

Microbial Interventions for Sustainable Agriculture: Plant Growth Promoting Rhizobacteria (PGPR) Activity of Isolates of Saline Soils from Karad Taluka, Maharashtra, India

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Key Words	Halotolerant bacteria, PGPR, Bioinoculants, Phosphorus solubilization, Potassium solubilization
DOI	https://doi.org/10.46488/NEPT.2026.v25i03.B4419 (DOI will be active only after the final publication of the paper)
Citation for the Paper	Patil, P.A., Pathade, A.G. and Pathade, G.R., 2026. Microbial interventions for sustainable agriculture: PGPR activity of isolates of saline soils from Karad Taluka, Maharashtra, India. <i>Nature Environment and Pollution Technology</i> , 25(3), B4419. https://doi.org/10.46488/NEPT.2026.v25i03.B4419

Abstract:

Soil salinity is a key abiotic factor that limits agricultural output worldwide, and the problem is getting worse in parts of Maharashtra, especially Karad Taluka. The current work examined saline soils from various locations in Karad, evaluating their physicochemical properties and isolating indigenous halotolerant bacterial strains with plant growth-promoting rhizobacterial (PGPR) attributes. Soil study revealed a strongly alkaline pH (8.8-9.4), high sodium levels, and shortages in organic carbon, nitrogen, and micronutrients. Five halotolerant bacterial isolates (Ko1, Va1, Be1, Vm1, and At1) were isolated and tested for PGPR activities including phosphate and potassium solubilisation, nitrogen fixation, indole-3-acetic acid (IAA) generation, and siderophore synthesis. All isolates showed positive activity in phosphate and

potassium solubilisation, nitrogen fixation, and IAA generation, with four additionally producing siderophores. Isolates Be1, Vm1, and At1 performed well across multiple traits, making them interesting candidates for bioinoculant development. The findings indicate that native halotolerant PGPR from Karad Taluka can help improve soil fertility, increase nutrient uptake, and promote plant development in salt-affected areas. Future field validation and molecular characterisation could help build environmentally friendly microbial formulations for saline agriculture.

1. Introduction

Soil salinity is a key abiotic factor that restricts agricultural productivity globally, impacting soil structure, nutrient access, microbial activity, and plant physiological functions (Butcher et al., 2016; Metternicht & Zinck, 2003; Wang et al., 2003). Salinity-induced osmotic stress, ion toxicity, and nutrient imbalance significantly reduce crop growth and yield, especially in arid and semi-arid regions, where poor irrigation practices and excessive fertilizer use accelerate soil degradation (Hailu & Mehari, 2021; Hussain et al., 2019; Sultana et al., 2020). In India, nearly 7 million hectares of land are affected by salinity and sodicity, with an increasing incidence of secondary salinization in irrigated agricultural regions (Mandal et al., 2009; Sharma & Chaudhari, 2012). Maharashtra has emerged as a region where intensive cultivation and inadequate drainage have led to soil alkalinity and nutrient depletion. Karad Taluka, located in the Krishna River basin and dominated by sugarcane-based agriculture, is increasingly marked by high soil pH, sodicity, low organic carbon, and micronutrient deficiencies, posing serious challenges to long-term soil fertility and crop productivity. Conventional approaches to managing saline soils rely heavily on chemical amendments and fertilizers, which often deliver only short-term benefits while further degrading soil biological health. In this context, plant growth-promoting rhizobacteria (PGPR) offer a sustainable alternative by enhancing nutrient availability and plant stress tolerance through mechanisms

such as nitrogen fixation, phosphorus and potassium solubilization, phytohormone production, and siderophore-mediated iron acquisition (Alori et al., 2017; Alori & Babalola, 2018; Ambrosini et al., 2016). Several studies have demonstrated the potential of halotolerant PGPR to improve crop performance under saline conditions (Jiang et al., 2018; Krishnamoorthy et al., 2016). However, most available studies focus on coastal, desert, or arid ecosystems, while inland saline–alkaline agricultural soils remain poorly explored, especially in Western Maharashtra. Importantly, indigenous microbial populations adapted to local soil conditions are more likely to survive, colonize the rhizosphere, and function effectively as bioinoculants compared to introduced strains (Trabelsi & Mhamdi, 2013). Despite worsening salinity issues in Karad Taluka, systematic studies linking soil physicochemical constraints to the functional screening of native halotolerant PGPR are lacking.

Therefore, the present study aims to (i) characterize the physicochemical properties of saline soils from Karad Taluka, Maharashtra, and (ii) isolate and evaluate indigenous halotolerant bacterial strains for key PGPR traits, including phosphate and potassium solubilization, nitrogen fixation, indole-3-acetic acid production, and siderophore synthesis. By focusing on locally adapted microorganisms, this work seeks to identify potential bioinoculants for the sustainable management and reclamation of inland saline agricultural soils.

2. Material and Methods

2.1. Study Area and Soil Sample Collection

Composite saline soil samples were collected from eight sites in Karad Taluka (Figure 1). Each site was georeferenced with precise latitude and longitude coordinates. Soil was collected at a depth of 15 cm using a borer. At each location, five subsamples—four from the corners and one from the center—were pooled, thoroughly mixed, and reduced to about 100 g by quartering. These samples were stored in sterile polythene bags at low temperature for later analysis (Ghare & Kumbhar, 2021), (Raut & Kurhe, 2020), (Kekane et al., 2015).



Fig 1: Map of Karad Taluka

2.2. Physicochemical Characterization of Soil Samples

The soil samples were examined for a range of essential physical and chemical parameters to assess their fertility and salinity. To determine soil alkalinity and salinity, pH and electrical conductivity (EC) were measured using calibrated digital meters. The dichromate oxidation method was used to assess organic carbon (OC), which provides insight into soil organic matter concentration and overall soil health. Nitrogen was assessed using the Kjeldahl method, phosphorus with the colorimetric molybdenum blue method, and potassium with Atomic Absorption Spectroscopy (AAS). To assess soil base saturation and cation balance, secondary nutrients such as calcium and magnesium were measured by EDTA titration. Furthermore, micronutrients such as iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), boron (B) and sulphur (S) were analysed using AAS or suitable colorimetric methods to assess their availability for plant uptake. These studies offered a thorough insight into the chemical composition and nutritional status of the soil samples (Ghare & Kumbhar, 2021), (Raut & Kurhe, 2020), (Panhalkar, 1994), (Kekane et al., 2015).

2.3. Enrichment, Isolation and Morphological Characterization of Bacterial Isolates

Soil samples were inoculated into nutrient broth supplemented with 7.5% (w/v) NaCl and incubated at 30 °C for 48 h under static conditions. Enriched cultures were serially diluted

and spread onto nutrient agar plates containing the same salt concentration, then incubated at 30 °C for 48 h (Vazquez et al., 2000). Morphologically distinct colonies were purified by repeated streaking. To determine the salt tolerance range, purified isolates were screened for growth on nutrient agar supplemented with increasing NaCl concentrations (0%, 2.5%, 5%, 7.5%, and 10% w/v). Plates were incubated at 30 °C for 48–72 h, and growth was recorded qualitatively by colony formation. Isolates showing consistent growth at $\geq 7.5\%$ NaCl were classified as halotolerant and selected for further PGPR screening. (Vazquez et al., 2000). Morphological characteristics such as colony shape, size, margin, elevation, texture, pigmentation, Gram staining, and motility were recorded.

