

# 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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## ABSTRACT

Soil salinity remains a major abiotic factor limiting agricultural productivity worldwide, with the situation worsening in parts of Maharashtra, particularly Karad Taluka. This study examined saline soils from various locations within Karad, assessing their physicochemical properties and isolating indigenous halotolerant bacterial strains that exhibit plant growth-promoting rhizobacterial (PGPR) traits. The soil analysis revealed highly alkaline pH levels (8.8-9.4), elevated sodium concentrations, and deficiencies in organic carbon, nitrogen, and micronutrients. Five halotolerant bacterial isolates (Ko1, Va1, Be1, Vm1, and At1) were obtained and evaluated for PGPR activities such as phosphate and potassium solubilization, nitrogen fixation, indole-3-acetic acid (IAA) production, and siderophore synthesis. All isolates demonstrated positive results in phosphate and potassium solubilization, nitrogen fixation, and IAA production, with four also producing siderophores. Notably, isolates Be1, Vm1, and At1 showed strong performance across multiple traits, highlighting their potential as bioinoculant candidates. These findings suggest that native halotolerant PGPR strains from Karad Taluka could enhance soil fertility, improve nutrient uptake, and support plant growth in saline conditions. Future research including field trials and molecular characterization could facilitate the development of environmentally sustainable microbial formulations for saline agriculture.

## 1. INTRODUCTION

Soil salinity is a major abiotic factor limiting agricultural productivity worldwide. It affects soil structure, nutrient availability, microbial activity, and plant physiological processes (Butcher et al. 2016, Metternicht & Zinck 2003, Wang et al. 2003). Salinity causes osmotic stress, ion toxicity, and nutrient imbalance, significantly reducing crop growth and yields, particularly in arid and semi-arid regions where poor irrigation practices and excessive fertilizer application accelerate soil degradation (Hailu & Mehari 2021, Hussain et al. 2019, Sultana et al. 2020). In India, approximately 7 million hectares are affected by salinity and sodicity, with secondary salinization increasing in irrigated areas (Mandal et al. 2009, Sharma & Chaudhari 2012). Maharashtra has become a hotspot where intensive farming and inadequate drainage have led to soil alkalinity and nutrient depletion. In Karad Taluka, situated in the Krishna River basin and primarily cultivating sugarcane, soils are increasingly characterized by high pH, sodicity, low organic carbon, and micronutrient deficiencies, threatening long-term soil fertility and crop productivity. Conventional management of saline soils relies heavily on chemical amendments and fertilizers, which often only offer short-term relief and may harm soil biological health further. Plant growth-promoting rhizobacteria (PGPR) present a sustainable alternative by boosting nutrient availability and helping plants withstand stress through mechanisms such as nitrogen fixation, solubilization



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of phosphorus and potassium, phytohormone production, and siderophore-mediated iron uptake (Alori et al. 2017, Alori & Babalola 2018, Ambrosini et al. 2016). Numerous studies have shown that halotolerant PGPR can enhance crop performance under saline conditions (Jiang et al. 2018, Krishnamoorthy et al. 2016). However, most research focuses on coastal, desert, or arid ecosystems, with inland saline-alkaline soils remaining underexplored, especially in Western Maharashtra. Additionally, native microbes adapted to local soil conditions are more likely to survive, colonize the rhizosphere, and function effectively as bioinoculants than introduced strains (Trabelsi & Mhamdi 2013, Patil et al. 2024). Despite increasing salinity problems in Karad Taluka, there is a lack of systematic studies connecting soil physicochemical constraints to the functional screening of indigenous halotolerant PGPR.

This study aims to characterize the physicochemical properties of saline soils in Karad Taluka, Maharashtra, and to isolate and assess indigenous halotolerant bacterial strains for key plant growth-promoting traits such as phosphate and potassium solubilization, nitrogen fixation, indole-3-acetic acid production, and siderophore synthesis. By focusing on locally adapted microorganisms, the research 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 gathered from eight locations in Karad Taluka (see Fig. 1). Each site was precisely georeferenced with latitude and longitude coordinates. Soil samples were extracted at a depth of 15 cm using a borer. At



Fig. 1: Map of Karad Taluka.

each site, five subsamples, four from the corners and one from the center, were combined, thoroughly mixed, and reduced to about 100 g by quartering. These samples were stored in sterile polythene bags at low temperature for further analysis (Ghare & Kumbhar 2021, Kekane et al. 2015, Raut & Kurhe 2020).

### 2.2. Physicochemical Characterization of Soil Samples

The soil samples were analyzed for a variety of essential physical and chemical parameters to evaluate their fertility and salinity. pH and electrical conductivity (EC) were measured using calibrated digital meters to determine alkalinity and salinity. Organic carbon (OC) was assessed through the dichromate oxidation method, offering insights into soil organic matter content and overall soil health. Nitrogen levels were measured using the Kjeldahl method, phosphorus via the colorimetric molybdenum blue method, and potassium with Atomic Absorption Spectroscopy (AAS). To evaluate soil base saturation and cation balance, secondary nutrients such as calcium and magnesium were determined through EDTA titration. Additionally, micronutrients including iron (Fe), copper (Cu), zinc (Zn), manganese (Mn), boron (B), and sulfur (S) were analyzed using AAS or suitable colorimetric techniques to assess their availability for plant uptake. These analyses provided a comprehensive understanding of 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 containing 7.5% (w/v) NaCl and incubated at 30°C for 48 h under static conditions. The resulting enriched cultures were serially diluted and plated onto nutrient agar with the same salt concentration, then incubated again at 30°C for 48 h (Vazquez et al. 2000). Morphologically distinct colonies were purified through repeated streaking. To assess salt tolerance, purified isolates were evaluated for growth on nutrient agar with increasing NaCl concentrations (0%, 2.5%, 5%, 7.5%, and 10% w/v). These plates were incubated at 30°C for 48–72 h, and growth was qualitatively assessed based on colony formation. Isolates that consistently grew at  $\geq 7.5\%$  NaCl were identified as halotolerant and selected for further screening of plant growth-promoting rhizobacteria (Vazquez et al. 2000). Morphological traits, including colony shape, size, margin, elevation, texture, pigmentation, Gram stain, and motility, were recorded.

### 2.4. Screening for PGPR Traits

The plant growth-promoting traits of the bacterial isolates

were assessed using established microbiological techniques. The Phosphate Solubilization Index (PSI) was determined by cultivating the isolates on Pikovskaya's agar plates for seven days at  $28 \pm 2^\circ\text{C}$  (Chakdar et al. 2018, Kirui et al. 2022, Mengesha & Legesse 2024). Potassium solubilization was evaluated on Aleksandrov agar medium supplemented with feldspar as a potassium source, and the Potassium Solubilization Index (KSI) was calculated based on the size of the solubilization zone (Verma et al. 2017). To determine nitrogen-fixing ability, isolates were grown on nitrogen-free Ashby's Mannitol agar to demonstrate diazotrophic activity, followed by the acetylene reduction assay for quantification (Tang et al. 2020). IAA production was measured using Salkowski reagent, with results compared to a standard curve for accurate quantification (Patten & Glick 2011). Lastly, siderophore production was assessed on Chrome Azurol S (CAS) agar, where the formation of clear halos around colonies indicated successful siderophore synthesis.

## 2.5. Controls and Experimental Validation

Uninoculated media were used as negative controls in all biochemical and PGPR assays to ensure their specificity. For qualitative comparisons, previously documented PGPR-positive laboratory strains served as reference standards for

phosphate solubilization, IAA production, and siderophore synthesis.

## 2.6. Molecular Characterization by Using 16S rRNA

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 conducted in triplicate, and results are expressed as mean  $\pm$  standard deviation. One-way analysis of variance (ANOVA) was used to assess differences in soil physicochemical parameters and PGPR indices, utilizing standard statistical software. A p-value below 0.05 was considered statistically significant.

