigation conditions.

All isolates in this study (WN-01 to WN-05) showed the ability to synthesize microbial IAA, with the highest concentration observed by isolate WN-02 at 81.9 µg ml^{-1} (Table 1). In high salinity situations, morphogenetic activities such as the stimulation of lateral roots, root elongation, and root hair growth by halotolerant rhizobacteria's synthesis of IAA are essential (Zhang et al., 2022), as well as triggering cellular responses in plant roots. IAA controls the expression of genes linked to aquaporins and cell proliferation, which enhances water transport efficiency under high osmotic pressure (Kang et al., 2019). The inoculation of *Bacillus haynesii* SFO145, *Staphylococcus petrasii* SFO132, *Pseudomonas soyae* R600, and *Salinicola halophilus* SFO075 at higher salinity levels (200 mM NaCl) significantly sustained and improved root and shoot length, fresh and dry biomass, and chlorophyll content (Alonazi et al., 2025). IAA and other hormones like ethylene and ABA create a complex hormonal signaling pathway that regulates stress responses, mitigates the accumulation of harmful ions (Na^+/Cl^-), and increases the development of ion transporters and antioxidant enzymes. Microbial IAA can inhibit stress ethylene and enhance detoxification by activating reactive oxygen species (ROS) pathways and the plant's cellular defense mechanisms (Hidri et al., 2022).

The results from PGP traits assessments in this study indicate that each halotolerant rhizobacteria strain exhibits several complementary qualities essential to plant growth. This multifaceted trait indicates an evolutionary response to extended exposure to salinity stress in their natural environments (Etesami and Maheshwari, 2018; Tang et al., 2020). Selective pressures in saline environments would favor microbes that are effective at utilizing various ways to sustain their metabolic activity and interactions with plants.

The wide variety of PGP characteristics within a single PGPR species can be linked to synergistic interactions and genetic material exchange among rhizobacteria (Poole et al., 2018; Wardell et al., 2022). Horizontal gene transfer, plasmid exchange, and the establishment of intricate microbial consortia can enhance the acquisition and retention of advantageous genes that encode diverse PGP properties. This dynamic genetic landscape enables rhizobacteria to quickly change and acquire novel features, augmenting their ability to promote plant development and endure adverse situations. It shows the significance of evaluating the collective capabilities of the microbial community rather than concentrating exclusively on individual strains to develop efficient bio-inoculants.

Rhizobacteria molecular identification

The five halotolerant isolates were identified as *B. subtilis* (WN-01), *P. megaterium* (WN-02), *P. segetis* (WN-03), *B. pumilus* (WN-04), and *B. cereus* (WN-05) (Table 1) using 16S rRNA gene sequencing for molecular identification (Table 2). These species are well-known PGPR, significantly contributing to plant development and resilience against environmental stressors, such as salinity (Dakshayini et al., 2025). The confirmation of this taxonomy is supported by the results of PGP trait tests, which can affirm the ability of these isolates to be relevant halotolerant PGPR. These isolates show varied and

Table 2. Identify sequences of halotolerant rhizobacteria from salinity-impacted soil in Pekalongan, based on GeneBank data by using BLAST

Isolate	Accession number	Species of PGPR homolog	Identity (%)	Query cover (%)
WN-01	MN456841.1	*B. subtilis* ATCC 19659	100.00	100.00
WN-02	CP117689.1	*P. megaterium* GEB3	100.00	100.00
WN-03	NR_043174.1	*P. segetis* FR1439	100.00	99.93
WN-04	KF933608.1	*B. pumilus* BDH12	99.03	99.00
WN-05	MK995636.1	*B. cereus* SCSB-13	99.93	100.00

complementary PGP traits, indicating functional redundancy as an ecological adaptation to salinity stress.

According to Rehan et al. (2023) and Thakur et al. (2024), *P. megaterium* (WN-02) is noteworthy for its capacity to solubilize phosphate by using gluconic acid and generate phytohormones, such as IAA, which greatly enhance nutrient absorption and plant biomass. Meanwhile, *B. subtilis* (WN-01) is widely used in commercial biofertilizers due to its synthesis of proteolytic enzymes and surfactants (Hashem et al., 2019), which are essential to promoting root health and suppressing infections (Kaspar et al., 2019). Regarding the limited exploration of *P. segetis* (WN-03), the *Pseudomonas* genus has demonstrated the ability to elicit systemic plant resistance via the jasmonate and ethylene signaling pathways (Rodríguez et al., 2020; Singh et al., 2022). The study of *B. pumilus* (WN-04) and *B. cereus* (WN-05) increases this potential through diverse PGP features, including nitrogen fixation, siderophore synthesis, and ACC deaminase activity (Kulkova et al., 2023). The fact that a single isolate possesses several PGP properties shows how valuable they could be as a powerful multigenic bioinoculant for revegetation and productivity enhancement in marginal soils.