2.4. Screening for PGPR Traits

The plant growth-promoting traits of the bacterial isolates were evaluated using standard microbiological methods. The Phosphate Solubilization Index (PSI) was determined after seven days of incubation at 28 ± 2 °C with Pikovskaya's agar plates (Chakdar et al., 2018), (Kirui et al., 2022), (V H et al., 2024), (Mengesha & Legesse, 2024). Potassium solubilization was tested on Aleksandrov agar medium (Verma et al., 2017) supplemented with feldspar as a potassium source, and the Potassium Solubilization Index (KSI) was calculated based on the solubilization zone. To assess the ability of isolates to fix atmospheric nitrogen, it was grown on nitrogen-free Ashby's Mannitol agar, which demonstrated diazotrophic activity followed by acetylene reduction test for quantitation (Tang et al., 2020). The production of Indole-3-acetic acid (IAA) was measured using the Salkowski reagent, and results were compared to a standard curve for quantitation (Patten & Glick, 2011). Lastly, siderophore production was evaluated on Chrome Azurol S (CAS) agar, with the formation of clear halos around colonies indicating successful siderophore synthesis.

2.5 Controls and Experimental Validation

Uninoculated media served as negative controls for all biochemical and PGPR assays, ensuring assay specificity. For qualitative comparison, previously reported PGPR-positive laboratory strains (where available) were used as reference standards for phosphate solubilization, IAA production, and siderophore synthesis.

2.6 Molecular Characterisation by using 16s RNA

The bacterial strains were identified via 16S rRNA gene sequencing. Genomic DNA was isolated and purified using the Thermo Scientific® GeneJet™ Genomic DNA Purification Kit. (Youseif, 2018).

2.7 Statistical Analysis

All experiments were performed in triplicate, with results presented as mean \pm standard deviation. To evaluate differences among soil physicochemical parameters and PGPR indices, one-way analysis of variance (ANOVA) was employed using standard statistical software. A p-value of less than 0.05 was deemed statistically significant.

3. RESULTS

3.1. Physicochemical Properties of Saline Soils

The soils from Karad Taluka show significant variation in texture, nutrients, and chemistry. Most are clay loam with 30–38% sand, indicating good water retention. As shown in Table 1(a), (b) (Fig. -2a, b, c, d), the soils are alkaline (pH 8.8–9.4), with high sodium (3.0–5.4), raising salinity concerns. Organic carbon (0.12–0.19%) is below ideal (0.4–0.6%), indicating low organic matter and microbial activity. Nitrogen (95–150 kg/ha) is below the recommended 280–420 kg/ha, but phosphorus (8.5–11.2 kg/ha) and potassium (132–162 kg/ha) are moderate. Copper (2.8–3.8 ppm) and iron (3.3–4.4 ppm) are adequate, while zinc (0.41–0.65 ppm) and manganese (0.23–0.38 ppm) are deficient. Sulphur (8.4–10.8 ppm) is near limits, and CaCO₃ (6–16%) is moderate. Water-holding capacity (20–29%) correlates with clay

content. Overall, the soils are alkaline, low in organic carbon and nitrogen, and moderately nutrient-rich, needing organic amendments and balanced fertilization to boost fertility and productivity.