## 3. RESULTS

### 3.1. Physicochemical Properties of Saline Soils

The soils of Karad Taluka display considerable variability in texture, nutrient composition, and chemical characteristics. Predominantly classified as clay loam with 30–38% sand content, these soils exhibit good water retention. As detailed

Table 1(a): Physicochemical properties of saline soils.

| Parameters                 | Permissible level | Belwade Budruk                     | Vanvasmachi                        | Dushere                        | Atake                              | Khodshi East                       | Khodshi West                       | Koparde                            | Gondi                              |
|----------------------------|-------------------|------------------------------------|------------------------------------|--------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| Soil Texture Composition   |                   | Sand 38%,<br>Silt 42%,<br>Clay 20% | Sand 32%,<br>Silt 40%,<br>Clay 28% | Sand ,<br>Silt 26%,<br>Clay 6% | Sand 30%,<br>Silt 34%,<br>Clay 36% | Sand 38%,<br>Silt 20%,<br>Clay 42% | Sand 30%,<br>Silt 36%,<br>Clay 34% | Sand 34%,<br>Silt 30%,<br>Clay 36% | Sand 32%,<br>Silt 28%,<br>Clay 40% |
| Texture                    |                   | Loam                               | Clay loam                          | Clay                           | Clay loam                          | Sandy clay                         | Clay loam                          | Clay loam                          | Clay loam                          |
| pH                         | 6.5-7.5           | 9.09                               | 9.2                                | 9.1                            | 8.9                                | 8.9                                | 9.2                                | 8.8                                | 9                                  |
| Na <sup>+</sup>            | 0-1               | 3                                  | 4.1                                | 5.4                            | 3.9                                | 3.3                                | 3.2                                | 3.8                                | 3.1                                |
| Organic Carbon (%)         | 0.40-0.60         | 0.12                               | 0.15                               | 0.14                           | 0.13                               | 0.18                               | 0.16                               | 0.15                               | 0.14                               |
| N (kg/ha)                  | 280-420           | 110                                | 120                                | 105                            | 100                                | 115                                | 118                                | 125                                | 108                                |
| P (kg/ha)                  | 14-21             | 10.5                               | 9.8                                | 9.5                            | 8.9                                | 9.5                                | 9.2                                | 9                                  | 8.7                                |
| K (kg/ha)                  | 150-200           | 142                                | 135                                | 138                            | 140                                | 145                                | 148                                | 150                                | 138                                |
| Cu (ppm)                   | 0.20-99.99        | 3.5                                | 3.2                                | 3.4                            | 3.3                                | 3.1                                | 3.2                                | 3                                  | 2.9                                |
| Fe (ppm)                   | 4.5-9.9           | 4                                  | 3.8                                | 3.7                            | 3.9                                | 3.6                                | 3.7                                | 3.5                                | 3.4                                |
| Zn (ppm)                   | 0.61-99.99        | 0.52                               | 0.48                               | 0.5                            | 0.46                               | 0.44                               | 0.45                               | 0.43                               | 0.41                               |
| Mn (ppm)                   | 2.0-99.99         | 0.28                               | 0.25                               | 0.26                           | 0.24                               | 0.27                               | 0.26                               | 0.25                               | 0.23                               |
| Br (ppm)                   | 0.6-0.99          | 0.75                               | 0.72                               | 0.74                           | 0.7                                | 0.68                               | 0.69                               | 0.72                               | 0.65                               |
| S (ppm)                    | 10.-20            | 9.8                                | 9.2                                | 9.5                            | 9                                  | 8.8                                | 8.9                                | 9.1                                | 8.6                                |
| CaCO <sub>3</sub> (%)      | 10                | 10                                 | 20                                 | 6.1                            | 13.4                               | 6.8                                | 6.6                                | 6.5                                | 6                                  |
| Water Holding Capacity (%) |                   | 20                                 | 20                                 | 27                             | 20                                 | 29                                 | 28                                 | 27                                 | 26                                 |

Table Cont....

in Table 1(a, b) and Fig. 2(a–d), they are alkaline (pH 8.8–9.4) with elevated sodium levels (3.0–5.4), raising salinity concerns. Organic carbon content ranges from 0.12% to 0.19%, below the optimal 0.4–0.6%, indicating low organic matter and diminished microbial activity. Nitrogen levels

(95–150 kg.ha<sup>-1</sup>) fall short of the recommended 280–420 kg.ha<sup>-1</sup>, while phosphorus (8.5–11.2 kg.ha<sup>-1</sup>) and potassium (132–162 kg.ha<sup>-1</sup>) are moderate. Copper (2.8–3.8 ppm) and iron (3.3–4.4 ppm) are adequate, whereas zinc (0.41–0.65 ppm) and manganese (0.23–0.38 ppm) are deficient. Sulfur

Table 1(b): Physicochemical properties of saline soils.

| Parameters                 | Permissible level | Karve                              | Vadgaon Haveli                     | Chore                              | Gharewadi                          | Nandgaon                           | Shenoli                            | Kodoli                             | Mean ± S.D    |
|----------------------------|-------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|---------------|
| Soil Texture Composition   |                   | Sand 30%,<br>Silt 34%,<br>Clay 36% | Sand 30%,<br>Silt 30%,<br>Clay 40% | Sand 36%,<br>Silt 34%,<br>Clay 30% | Sand 30%,<br>Silt 30%,<br>Clay 40% | Sand 36%,<br>Silt 32%,<br>Clay 32% | Sand 30%,<br>Silt 38%,<br>Clay 32% | Sand 30%,<br>Silt 30%,<br>Clay 40% |               |
| Texture                    |                   | Clay loam                          | Clay loam                          | Clay loam                          | Clay loam                          | Clay loam                          | Clay loam                          | Clay loam                          |               |
| pH                         | 6.5-7.5           | 9.4                                | 9.1                                | 9                                  | 8.9                                | 9.2                                | 9.3                                | 9.1                                | 9.08 ± 0.17   |
| Na <sup>+</sup>            | 0-1               | 3.4                                | 3.2                                | 4.1                                | 4.9                                | 5.3                                | 3.4                                | 4.2                                | 3.89 ± 0.79   |
| Organic Carbon (%)         | 0.40-0.60         | 0.19                               | 0.17                               | 0.15                               | 0.13                               | 0.14                               | 0.15                               | 0.16                               | 0.15 ± 0.02   |
| N (kg/ha)                  | 280-420           | 95                                 | 100                                | 102                                | 130                                | 145                                | 138                                | 150                                | 117.4 ± 17.15 |
| P (kg/ha)                  | 14-21             | 8.5                                | 9                                  | 8.8                                | 10.8                               | 11.2                               | 10.5                               | 10.2                               | 9.61 ± 0.85   |
| K (kg/ha)                  | 150-200           | 132                                | 141                                | 139                                | 155                                | 160                                | 158                                | 162                                | 145.53 ± 9.51 |
| Cu (ppm)                   | 0.20-99.99        | 2.8                                | 3.2                                | 3.1                                | 3.6                                | 3.8                                | 3.5                                | 3.7                                | 3.29 ± 0.29   |
| Fe (ppm)                   | 4.5-9.9           | 3.3                                | 3.5                                | 3.6                                | 4.3                                | 4.4                                | 4.2                                | 4.1                                | 3.80 ± 0.34   |
| Zn (ppm)                   | 0.61-99.99        | 0.42                               | 0.44                               | 0.45                               | 0.6                                | 0.65                               | 0.58                               | 0.62                               | 0.50 ± 0.08   |
| Mn (ppm)                   | 2.0-99.99         | 0.24                               | 0.25                               | 0.26                               | 0.35                               | 0.38                               | 0.36                               | 0.34                               | 0.28 ± 0.05   |
| Br (ppm)                   | 0.6-0.99          | 0.63                               | 0.68                               | 0.67                               | 0.8                                | 0.82                               | 0.78                               | 0.79                               | 0.72 ± 0.06   |
| S (ppm)                    | 10.-20            | 8.4                                | 9                                  | 8.7                                | 10.2                               | 10.8                               | 10.4                               | 10.1                               | 9.37 ± 0.73   |
| CaCO <sub>3</sub> (%)      | 10                | 6.2                                | 16.4                               | 6.3                                | 7                                  | 7.2                                | 6.8                                | 7.1                                | 8.83 ± 4.32   |
| Water Holding Capacity (%) |                   | 20                                 | 29                                 | 27                                 | 26                                 | 25                                 | 29                                 | 28                                 | 25.40 ± 3.56  |