Rhizobacteria isolates compatibility

The compatibility test showed that the five halotolerant isolates exhibited growth without inhibitory zones, indicating the absence of antagonism (Figure 2). This ability is crucial for formulating efficient multispecies bioinoculants since negative interactions could reduce efficacy (Santoyo et al., 2021; Tienda et al., 2024). These data indicate that these isolates can establish stable and functional microbial communities in the rhizosphere, consistent with the value of microbial synergy for successful inoculation (Santoyo et al., 2021). Previous research suggests that PGPR consortia, especially those combining *Bacillus* and *Pseudomonas*, are superior to individual inoculants in stimulating the growth of

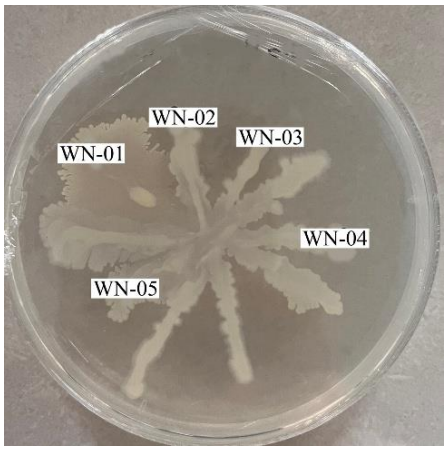

Figure 2. The result of the compatibility assessment among all isolates

Note: All isolates showed growth overlap, and the lack of an inhibitory zone indicates compatibility between all isolates

plants and their ability to withstand salt stress (Ali et al., 2025). Consequently, the interspecific compatibility of these isolates strengthens the foundation for developing multifunctional bioinoculants, making them promising biostimulant agents for productivity improvement in saline environments (Santoyo et al., 2021).

PGP experiment

In vivo testing on the sweet corn model plant (*Z. mays saccharata* var. Bonanza F1) showed that applying a halotolerant rhizobacteria consortium significantly induced growth enhancement under moderate salinity stress conditions (4 dS m^{-1}). Treatment with a cell density of 10^8 CFU ml^{-1} consistently resulted in the highest vegetative growth parameters, including plant height (104.70±3.7 cm) and leaf surface area (341.412±11.32 cm^2) (Table 3). This increase indicates that optimal microbial inoculum density facilitates more efficient rhizosphere colonization and leads to the amplification of growth stimulation through synergistic mechanisms such as phytohormone synthesis (e.g., IAA), phosphate solubilization, and atmospheric nitrogen fixation (Backer et al., 2018; Hu et al., 2021). The consistency of these

Table 3. Plant height, leaf surface area, and total chlorophyll content of *Z. mays saccharata* Sturt at 20 days after planting

Parameters	Treatment group							
	C-	C+	10^4	10^5	10^6	10^7	10^8
Plant height (cm)	68.18±6.15^{e}	70.32±5.52^{de}	76.20±5.17^{d}	89.18±6.65^{c}	97±2.68^{b}	98±1.87^{b}	104.70±3.7^{a}
Leaf surface area (cm^2)	134.953±7.72^{f}	197.543±7.56^{e}	194.719±12.11^{e}	241.748±14.35^{d}	268.607±18.44^{c}	295.207±16.77^{b}	341.412±11.32^{a}
Chlorophyll content (mg g^{-1} FW)	163.744±50.5^{b}	173.464±13.5^{b}	175.251±2.5^{b}	176.701±7.17^{b}	189.098±4.89^{ab}	191.169±11.67^{ab}	208.439±10.29^{a}

Table 4. Total soil microorganism count in NA media from saline soil treated with PGPR inoculants at 20 days after planting

Parameters	Treatment group							
	Before	C-	C+	10^4	10^5	10^6	10^7	10^8
Total count of soil microorganisms (CFU g^{-1})	$1.5x10^3$	$3.0x10^4$	$2.0x10^6$	$3.7x10^6$	$6.9x10^6$	$1.3x10^7$	$2.4x10^7$	$1x10^8$

findings with the results of the PGP trait tests (Table 1) reinforces the hypothesis that the broad PGP spectrum of these isolates contributes to the holistic mitigation of salinity stress. The crucial physiological aspect observed was a significant increase in total chlorophyll content (208.439±10.29 mg g^{-1} FW) at the treatment of 10^8 CFU ml^{-1}, indicating an increase in plant photosynthesis efficiency under salinity stress (Table 3). This effect is likely caused by PGPR's capacity to increase the bioavailability of vital minerals like Fe and decrease oxidative stress by producing siderophores (Verma et al., 2021). The study by Desoky et al. (2020) confirmed that PGPR can enhance the expression of antioxidant genes and effectively reduce cell damage caused by oxidative stress induced by salinity in tomato plants.