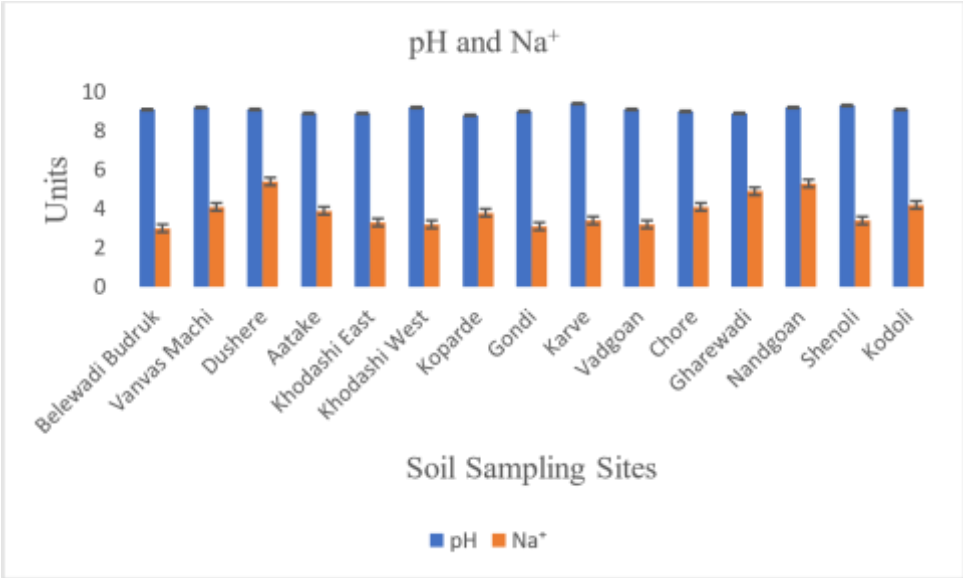


Fig. 2a : pH and Na+ of soil samples

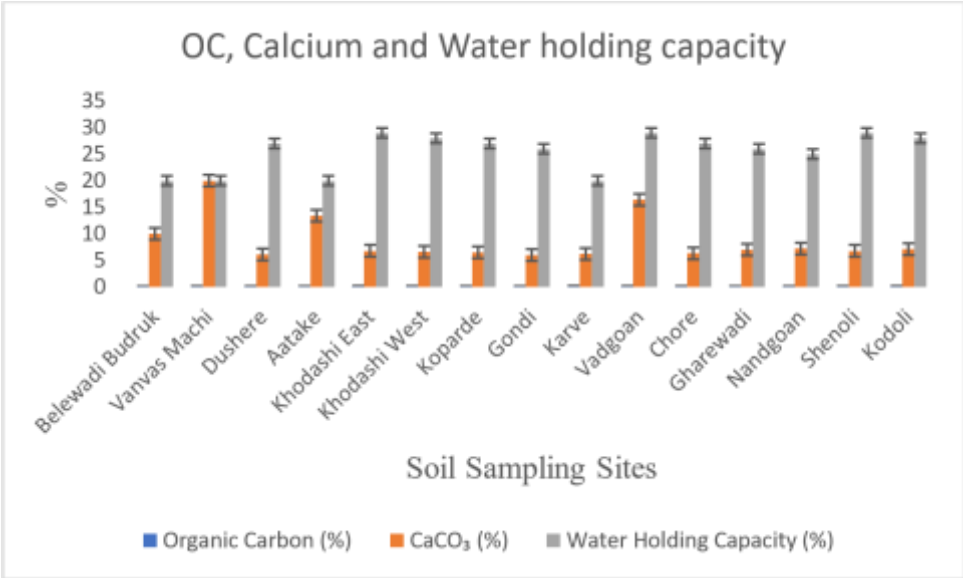


Fig 2b: OC, CaCO₃ and water holding capacity of soil samples sites

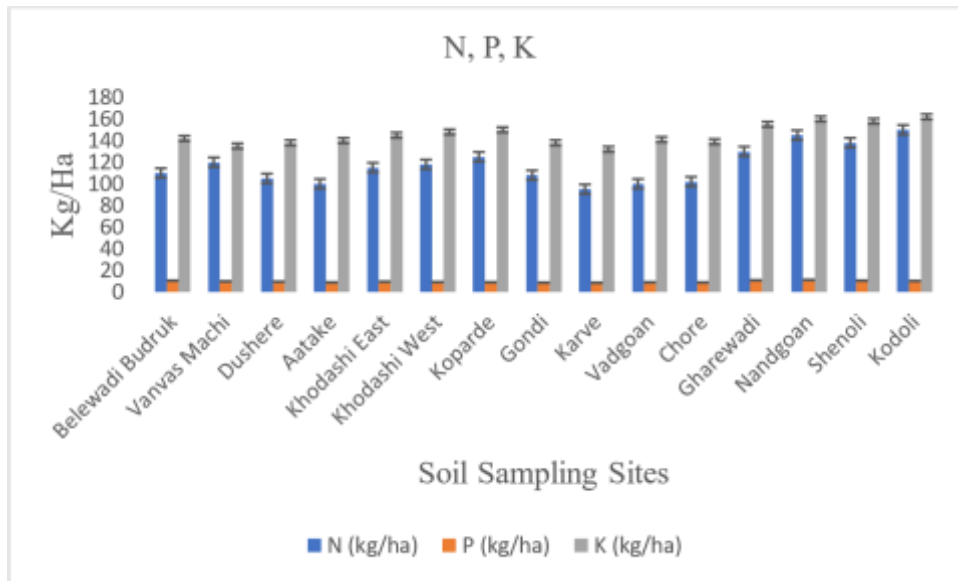


Fig. 2c: N, P, K of soil samples

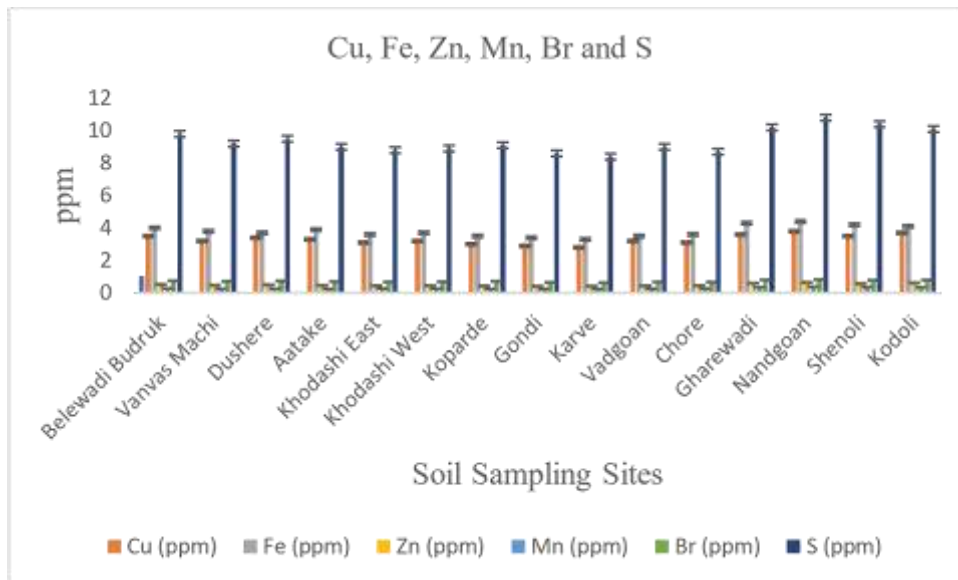


Fig. 2d: Cu, Fe, Zn, Mn, Br and S of soil samples

The soils had a mean pH of 9.09 with low variability ($CV = 1.76\%$), indicating consistent alkalinity across locations. Sodium levels showed moderate variability ($CV = 20.31\%$), confirming heterogenous sodicity. Organic carbon was low, reflecting poor organic matter in saline soils. Potassium was high and stable, while nitrogen and phosphorus varied moderately. Micronutrients such as zinc and magnesium also varied, suggesting possible deficiencies. Calcium carbonate content was highly variable ($CV = 48.24\%$) due to uneven calcareousness,

which affected alkalinity and nutrient concentrations. Sodicity and texture moderately influenced water-holding capacity.

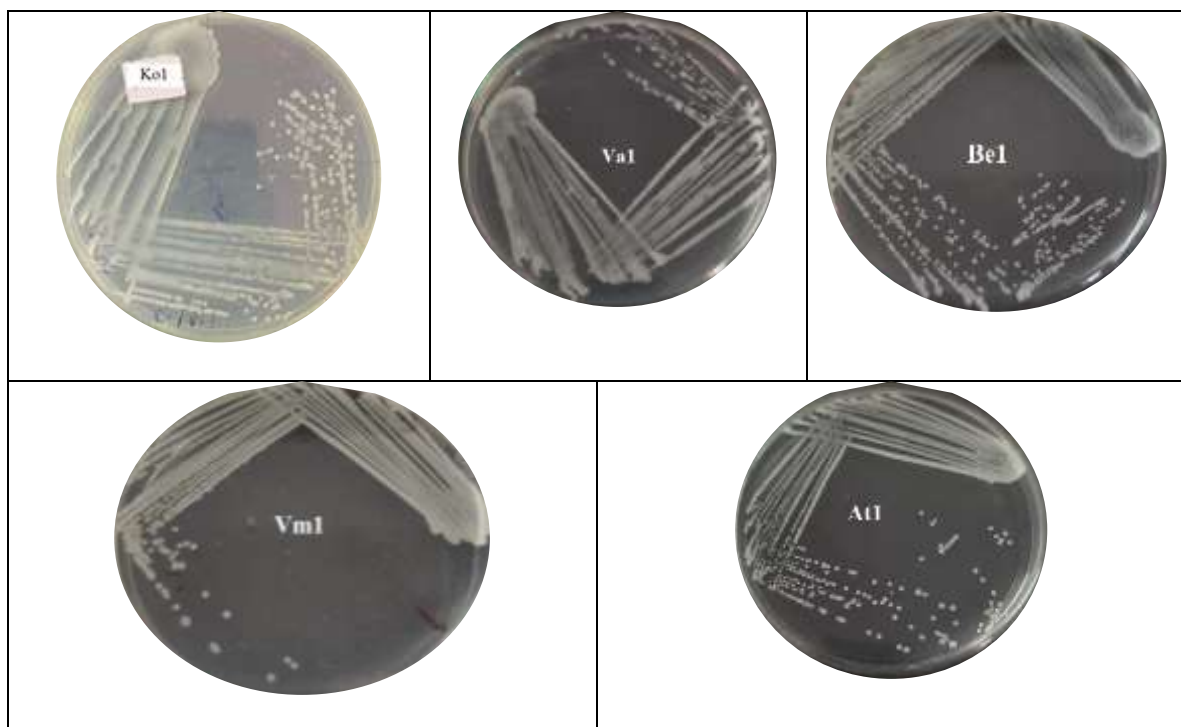
Based on the coefficient of variation (CV), soil pH and nutrients P, K, Cu, Fe, Br, and S showed low variability (<10%), indicating a uniform distribution across sites. Organic carbon (OC), nitrogen (N), zinc (Zn), manganese (Mn), and water holding capacity (WHC) had moderate variability (10–20%), suggesting some spatial variation influenced by soil management and organic matter. In contrast, sodium (Na^+) and calcium carbonate (CaCO_3) showed high variability (>20%), reflecting heterogeneity in salinity and calcareous nature, possibly due to local environmental conditions and parent material. One-way ANOVA for soil pH showed no significant difference between groups, with $F=2.08$ and $p=0.173$ (>0.05), indicating pH remained relatively consistent across locations.