ppm: Parts per million, kg/ha: Kilogram per hectare,

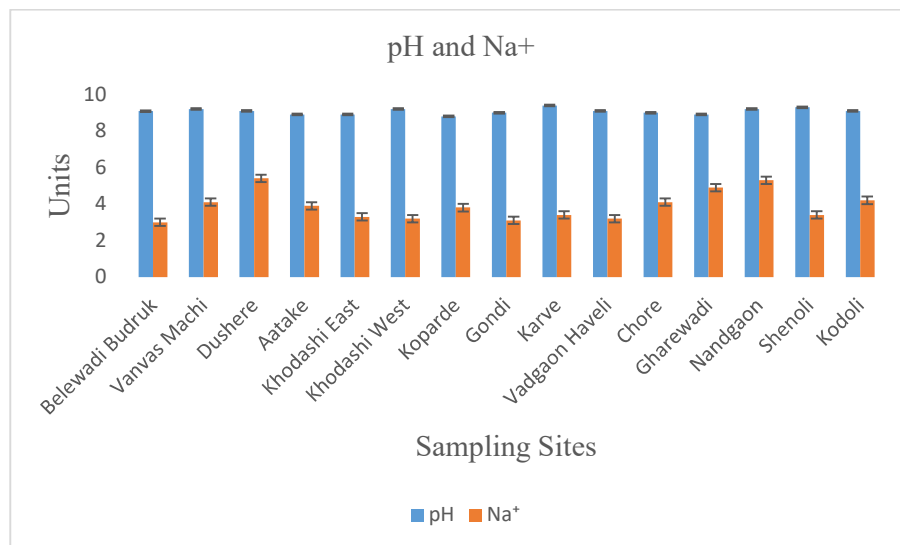


Fig. 2a : pH and Na<sup>+</sup> of soil samples.

Figure Cont....

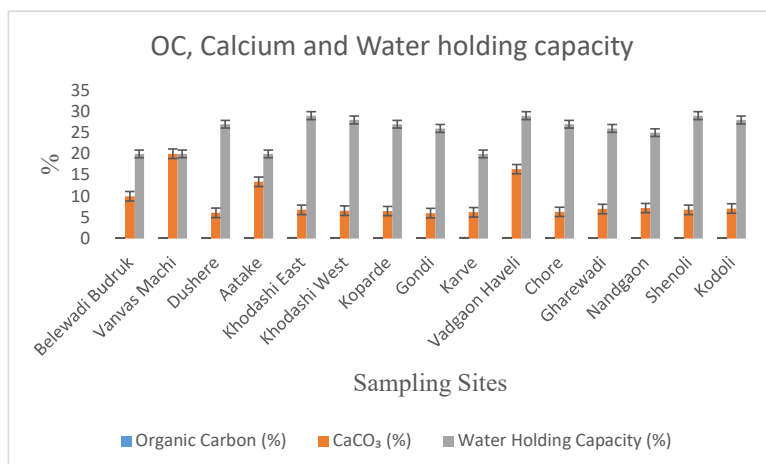
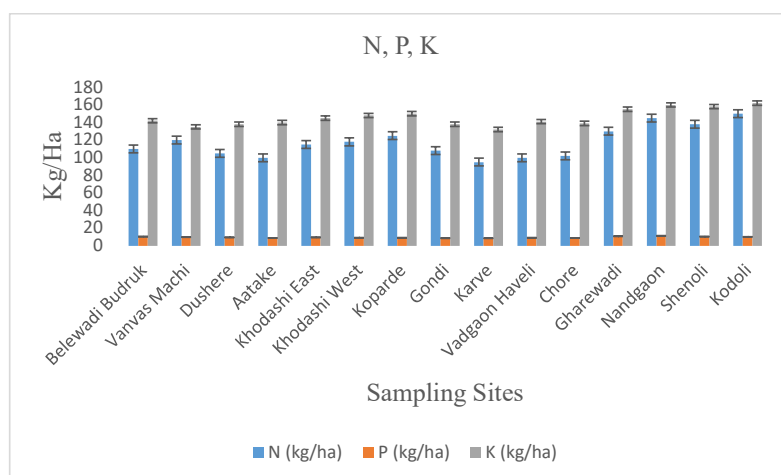
Fig 2b: OC, CaCO<sub>3</sub> and water holding capacity of soil samples sites.

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

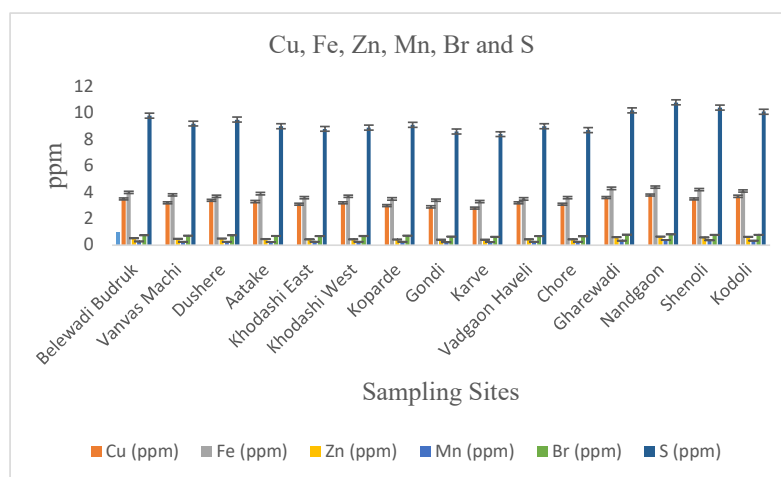


Fig. 2d: Cu, Fe, Zn, Mn, Br and S of soil samples.

(8.4–10.8 ppm) is near the lower threshold, and calcium carbonate (6–16%) is present at moderate levels. Water-holding capacity, ranging from 20% to 29%, correlates with clay content. Overall, these soils are alkaline, low in organic carbon and nitrogen, and moderately rich in nutrients. To enhance soil fertility and productivity, organic amendments and balanced fertilization are essential.

The soils exhibited an average pH of 9.09 with minimal variability (CV = 1.76%), indicating consistent alkalinity across the sampling sites. Sodium levels demonstrated moderate variability (CV = 20.31%), confirming heterogeneous sodicity. Organic carbon content was low, reflecting poor organic matter in the saline soils. Potassium levels remained high and stable, while nitrogen and phosphorus showed moderate fluctuations. Micronutrients such as zinc and manganese also varied, indicating potential deficiencies. Calcium carbonate content was highly variable (CV = 48.24%) due to uneven calcareousness, influencing alkalinity and nutrient availability. Sodicity and soil texture moderately impacted water-holding capacity.

Based on the coefficient of variation (CV), soil pH and the nutrients P, K, Cu, Fe, Br, and S showed low variability (<10%), indicating a uniform distribution across the sites. Organic carbon (OC), nitrogen (N), zinc (Zn), manganese (Mn), and water-holding capacity (WHC) exhibited moderate variability (10–20%), suggesting spatial variation influenced by soil management practices and organic matter content. In

contrast, sodium ( $\text{Na}^+$ ) and calcium carbonate ( $\text{CaCO}_3$ ) showed high variability (>20%), reflecting heterogeneity in salinity and calcareousness, possibly due to local environmental conditions and parent material. One-way ANOVA for soil pH showed no significant differences among the groups ( $F = 2.08$ ,  $p = 0.173$ ,  $p > 0.05$ ), indicating that pH remained relatively consistent across the sampling locations.