Statistical tests confirm that the increase in inoculum cell density from 10^4 to 10^8 CFU ml^{-1} is positively and significantly correlated with the improvement of all plant development and physiological characteristics. This positive correlation underscores that higher doses of PGPR facilitate more extensive rhizosphere colonization, thereby inducing plant metabolic mechanisms more optimally. It includes increased synthesis of phytohormones, nitrogen fixation efficiency, and better absorption of macro- and micronutrients (Hu et al., 2021; Marasco et al., 2022). Applying a multi-strain consortium in this study provides a significant comparative advantage over single inoculation. Each strain in this consortium, which has proven to be compatible (Figure 2), offers complementary metabolic contributions, strengthening plant resilience through stable and synergistic microbial interactions (Santoyo et al., 2021; Tienda et al., 2024). Ali et al. (2025), for instance, stated that the consortium of *Serratia fonticola* and *Pseudomonas koreensis* significantly enhanced the growth and salinity tolerance of *Cucumis sativus* L. compared to single-strain applications. The study by Khan et al. (2022) further supports this concept, showing that a PGPR consortium consisting of *Ensifer adhaerens*, *Pseudomonas fluorescens*, and *Bacillus megaterium* significantly increases biomass and physiological parameters (e.g., reduced electrolyte leakage, increased K/Na ratio, relative water content, and chlorophyll concentration) in wheat under high salinity conditions. Another study by Setiaji et al. (2025) observed that native isolates from Indonesian saline fields could produce IAA, solubilize phosphate, and enhance shallot growth

under salt stress conditions up to 100%. This consistency suggests that the beneficial effects of halotolerant rhizobacteria are not limited to a specific crop or location, but may represent a broadly applicable strategy for sustainable agriculture in salt-impacted areas.

Dynamics of soil microbial communities

The soil microbial density was considerably raised when a group of halotolerant rhizobacteria was applied to saline soil, which was initially low (1.5×10^3 CFU g^{-1}), to 1.0×10^8 CFU g^{-1} in the treatment with a cell density of 10^8 CFU ml^{-1}. In contrast, the negative control (C–) only reached 3.0×10^4 CFU g^{-1}, indicating that without PGPR inoculation, the soil microbiome did not show significant recovery until 20 days after planting. The positive control treatment (C+), although using a commercial biofertilizer not derived from saline soil isolation, successfully reached 2.0×10^6 CFU g^{-1}, affirming that inoculation, even on a small scale, can facilitate the recovery of the saline soil microbial community (Table 4).

The drastic increase in soil microbial populations in this treatment group is consistent with the ecological mechanisms of halotolerant PGPR. These microorganisms can synthesize secondary metabolites such as EPS, siderophores, and osmolytes, which are crucial for their adaptation to osmotic and ionic stress induced by salinity (Arora et al., 2020; Sunita et al., 2020; Kumar et al., 2023). Furthermore, PGPR modulates chemical communication between microbes through quorum sensing, promoting effective colonization in the rhizosphere and stimulating the growth and activity of beneficial native microbiomes (Etesami and Maheshwari, 2018; Dey et al., 2021). The achievement of 1.0×10^8 CFU g^{-1} in the 10^8 treatment is a strong indicator of the stability and efficiency of consortium colonization in the root zone. Similar research supports these conclusions. According to Yan et al. (2024), the PGPR consortium, which included the inoculants *S. fonticola* GSCK6, *Bacillus atrophaeus*, and *Priestia endophyticus* GSCK1, not only strengthened the soil microbial community but also enhanced the activity of essential soil enzymes such as arylsulfatase and urease under salinity conditions. The increase in enzyme activity directly correlates with higher soil fertility and nutrient retention efficiency.

Thus, the cooperative bioinoculant technology created from these local isolates shows tremendous strategic promise for restoring salinity-degraded land. This strategy thoroughly incorporates resilient microbial colonization, improved soil biological activities, and enhanced plant productivity, delivering an integrated solution for sustainable agriculture in marginal areas.

CONCLUSIONS

This study successfully identified and characterized five halotolerant rhizobacteria isolates from saline soil in Pekalongan, Indonesia, namely *B. subtilis*, *P. megaterium*, *P. segetis*, *B. pumilus*, and *B. cereus*. All isolates exhibited diverse PGP traits and high salinity tolerance. Pot tests on sweet corn (*Z. mays saccharata*) showed that combining the five isolates significantly increased plant growth under 4 dS m^{-1} salinity stress and enriched the soil microbial population. These results highlight the noteworthy possibilities of halotolerant rhizobacteria consortia as bioinoculants to enhance agricultural productivity in marginal lands degraded by salinity. Therefore, large-scale field trials in various agroecological conditions in Indonesia are recommended to ensure this bioinoculant's consistency and applicability.

ACKNOWLEDGEMENT

The authors sincerely express their gratitude for the generous financial support provided by the Indonesia Endowment Fund for Education (LPDP), which has been instrumental in facilitating the research activities. The assistance from this institution has been crucial in achieving the research objectives and enhancing the quality of this work.

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