Performance variation among halotolerant bacteria relates to their origin in saline–alkaline, nutrient-poor soils. These soils, with high pH, sodium, and low organic carbon, select for microbes that can withstand osmotic and ionic stresses. Isolates Be1, Vm1, and At1 showed enhanced phosphate, potassium solubilization, nitrogen fixation, IAA, and siderophore production, indicating adaptations for nutrient mobilization. In alkaline soils, insoluble phosphorus and potassium give these microbes an advantage by improving nutrient uptake. Siderophores likely help with iron limitations, and IAA promotes root growth, aiding microbe-plant interactions. Ko1 did not produce siderophores, indicating niche specialization. Overall, trait differences driven by soil conditions suggest locally adapted, multifunctional isolates are more effective as bioinoculants in saline–alkaline environments, emphasizing the importance of region-specific microbes selection.

3.2. Morphological Characteristics of Bacterial Isolates

The morphological and microscopic characteristics of five bacterial isolates (Ko1, Va1, Be1, Vm1, and At1) (Photoplate 1) were obtained from various samples. All colonies are round

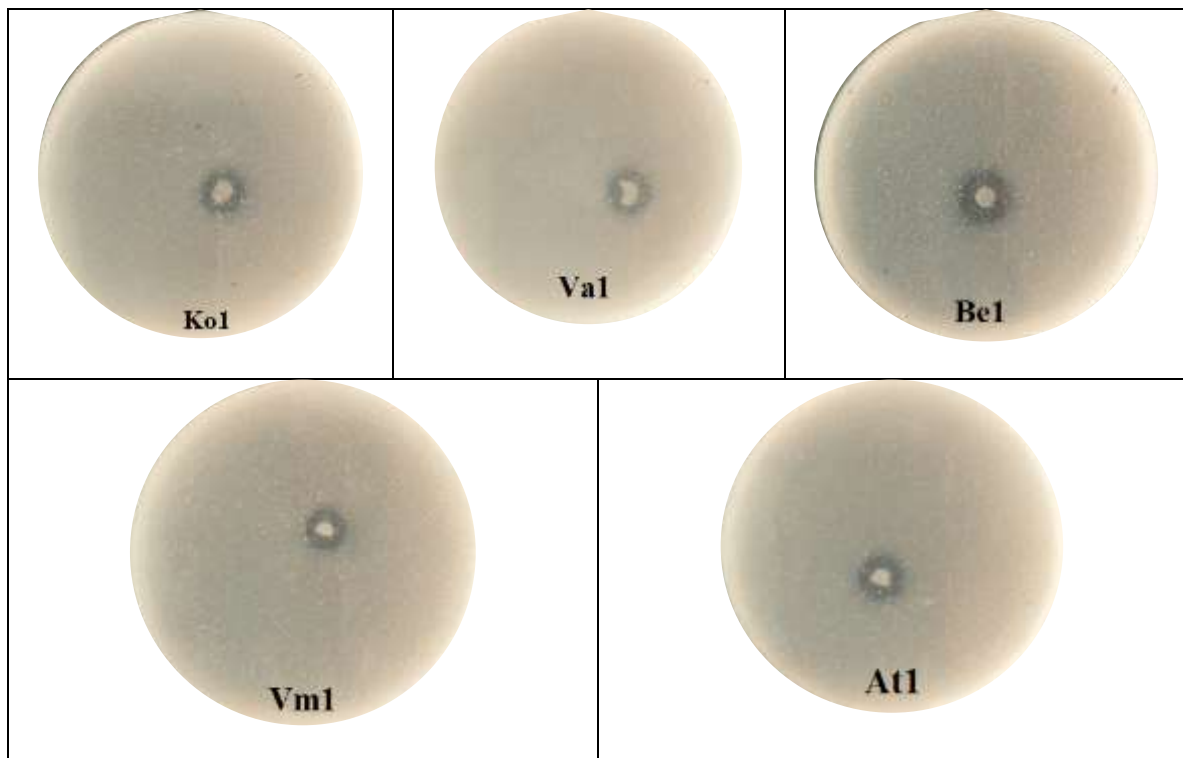
or spherical, with diameters ranging from 1 mm to pinpoint, reflecting varying growth rates and colony compactness. The colours range from white to off-white or transparent, while the textures include moist, slimy, and dry-moist, indicating changes in surface hydration. Most colonies have flat to convex elevation with whole (smooth) margins, indicating consistent colony boundaries without abnormalities. Microscopic analysis reveals that all isolates are Gram-negative and appear as short rods, indicating a predominance of non-spore-forming Gram-negative bacteria in these samples.



Photoplate 1: Bacterial isolates from saline soil samples grown on nutrient agar media at 30°C for 48 hr.

3.3. PGPR Activities of Isolates

When the bacterial isolates were tested in the laboratory, they showed a variety of plant growth-promoting properties. Phosphate solubilisation was confirmed for all isolates, as demonstrated by the formation of halo zones on Pikovskaya's agar, however Phosphate Solubilisation Index (PSI) values varied across them (Photoplate 2).