The performance variation among the halotolerant bacterial isolates was linked to their origin in saline-alkaline, nutrient-deficient soils. These soils, characterized by high pH, elevated sodium content, and low organic carbon, favor microorganisms capable of enduring osmotic and ionic stress. Isolates Be1, Vm1, and At1 exhibited enhanced phosphate and potassium solubilization, nitrogen fixation, IAA production, and siderophore secretion, indicating adaptations for nutrient mobilization. In alkaline soils, where phosphorus and potassium often exist in insoluble forms, these microbes have an advantage by increasing nutrient bioavailability. Siderophores likely assist in overcoming iron limitations, while IAA promotes root growth, strengthening plant-microbe interactions. Ko1 did not produce siderophores, suggesting niche specialization. Overall, the variation in functional traits driven by soil conditions indicates that locally adapted, multifunctional isolates are more effective as bioinoculants in saline-alkaline environments, highlighting the importance of selecting region-specific microbial strains.

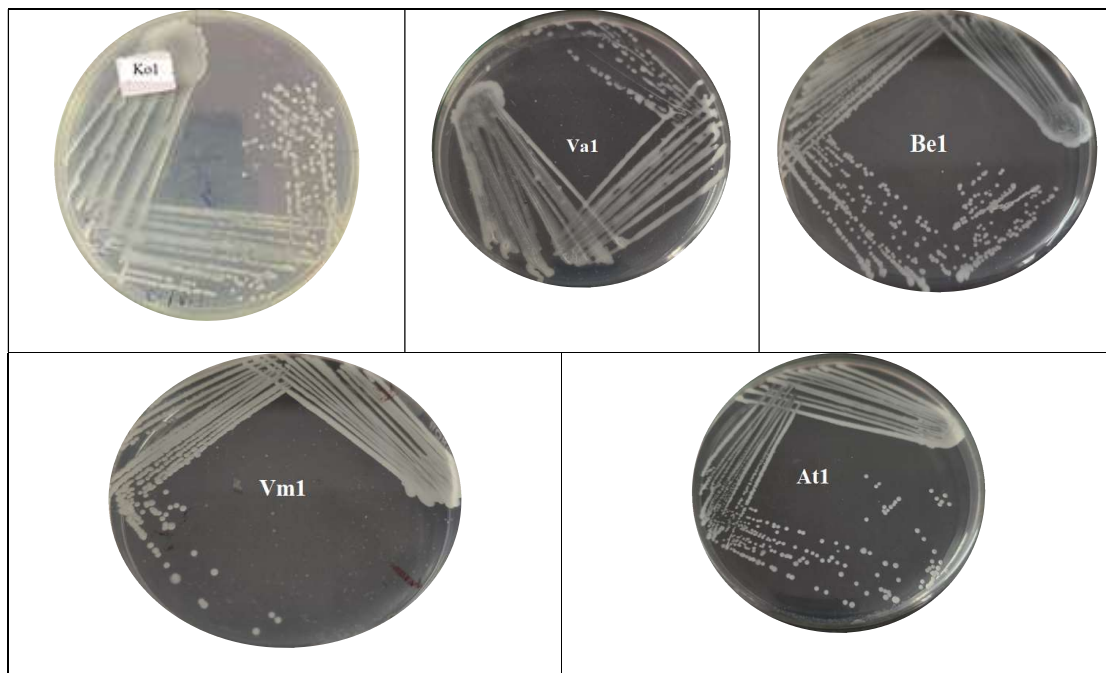


Fig. 3: Bacterial isolates from saline soil samples grown on nutrient agar media at 30°C for 48 hr.

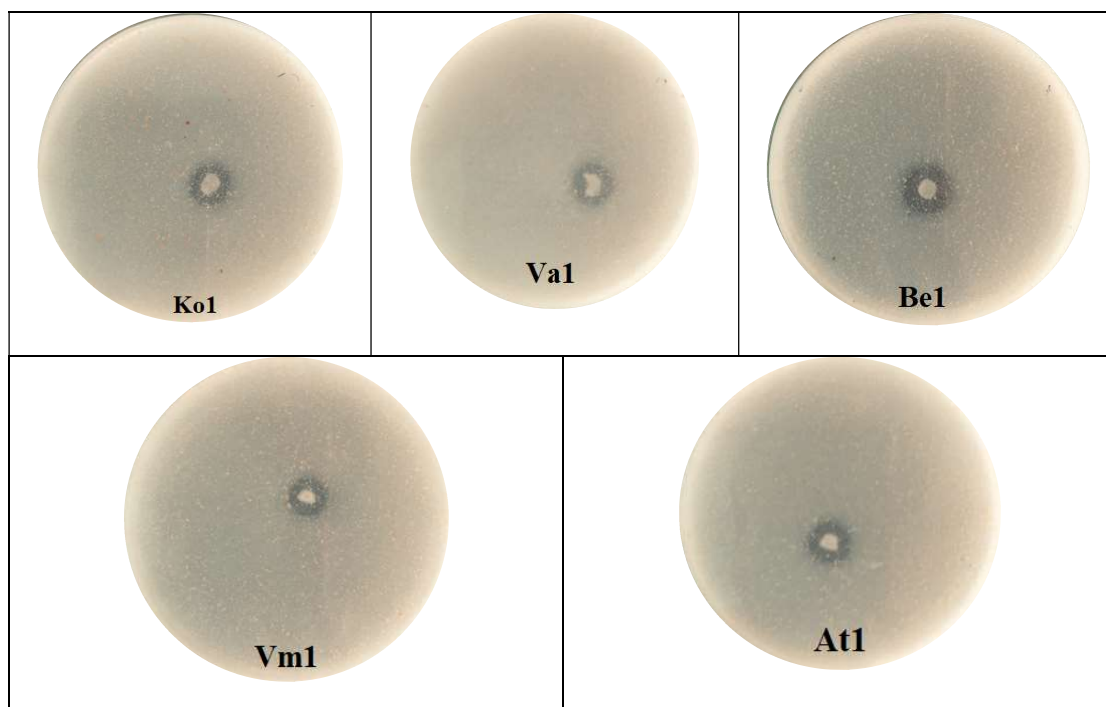


Fig. 4: Phosphate solubilization by isolates on Pikovskaya's medium.

### 3.2. Morphological Characteristics of Bacterial Isolates

The morphological and microscopic features of five bacterial isolates, i.e., Ko1, Va1, Be1, Vm1, and At1 are detailed in Fig. 3. These isolates were collected from diverse soil samples. All colonies appeared round or circular, varying in size from pinpoint to 1 mm, which reflects differences in growth rates and colony density. Colony colors ranged from white to off-white or transparent, with textures fluctuating from moist and slimy to dry-moist, indicating surface hydration variability. Most colonies were flat to convex with smooth, entire margins, suggesting uniform boundaries without irregularities. Microscopic examination showed that all isolates were Gram-negative, short rods, indicating a dominance of non-spore-forming Gram-negative bacteria in these samples.

### 3.3. PGPR Activities of Isolates

In laboratory tests, the bacterial isolates displayed a range of plant growth-promoting properties. All isolates demonstrated phosphate solubilisation, evidenced by halo zone formation on Pikovskaya's agar, although their Phosphate Solubilisation Index (PSI) values varied (Fig. 4).

#### 3.3.1. Determination of Phosphate Solubilisation Index

The isolates demonstrating phosphate-solubilizing activity were later assessed for the Phosphate Solubilization Index

(PSI) on Pikovskaya's agar. Each bacterial isolate was placed at the center of a Pikovskaya's agar plate and incubated at  $28 \pm 2^\circ\text{C}$  for five days (Chakdar et al. 2018, Kirui et al. 2022). On the fifth day, the diameters of the bacterial colony and the clear zone surrounding it were measured and used to calculate the PSI using the specified equation (see Table 2).