Photoplate 2: Phosphate solubilization by isolates on Pikovskaya's medium

Determination of phosphate solubilisation index

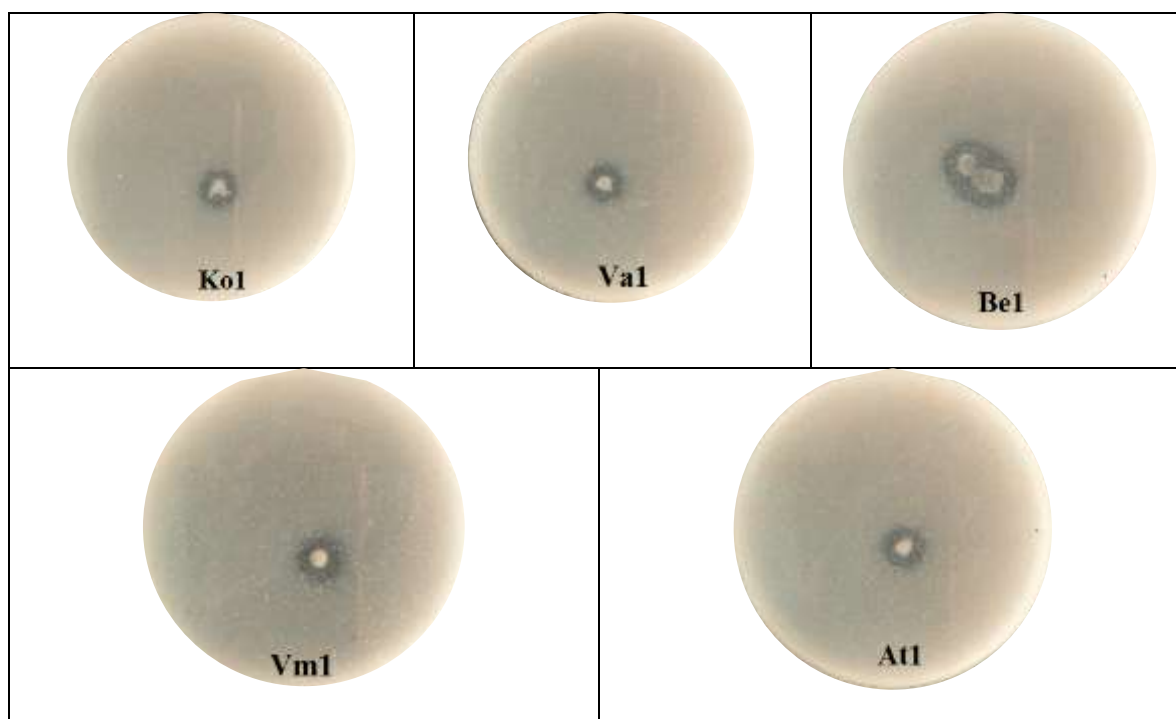
The isolates that exhibited phosphate solubilisation activity were then tested for the phosphate solubilisation index on Pikovskaya's agar. Each bacterial isolate was inoculated into the centre of a Pikovskaya's agar plate and cultivated for 5 days at $28 \pm 2^\circ\text{C}$ (Chakdar et al., 2018), (Kirui et al., 2022). On the fifth day, the diameter of the bacterial colony and the cleared zone were measured and used to calculate the Phosphate Solubilisation Index (PSI) using the following equation: (Table 2).

Phosphate solubilizing index (PSI) = Halo zone diameter + colony diameter / colony diameter

Table 2: Phosphate Solubilization Index of Isolates

Sr. no.	Bacterial Isolate	Colony diameter (mm)	Colony +Halozone diameter (mm)	Phosphate Solubilization Index
1	Ko1	2	9	5.5
2	Va1	3	9	4.0
3	Be1	3	15	6.0
4	Vm1	3	10	4.33
5	At1	3	11	4.6

Potassium solubilisation was similarly positive, with distinct solubilisation zones visible on the Aleksandrov medium and quantifiable Potassium Solubilisation Index (KSI) values measured (photoplate 3).



Photoplate 3: Potassium solubilization by bacterial isolates on Aleksandrov medium

Determination of Potassium solubilisation index

The isolates that exhibited phosphate solubilisation activity were then tested for the Potassium solubilisation index on Alexandrov agar. Each bacterial isolate was inoculated into the centre of a Alexandrov agar plate and cultivated for 4 days at 30°C C (Fatharani & Rahayu, 2018) (Photoplate-.3), (Table 3).

Table 3: Potassium Solubilization Index of Isolates

Sr. no.	Bacterial Isolate	Colony diameter (mm)	Colony + Halozone diameter (mm)	Potassium Solubilization Index (PSI)
1	Ko1	3	9	3.25
2	Va1	3	10	4.33
3	Be1	4	13	4.35
4	Vm1	3	10	4.33
5	At1	3	12	5.0