Phosphate Solubilizing Index (PSI) = Halo zone diameter + Colony diameter/Colony diameter

#### 3.3.2. Determination of Potassium Solubilization Index

Potassium solubilisation was equally effective, evidenced by distinct solubilisation zones on the Aleksandrov medium and measurable Potassium Solubilisation Index (KSI) values (Fig. 5). The isolates demonstrating phosphate-solubilizing activity were further assessed for the Phosphate Solubilization Index (PSI) on Pikovskaya's agar. Each

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                            |

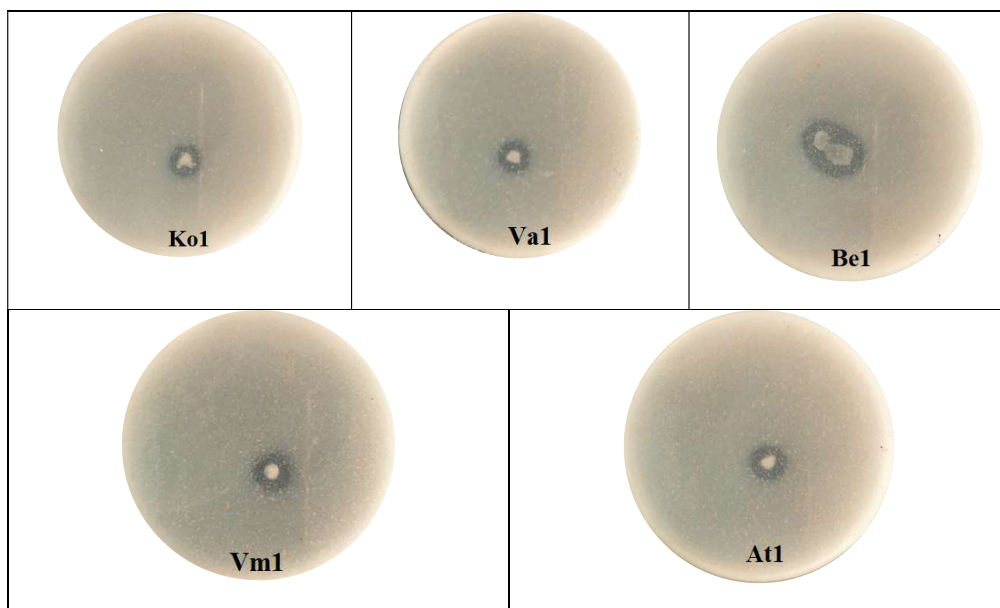


Fig. 5: Potassium solubilization by bacterial isolates on Alexandrov medium.

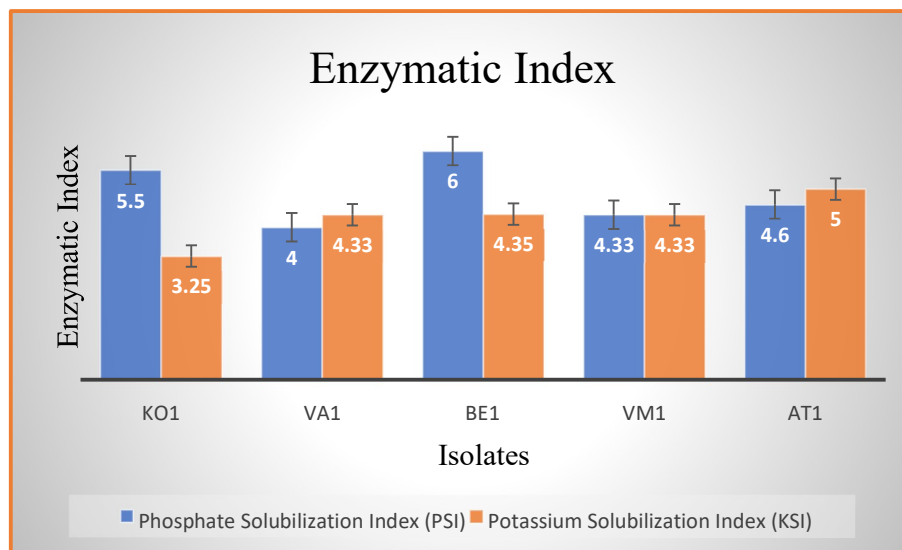


Fig. 6: Enzymatic index of selected isolates.

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                                  |

bacterial isolate was placed at the center of a Pikovskaya's agar plate and incubated at  $28 \pm 2^\circ\text{C}$  for five days (Chakdar et al. 2018, Kirui et al. 2022). On the fifth day, the diameters of the bacterial colonies and the clear zones surrounding them were measured to determine the PSI using the specified equation (Table 3 and Fig. 6).

The isolates demonstrated their ability to fix atmospheric nitrogen through growth on Ashby's medium, indicating diazotrophic potential (Fig. 7). Additionally, the development of a distinctive red coloration in the presence of Salkowski

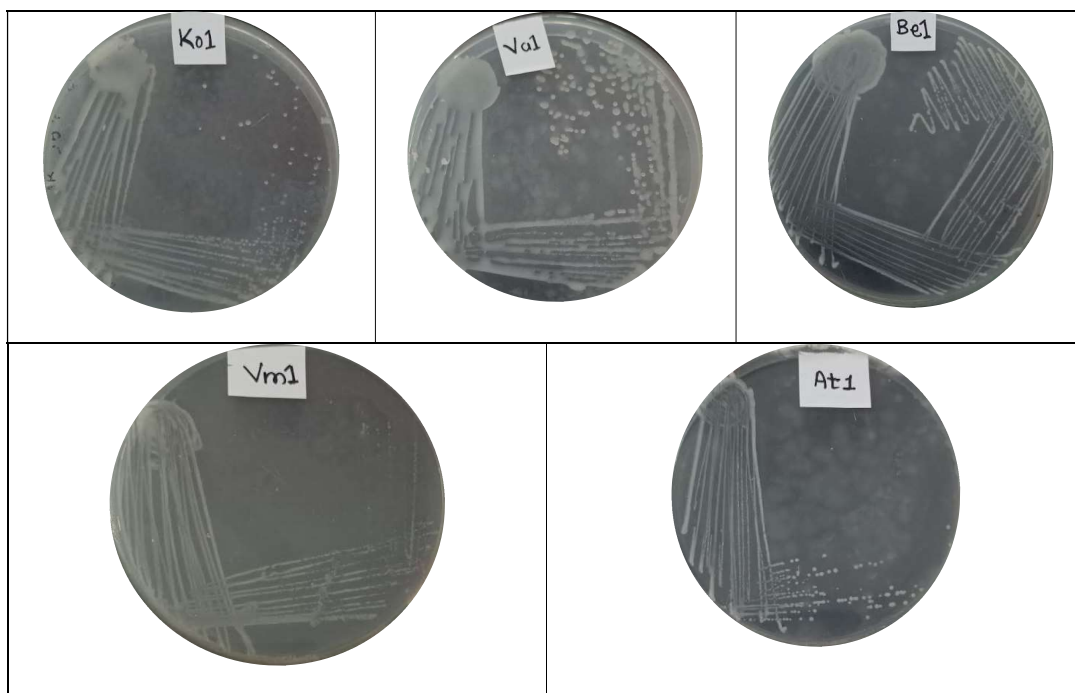


Fig. 7:  $N_2$  Fixation by bacterial isolates on Ashby's medium.

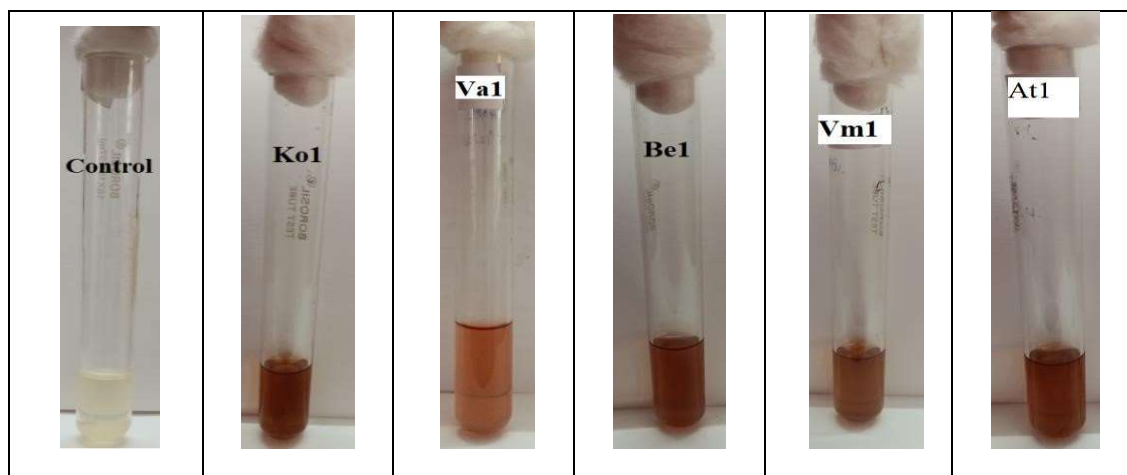


Fig. 8: IAA production by bacterial isolates on LB Broth supplemented with L-tryptophan (0.1%).

reagent confirmed that all isolates tested positive for indole-3-acetic acid (IAA) production (Fig. 8). Finally, four isolates (Va1, Be1, Vm1, and At1) produced siderophores, whereas Ko1 showed no siderophore activity (Fig. 9).

Isolate Be1, Vm1, and At1 among the tested strains exhibited strong multi-trait PGPR activity, including phosphate and potassium solubilization, nitrogen fixation, IAA production, and siderophore production. They emerged as promising candidates for bioinoculant development. The plant growth-promoting rhizobacteria (PGPR) traits of five

halotolerant bacterial isolates were systematically evaluated, with results summarized in Table 4. All isolates showed positive activity for phosphate and potassium solubilization, nitrogen fixation, and IAA production, indicating their potential to enhance nutrient availability and promote plant growth in saline conditions. Ko1 displayed activity for all traits except siderophore production, suggesting it supports plant growth through nutrient mobilization and hormone production but may have a limited role in iron chelation. Conversely, Va1, Be1, Vm1, and At1 tested positive for

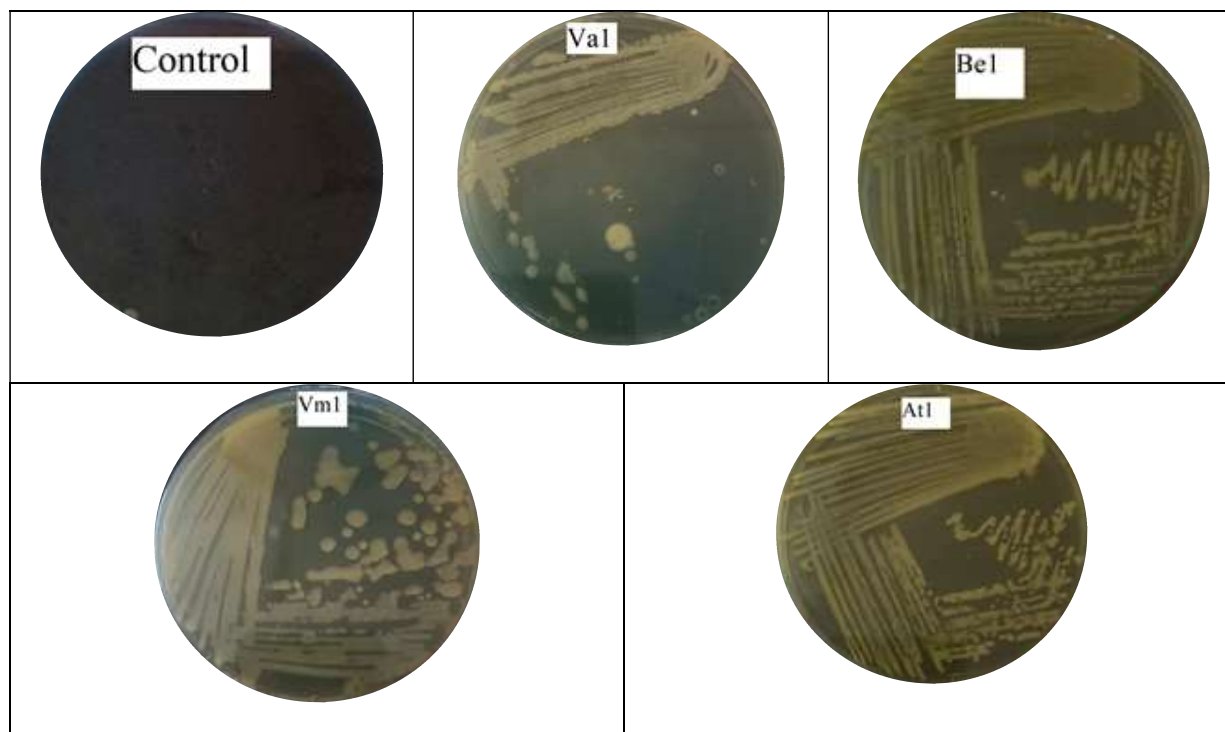


Fig. 9: Production of Siderophores by bacterial isolates on Chrome Azurol S (CAS) agar.

Table 4: Summarized PGPR activity.

| Sr. No. | Isolates | P Solubilization | K Solubilization | N <sub>2</sub> Fixation | IAA Production | Siderophore Production |
|---------|----------|------------------|------------------|-------------------------|----------------|------------------------|
| 1       | Ko1      | +                | +                | +                       | +              | -                      |
| 2       | Va1      | +                | +                | +                       | +              | +                      |
| 3       | Be1      | +                | +                | +                       | +              | +                      |
| 4       | Vm1      | +                | +                | +                       | +              | +                      |
| 5       | At1      | +                | +                | +                       | +              | +                      |

all five PGPR traits, with siderophore production adding to their multifunctional potential and making them strong bioinoculant candidates. Overall, while all isolates show promise, Be1, Vm1, and At1 stand out for their consistent performance across all traits, highlighting their potential in developing microbial bioformulations to boost crop productivity and soil fertility in saline-affected areas.

### 3.4. Molecular Identification

The genomic DNA of three isolates, At1, Vm1 and Be1, was identified as *Pantoea dispersa*, *Solibacillus isronensis* and *Pseudomonas putida*, respectively, using the BLASTN tool for 16s rDNA partial gene sequencing (Fig. 10).

## 4. DISCUSSION

The study isolated indigenous halotolerant bacteria from

saline soils in Karad, Maharashtra, demonstrating effective plant growth-promoting capabilities. All strains exhibited traits such as phosphate and potassium solubilization, nitrogen fixation, and IAA production. Additionally, four strains (Va1, Be1, Vm1, and At1) produced siderophores. These salt-adapted microorganisms enhance plant growth under saline conditions.