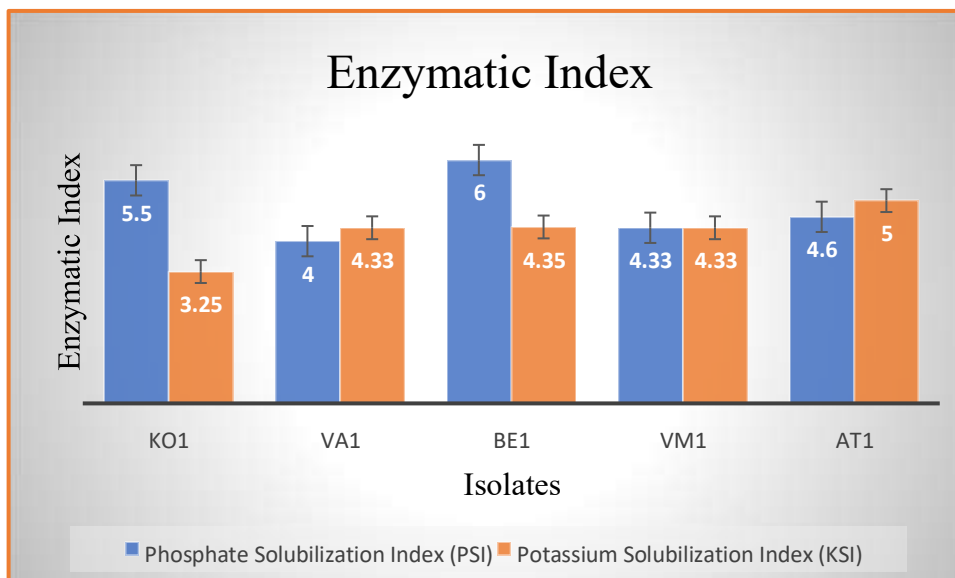
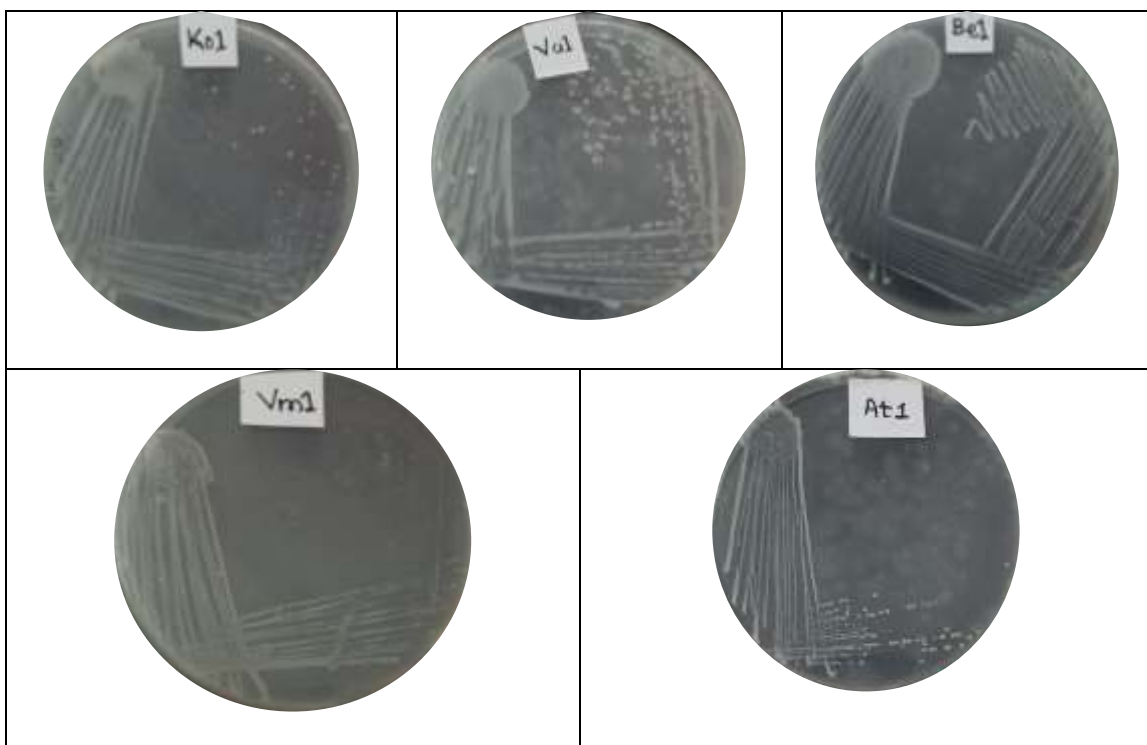
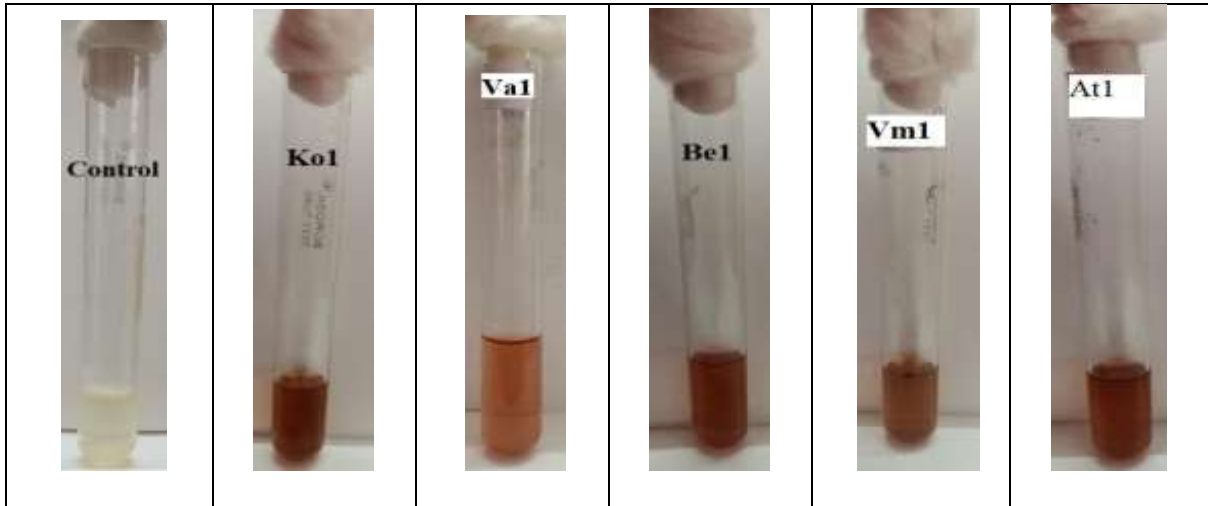


Fig. 3: Enzymatic index of selected isolates

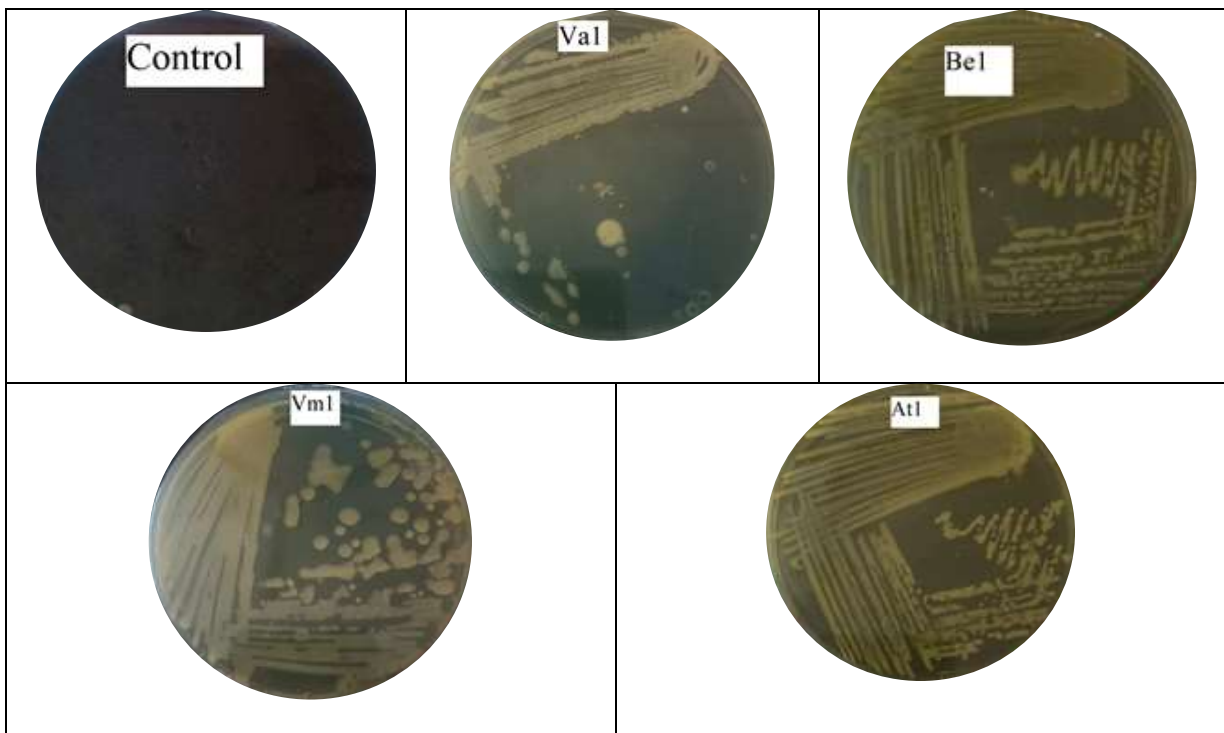
The isolates' ability to fix atmospheric nitrogen was demonstrated by their growth on Ashby's medium, indicating diazotrophic potential (Photoplate 4). Furthermore, the emergence of a distinctive red colour in the presence of Salkowski reagent demonstrated that all isolates tested positive for the synthesis of indole-3-acetic acid (IAA) (Photoplate 5). Lastly, four isolates (Va1, Be1, Vm1, and At1) produced siderophores, but Ko1 did not exhibit any siderophore activity (Photoplate 6).