Soil salinity and sodicity threaten agricultural sustainability by causing land degradation and adversely affecting the soil properties essential for plant growth. Metternicht and Zinck (2003) and Butcher et al. (2016) identified soil salinity as a major constraint on soil fertility. The strongly alkaline and 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

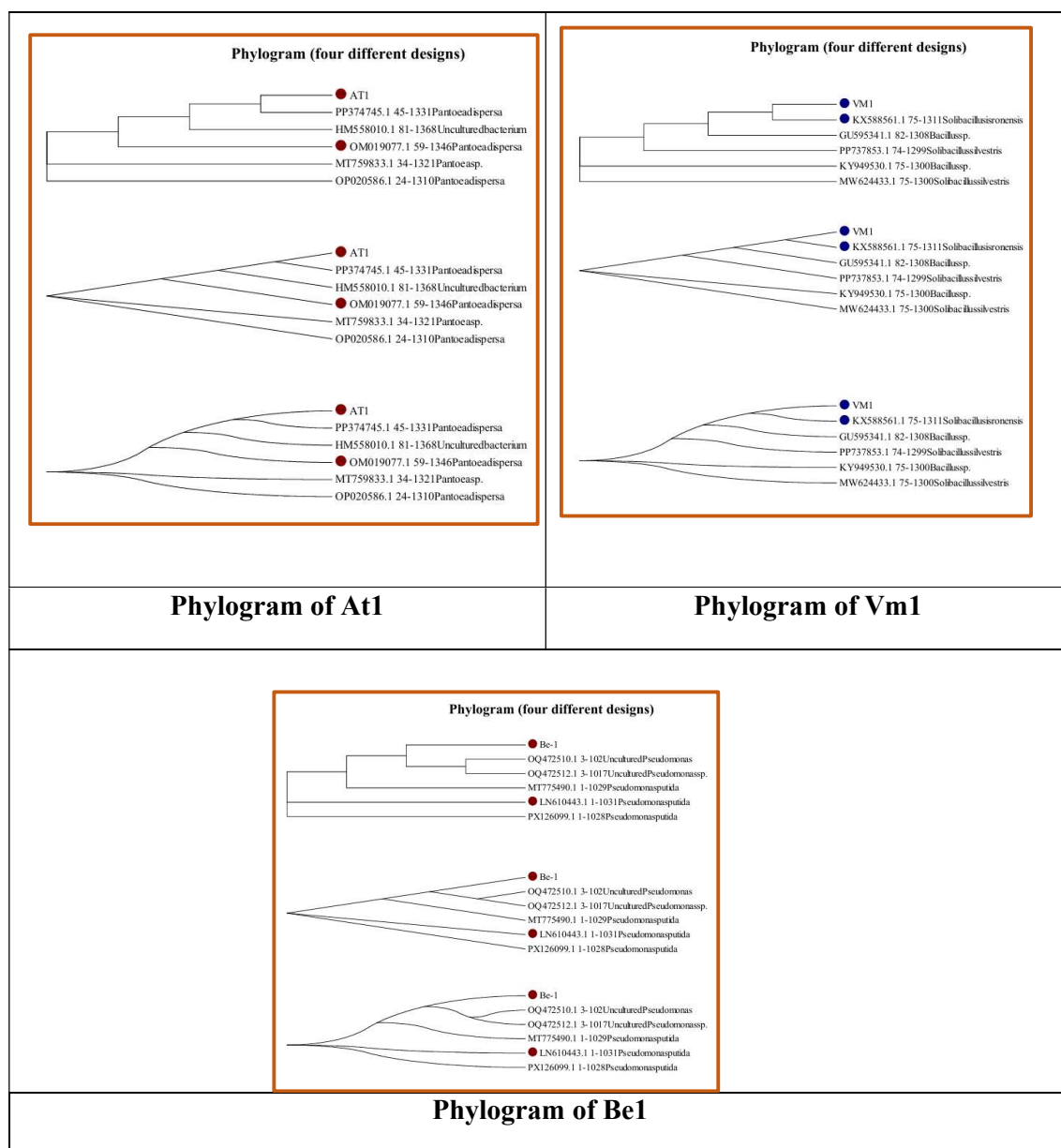


Fig. 10: Phylogenoms of isolates.

all isolates reflects the 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 rather a necessary adaptive trait for microbial persistence in these soils.

Jiang et al. (2018) observed that halotolerant microorganisms from saline soils often demonstrate enhanced mineral solubilization. This accounts for the superior performance of isolates Be1, Vm1, and At1 in phosphate and potassium solubilization tests. These isolates likely have more effective mechanisms for producing organic

acids or chelating metals, giving them a competitive edge under nutrient-limited conditions. The narrower functional range of isolate Ko1 suggests niche specialization rather than diminished viability, highlighting the functional diversity within native microbial communities.

Krishnamoorthy et al. (2016) showed that nitrogen-fixing bacteria are more prevalent in salt-affected soils with low organic carbon content. All isolates maintained their diazotrophic ability, indicating an adaptive advantage. In these challenging soils, nitrogen fixation likely compensates for nitrogen scarcity, supporting microbial survival and

maintaining rhizosphere stability. This strategy enhances the ecological importance of these isolates for sustainable nutrient cycling in saline agricultural environments.

Alori & Babalola (2018) highlighted the crucial role of phytohormone-producing PGPR in enhancing root architecture under stress conditions, aligning with the universal IAA production observed in this study. IAA-driven root elongation and branching likely promote increased contact between roots and microbes, as well as more efficient nutrient uptake, thereby supporting stronger microbial colonization in challenging soil environments. This function is especially vital in saline soils, where root growth is often hindered by osmotic stress.

Ambrosini et al. (2016) found that siderophore production enhances microbial competitiveness and promotes iron uptake in plants within alkaline soils, explaining why four isolates exhibited this trait. Due to the significantly reduced iron availability at high pH levels, siderophore synthesis likely boosts both microbial survival and plant nutrition. The lack of siderophore production in isolate Ko1 further indicates a case of functional specialization rather than a shared trait among the 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 for saline-alkaline systems.

Trabelsi & Mhamdi (2013) cautioned 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 the saline soils of Karad Taluka, identified as *Pantoea dispersa* (At1), *Solibacillus isronensis* (Vm1), and *Pseudomonas putida* (Be1) through partial 16S rDNA sequencing. All three species are known for their plant growth-promoting abilities or salt-stress tolerance, positioning them as promising bioinoculants for saline soils. Notably, members of the *Pantoea* genus, including *P. dispersa*, are increasingly recognized as salt-tolerant PGPR that enhance plant growth via mechanisms such as phytohormone (e.g., IAA) production, phosphate solubilization, siderophore synthesis, and ACC deaminase activity, which mitigates ethylene-induced stress under saline conditions (Lv 2022).

Costa-Gutierrez et al. (2022) investigated *Pseudomonas putida* strains and emphasized their significance as plant growth-promoting rhizobacteria in agriculture. Some *P. putida* strains demonstrate traits such as producing phytohormones, solubilizing nutrients, and effectively colonizing roots. Their review summarized current understanding of *P. putida* and related strains, highlighting their plant growth-promoting abilities. Overall, the results indicate that soil-driven selection influences PGPR functionality and that native halotolerant bacteria from inland saline-alkaline soils offer a valuable, ecologically compatible resource for sustainable soil management. By directly associating microbial traits with local soil conditions, this study advances the development of practical bioinoculants for saline agriculture, moving beyond generalized PGPR functions.

This study isolated native, salt-tolerant plant growth-promoting rhizobacteria (PGPR) from inland saline-alkaline soils in Maharashtra, a region with limited microbiological research. It found that soils with high alkalinity (pH 8.8–9.4), sodicity, and low organic carbon and nutrient content harbor bacteria that exhibit multiple plant growth-promoting traits. Unlike many previous studies that focused on single traits or non-native strains, these microorganisms demonstrated abilities such as phosphate and potassium solubilization, nitrogen fixation, indole-3-acetic acid production, and siderophore synthesis. Three isolates—Be1, Vm1, and At1—showed all five traits, indicating strong ecological adaptation and resilience. By combining soil physicochemical analysis with microbial functional screening, this research highlights how inland saline soils select for multifunctional PGPR and lays the groundwork for region-specific bioinoculants aimed at enhancing soil fertility and crop yields in salt-affected areas.