Photoplate 4: N₂ Fixation by bacterial isolates on Ashbyes medium



Photoplate 5: IAA production by bacterial isolates on LB Broth supplemented with L-tryptophan (0.1%)



Photoplate 6: Production of Siderophore by bacterial isolates Chrome Azurol S (CAS) agar

Isolate Be1, Vm1, and At1 among the tested strains showed strong multi-trait PGPR activity, including phosphate and potassium solubilization, nitrogen fixation, IAA production, and siderophore production. They were recognized as promising candidates for bioinoculant

development. The plant growth-promoting rhizobacteria (PGPR) traits of the five halotolerant bacterial isolates were systematically evaluated, and the results are summarized in Table 4. All isolates demonstrated positive activity for phosphate solubilization, potassium solubilization, nitrogen fixation, and indole-3-acetic acid (IAA) production, indicating their potential to enhance nutrient availability and promote plant growth under saline conditions. Among the isolates, Ko1 exhibited activity for all traits except siderophore production, suggesting that although it can support plant growth through nutrient mobilization and hormone production, it may not contribute significantly to iron chelation in the rhizosphere. In contrast, the other four isolates—Va1, Be1, Vm1, and At1—were positive for all five PGPR traits tested. The presence of siderophore production in these isolates, in addition to other growth-promoting traits, highlights their multifunctional potential and superior capability as candidate bioinoculants. Overall, the results indicate that while all isolates hold promise as plant growth-promoting bacteria, Be1, Vm1, and At1 stand out as particularly effective strains due to their consistent performance across all evaluated traits. These findings support their use in developing microbial bioformulations to improve crop productivity and soil fertility in saline-affected regions.

Table 4: Summarized PGPR Activity

Sr. No.	Isolates	P Solubilization	K Solubilization	N₂ Fixation	IAA Production	Siderophore Production
1	Ko1	+	+	+	+	-
2	Va1	+	+	+	+	+
3	Be1	+	+	+	+	+
4	Vm1	+	+	+	+	+
5	At1	+	+	+	+	+

3.4. Molecular Identification

The genomic DNA of three isolates, At1, Vm1 and Be1, were identified as *Pantoea dispersa*, *Solibacillus isronensis* and *Pseudomonas putida* respectively using the BLASTN tool for 16S rDNA partial gene sequencing.