## 5. CONCLUSIONS

The study demonstrated that the saline soils of Karad Taluka are highly alkaline and deficient in nutrients, which restricts agricultural productivity. Nonetheless, 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 key PGPR traits, highlighting their strong potential as sustainable bioinoculants. Their application could improve soil fertility and enhance plant tolerance to salinity, offering an eco-friendly alternative to chemical fertilizers. The soils were markedly alkaline, highly sodic, low in organic carbon, and showed variable nutrient levels. Elevated sodium and

calcium carbonate levels primarily compromised soil physical properties and nutrient availability. Sustainable management strategies should include gypsum application, organic matter addition, and site-specific nutrient management. Future research should focus on molecular characterization and field trials to assess these isolates' effectiveness, thereby advancing soil salinity management and boosting crop productivity sustainably.

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## 7. REFERENCES

- Alori, E.T. and Babalola, O.O., 2018. Microbial inoculants for improving crop quality and human health in Africa. *Frontiers in Microbiology*, 9, pp.1–12. [DOI]
- Alori, E.T., Dare, M.O. and Babalola, O.O., 2017. Microbial inoculants for soil quality and plant health. In: *Microorganisms for Sustainability*, Springer, pp.281–307. [DOI]
- Ambrosini, A., de Souza, R. and Passaglia, L.M.P., 2016. Ecological role of bacterial inoculants and their potential impact on soil microbial diversity. *Plant and Soil*, 400(1–2), pp.193–207. [DOI]
- Butcher, K., Wick, A.F., Desutter, T., Chatterjee, A. and Harmon, J., 2016. Soil salinity: A threat to global food security. *Agronomy Journal*, 108(6), pp.2189–2200. [DOI]
- Chakdar, H., Dastager, S.G., Khire, J.M., Rane, D. and Dharne, M.S., 2018. Characterization of mineral phosphate solubilizing and plant growth promoting bacteria from termite soil of arid region. *3 Biotech*, 8(11), pp.1–11. [DOI]
- Costa-Gutierrez, S.B., Adler, C., Espinosa-Urgel, M. and de Cristóbal, R.E., 2022. *Pseudomonas putida* and its close relatives: mixing and mastering the perfect tune for plants. *Applied Microbiology and Biotechnology*, 106(9), pp.3351–3367. [DOI]
- Fahad, S., Hasanuzzaman, M., Alam, M., Ullah, H., Saeed, M., Khan, I.A. and Adnan, M., 2020. Environment, climate, plant and vegetation growth. In: *Environment, Climate, Plant and Vegetation Growth*, Springer. [DOI]
- Ghare, P.M. and Kumbhar, A.P., 2021. Study on physico chemical parameters of soil sample. *IARJSET*, 8(9), pp.171–187. [DOI]
- Hailu, B. and Mehari, H., 2021. Impacts of soil salinity/sodicity on soil-water relations and plant growth in dry land areas: A review. *Journal of Natural Sciences Research*, 12(3), pp.1–10. [DOI]
- Hussain, S., Shaukat, M., Ashraf, M., Zhu, C., Jin, Q. and Zhang, J., 2019. Salinity stress in arid and semi-arid climates: Effects and management in field crops. In: *Climate Change and Agriculture*. [DOI]
- Jiang, H., Qi, P., Wang, T., Wang, M., Chen, M., Chen, N., Pan, L. and Chi, X., 2018. Isolation and characterization of halotolerant phosphate-solubilizing microorganisms from saline soils. *3 Biotech*, 8(11), pp.1–8. [DOI]
- Kekane, S.S., Chavan, R.P., Shinde, D.N., Patil, C.L. and Sagar, S.S., 2015. A review on physico-chemical properties of soil. *International Journal of Chemical Studies*, 3(4), pp.29–32.
- Kirui, C.K., Njeru, E.M. and Runo, S., 2022. Diversity and phosphate solubilization efficiency of phosphate solubilizing bacteria isolated from semi-arid agroecosystems of Eastern Kenya. *Microbiology Insights*, 15, pp.1–14. [DOI]
- Krishnamoorthy, R., Kim, K., Subramanian, P., Senthilkumar, M., Anandham, R. and Sa, T., 2016. Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. *Agriculture, Ecosystems and Environment*, 231, pp.233–239. [DOI]
- Lv, L., Luo, J., Ahmed, T., Zaki, H.E., Tian, Y., Shahid, M.S., Chen, J. and Li, B., 2022. Beneficial effect and potential risk of *Pantoea* on rice production. *Plants*, 11(19), p.2608. [DOI]
- Mandal, A.K., Sharma, R.C. and Singh, G., 2009. Assessment of salt affected soils in India using GIS. *Geocarto International*, 24(6), pp.437–456. [DOI]
- Mengesha, A.S. and Legesse, N.H., 2024. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of lentil (*Lens culinaris* M.). *PLoS ONE*, 19(11), pp.1–17. [DOI]
- Metternicht, G.I. and Zinck, J.A., 2003. Remote sensing of soil salinity: Potentials and constraints. *Remote Sensing of Environment*, 85(1), pp.1–20. [DOI]
- Panhalkar, S.S., 1994. Assessment of soil salinity in southern Shirol Tahsil of Maharashtra (India) using IDW geospatial techniques. *Journal of Environmental Studies*, 2(3), pp.1–10.
- Patil, A., Abhang, R. and Pathade, G., 2024. Development of microbial bioinoculant of soil isolates for the reclamation of salt affected soils from Karad Taluka, Maharashtra, India: A mini review. *Ecology, Environment and Conservation*, 30(Suppl), pp.243–246. [DOI]
- Patten, C.L. and Glick, B.R., 2011. Role of bacterial indole-3-acetic acid in plant growth promotion. *Applied and Environmental Microbiology*, 68(8), pp.3795–3801. [DOI]
- Raut, R.R. and Kurhe, A., 2020. Studies on soil quality parameters in relation to cropping patterns, micronutrients and pH from Goagalgaon area in Ahmednagar District. *International Journal of Innovative Research in Multidisciplinary Fields*, 30(4), pp.1–9.
- Sharma, D.K. and Chaudhari, S.K., 2012. Agronomic research in salt affected soils of India: An overview. *Indian Journal of Agronomy*, 57(3), pp.175–185.
- Sultana, S., Paul, S.C., Parveen, S., Alam, S., Rahman, N., Jannat, B., Hoque, S., Rahman, M.T. and Karim, M.M., 2020. Isolation and identification of salt-tolerant plant-growth-promoting rhizobacteria and their application for rice cultivation under salt stress. *Canadian Journal of Microbiology*, 66(2), pp.1–12. [DOI]
- Tang, A., Haruna, A.O., Majid, N.M.A. and Jalloh, M.B., 2020. Potential PGPR properties of cellulolytic, nitrogen-fixing, phosphate-solubilizing bacteria in rehabilitated tropical forest soil. *Microorganisms*, 8(3), pp.1–22. [DOI]
- Trabelsi, D. and Mhamdi, R., 2013. Microbial inoculants and their impact on soil microbial communities: A review. *BioMed Research International*, 2013, pp.1–11. [DOI]
- Vazquez, P., Holguin, G., Puente, M.E., Lopez-Cortes, A. and Bashan, Y., 2000. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Biology and Fertility of Soils*, 30(5–6), pp.460–468. [DOI]
- Verma, P., Yadav, A.N., Khannam, K.S., Saxena, A.K. and Suman, A., 2017. Potassium-solubilizing microbes: Diversity, distribution and role in plant growth promotion. *Microorganisms for Sustainability*, 6, pp.125–149. [DOI]
- Wang, W., Vinocur, B. and Altman, A., 2003. Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, 218(1), pp.1–14. [DOI]
- Youseif, S.H., 2018. Genetic diversity of plant growth promoting rhizobacteria and their effects on the growth of maize plants under greenhouse conditions. *Annals of Agricultural Sciences*, 63(1), pp.25–35. [DOI]