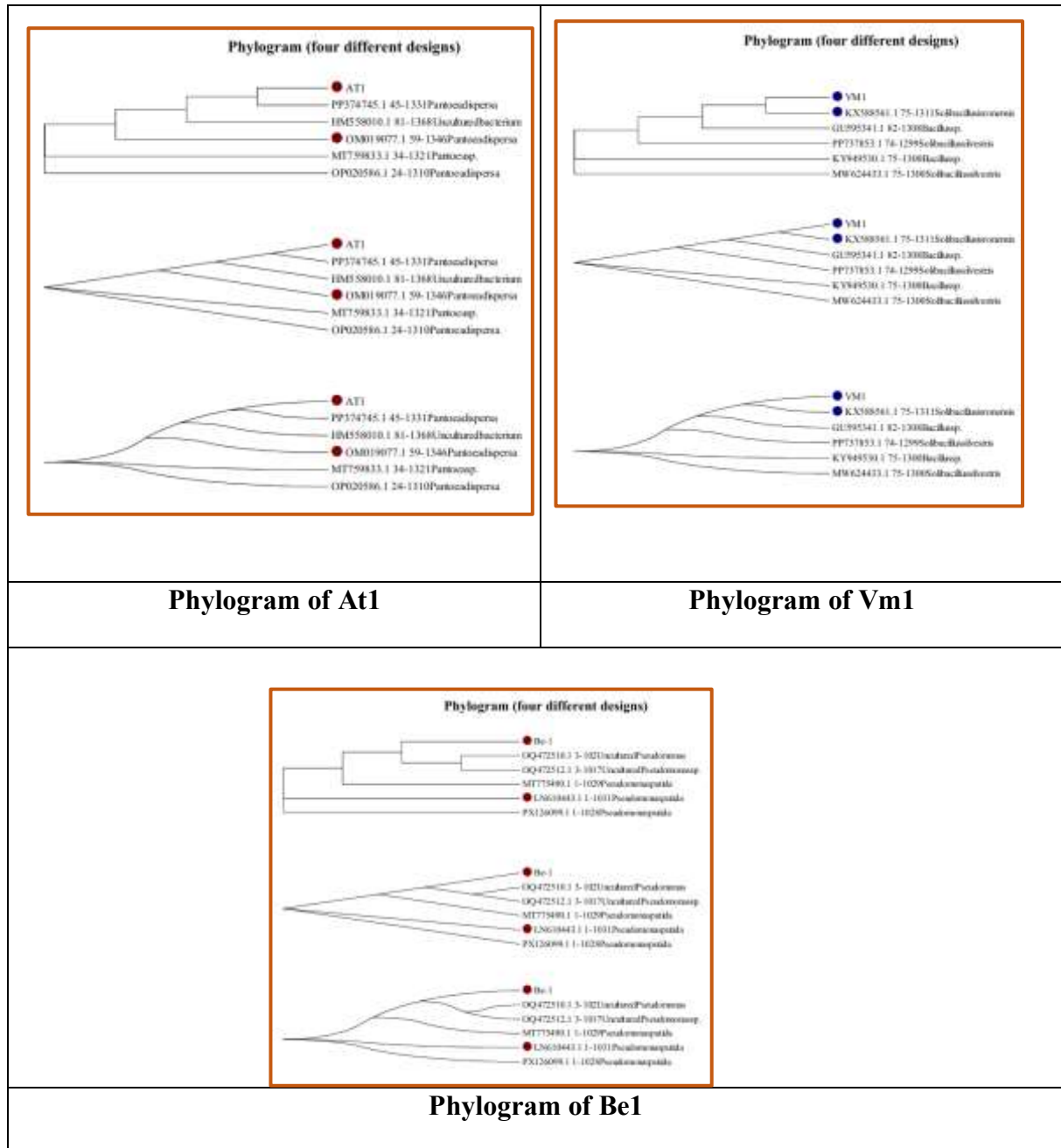


Fig. 4: Phylograms of Isolates

4. DISCUSSION

The study identifies indigenous halotolerant bacteria from saline soils in Karad, Maharashtra, as effective PGPR. All isolates display traits like phosphate and potassium solubilization, nitrogen fixation, and IAA production; four (Va1, Be1, Vm1, At1) also produce siderophores. These microbes adapt to saltwater environments, aiding plant growth. Soil salinity and sodicity threaten agricultural sustainability, causing land degradation and harming soil's vital properties for plant growth. (Metternicht & Zinck, 2003) and (Butcher et al., 2016) highlight soil salinity as a dominant constraint on soil fertility, and the strongly alkaline, sodic conditions observed in Karad Taluka provide a clear ecological context for the functional traits expressed by the isolated bacteria. The consistent expression of phosphate and potassium solubilization across all isolates reflects selective pressure imposed by alkaline soils, where essential nutrients are largely immobilized and inaccessible to plants. This suggests that nutrient solubilization is not incidental but a necessary adaptive trait for microbial persistence in these soils. (Jiang et al., 2018) reported that halotolerant microorganisms from saline soils often exhibit enhanced mineral solubilization, which explains the superior performance of isolates Be1, Vm1, and At1 in phosphate and potassium solubilization assays. These isolates likely possess more efficient mechanisms for producing organic acids or chelating metals, conferring a competitive advantage under nutrient-limited conditions. The comparatively lower functional breadth of isolate Ko1 indicates niche specialization rather than reduced viability, underscoring functional differentiation within indigenous microbial communities. (Krishnamoorthy et al., 2016) demonstrates that nitrogen-fixing bacteria are favored in salt-affected soils with low organic carbon, consistent with the observation that all isolates retained diazotrophic potential. Nitrogen fixation in these soils likely compensates for the limited availability of nitrogen and supports microbial survival and rhizosphere stability. This adaptive strategy enhances the ecological relevance of the isolates for sustainable nutrient cycling in

saline agricultural systems. (Alori & Babalola, 2018) emphasize the role of phytohormone-producing PGPR in improving root architecture under stress, consistent with the universal IAA production observed in this study. IAA-mediated root elongation and branching may increase root–microbe contact and nutrient uptake efficiency, thereby reinforcing microbial colonization in hostile soil environments. This function is particularly important in saline soils, where root growth is often restricted by osmotic stress. (Ambrosini et al., 2016) report that siderophore production improves microbial competitiveness and plant iron acquisition in alkaline soils, which explains why four isolates expressed this trait. Iron availability is severely limited at high pH, and siderophore synthesis likely enhances both microbial survival and plant nutrition. The absence of siderophore production in Ko1 further supports functional specialization rather than uniform trait distribution among isolates. (Hailu & Mehari, 2021) and (Fahad et al., 2020) noted that salinity stress imposes multiple simultaneous constraints on plants, underscoring the importance of multifunctional PGPR. The superior multi-trait performance of Be1, Vm1, and At1 suggests that functional redundancy across nutrient mobilization, hormone production, and iron acquisition enhances their reliability as bioinoculant candidates in saline–alkaline systems. (Trabelsi & Mhamdi, 2013) caution that non-native microbial inoculants may disrupt native soil communities, underscoring the importance of using indigenous strains. The isolates identified in this study are adapted to the specific physicochemical constraints of Karad soils, increasing their likelihood of persistence, compatibility, and effectiveness under field conditions.

This study isolated three bacterial strains from saline soils of Karad Taluka, identified as *Pantoea dispersa* (At1), *Solibacillus isronensis* (Vm1), and *Pseudomonas putida* (Be1) by partial 16S rDNA sequencing. All are reported to have plant-growth-promoting potential or salt-stress tolerance, making them potential saline-soil bioinoculants. Members of genus *Pantoea*, including *P. dispersa*, are increasingly reported as salt-tolerant PGPR that promote

plant growth via mechanisms like phytohormone production (e.g., IAA), phosphate solubilization, siderophore production, and ACC-deaminase activity reducing ethylene stress under salinity (Lv, L., 2022). (Bernardette et al., 2022) worked on *Pseudomonas putida* strain. Plant growth promoting rhizobacteria (PGPR) are vital in agriculture for their benefits. Some *Pseudomonas putida* strains exhibit traits such as hormone production, nutrient solubilization, and root colonization. This review covers current knowledge of *P. putida* and related strains, listing their growth-promoting traits. Overall, the findings show that soil-driven selection shapes PGPR functionality and that indigenous halotolerant bacteria from inland saline–alkaline soils are a valuable, ecologically compatible resource for sustainable soil management. By directly linking microbial trait expression to local soil conditions, this study advances practical bioinoculant development for saline agriculture rather than reiterating generalized PGPR functions.

This research isolates native halotolerant PGPR from inland saline–alkaline soils in Maharashtra, a region with limited microbiological studies. It shows soils with high alkalinity (pH 8.8–9.4), sodicity, and low organic carbon and nutrients host bacteria with multiple traits, unlike many studies focusing on single traits or non-local strains. These microbes can solubilize phosphate and potassium, fix nitrogen, produce indole-3-acetic acid, and synthesize siderophores. Three isolates - Be1, Vm1, and At1 - exhibit all five traits, showing ecological adaptation and resilience. Combining soil analysis with microbial screening, the study reveals how inland saline soils select for multifunctional PGPR and aids in developing region-specific bioinoculants to improve soil fertility and crop yields in salt-affected regions.

5. CONCLUSION

The study reveals that saline soils in Karad Taluka are highly alkaline and nutrient-deficient, which hampers agricultural productivity. However, indigenous halotolerant bacterial isolates with plant growth-promoting rhizobacteria (PGPR) traits were successfully identified.

These isolates effectively solubilized phosphorus and potassium, fixed nitrogen, and produced indole-3-acetic acid (IAA). Three strains (Be1, Vm1, and At1) exhibited all five essential PGPR traits, indicating great potential as sustainable bioinoculants. Their use could enhance soil fertility and plant salinity tolerance, serving as an environmentally friendly alternative to chemical fertilizers. The soils are strongly alkaline, highly sodic, low in organic carbon, and variable in nutrient content. Elevated sodium and calcium carbonate mainly limit soil physical state and nutrient flow. Sustainable management requires gypsum, organic matter, and site-specific nutrient practices. Future research should include molecular characterization and field trials to assess efficacy, thereby advancing soil salinity management and improving crop productivity sustainably.

6. Author Contributions All authors contributed equally.

7. Funding:

This research received no external funding

8. Informed Consent Statement:

Not applicable

9. Acknowledgments

The author is thankful to Krishna Institute of Science and Technology (formerly known as Krishna Institute of Allied Sciences), Krishna Vishwa Vidyapeeth.

10. Conflicts of Interest

The authors declare no conflicts of interest.

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