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Article

Isolation and Identification of Bacterial Isolates from Saline Soil and Evaluation of Plant Growth-Promoting Traits under Salt Stress

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Abstract: Stenotrophomonas spp., identified as rhizospheric bacteria, demonstrate considerable potential in enhancing plant growth under saline stress via mineral solubilization and the synthesis of bioactive metabolites. This study involved the acquisition of bacterial isolates from salt-affected soils, which were then subjected to thorough morphological, biochemical, and molecular characterization. Molecular identification was performed through amplification and sequencing of the 16S rRNA gene; analysis of the resultant sequences verified the isolates as Stenotrophomonas rhizophila. The sequences have been deposited in the GenBank database with accession numbers PQ438080 (strain SprA) and PQ438113 (strain SprB). Both isolates displayed optimal growth at 37 °C and exhibited extensive tolerance to salinity, pH, and temperature, sustaining viability up to 50 °C. Functional assays demonstrated their capacity to solubilize phosphate and synthesize siderophores, indole-3-acetic acid (IAA), and hydrogen cyanide (HCN). Strain SprA demonstrated superior growth efficiency across various NaCl concentrations (OD600: 0.112-0.900), whereas strain SprB achieved the highest IAA production (83.73 µg/mL). The physiological and biochemical characteristics, along with their established taxonomic classification, highlight the potential of S. rhizophila strains as bioinoculants for improving plant resilience and productivity in saline agroecosystems.

Key words: *Stenotrophomonas* spp., halotolerant plant growth-promoting rhizobacteria (HT-PGPR), 16S rRNA gene.

1. Introduction

Soil salinization is a critical environmental and agricultural issue, particularly in semi-arid regions such as Egypt, where low precipitation, high temperatures, and high evaporation rates exacerbate salt accumulation in the soil (**Moursyet al., 2025**). The intrusion of saline water into irrigated lands and increasing drought events further intensify the problem, resulting in reduced agricultural productivity

and threatening food security. Salinity negatively affects plant growth by disrupting the availability, distribution, and mobility of essential nutrients (**Alexander** *et al.*, **2019a**). High concentrations of sodium (Na⁺) and chloride (Cl⁻) ions lead to osmotic stress and ionic imbalances, which impair plant physiological functions and nutrient uptake.

In response to these challenges, recent studies have explored the use of halotolerant plant growth-promoting rhizobacteria (HT-PGPR) as a sustainable approach to improving crop tolerance under saline conditions. Halotolerant bacteria are capable of surviving in both saline and non-saline environments. They maintain osmotic balance by synthesizing compatible solutes and regulating intracellular ion concentrations, allowing them to adapt to harsh environments such as salt-affected soils and saline irrigation systems (Rahman et al., 2017 and Roberts, 2005).

Among HT-PGPR, *Stenotrophomonas rhizophila* has gained attention for its potential role in enhancing plant growth under salt stress. Species within the *Stenotrophomonas* genus have been identified in the rhizosphere of various crops and are known for their diverse plant growth-promoting mechanisms, including the production of ACC deaminase, phosphate solubilization, and biological nitrogen fixation (**Alemneh***et al.*, **2021**). Notably, *S. rhizophila* has been isolated from saline soils and exhibits a high tolerance to salt due to the presence of stress-protective compounds such as trehalose and glucosylglycerol (Singh et al., 2013).

To study and utilize these beneficial bacteria effectively, accurate species identification and characterization are essential. One of the most widely used molecular tools for bacterial identification is 16S ribosomal RNA (rRNA) gene sequencing. The 16S rRNA gene, which encodes the RNA component of the 30S ribosomal subunit, contains conserved and hypervariable regions that enable reliable phylogenetic analysis and species-level classification (Clarridge, 2004 andWoeseand Fox, 1977). This method is particularly valuable for identifying both culturable and unculturable bacteria in environmental samples (Janda and Abbott, 2007), and has become a standard technique in microbial ecology, agricultural microbiology, and biotechnology.

Despite its usefulness, 16S rRNA sequencing has limitations in differentiating closely related species, such as *S. maltophilia* and *S. rhizophila*, due to their high sequence similarity. Therefore, complementary genetic markers (e.g., *gyrB*, *recA*, *atpD*) or whole-genome sequencing may be required for more accurate classification (**Alaviet al., 2014**). Nevertheless, 16S rRNA remains a foundational tool in microbial identification and community profiling, especially when combined with next-generation sequencing (**Zhang et al., 2023**).

This study aims to isolate and characterize halotolerant strains of *Stenotrophomonas rhizophila* from saline environments and to evaluate their plant growth-promoting traits under salt stress. The research further seeks to genetically identify the isolates using 16S rRNA gene sequencing.

2. Materials and Methods

A series of experiments were conducted to achieve the study's objectives. These included the isolation and characterization of halophilic bacteria from saline soil, in vitro evaluation of their tolerance to salt stress, assessment of plant growth-promoting (PGP) traits under salinity, and molecular identification using 16S rRNA gene sequencing.

2.1. Sample Collection

Saline soil samples were collected from the rhizospheric region of a previously cultivated field at the Research Station – Sahel El Husinia. The physical and chemical properties of the soil were analyzed and are presented in Table 1.

Table (1). Physical and chemical characteristics of the saline soil sample

Soil Characteristic	Value
Clay (%)	65.52
Silt (%)	25.33
Fine Coarse (%)	6.23
Fine Sand (%)	2.92
Soil Texture	Clay
Electrical Conductivity (EC, dS/m)	9.27
Soil pH	8.13
Organic Matter (%)	0.26
Exchangeable Sodium Percentage (ESP)	21.42

2.2. Isolation of Halophilic Bacteria

Ten grams of soil were suspended in 90 mL of sterile distilled water in a 250 mL conical flask. Additionally, 1 g of rhizospheric soil was suspended in 9 mL of sterile water in test tubes. Both suspensions were shaken vigorously for 10 minutes and serially diluted up to 10⁻⁵. One milliliter from each dilution was plated (in triplicate) onto nutrient agar supplemented with NaCl. Plates were incubated at 28 °C for 3–5 days. Colonies with distinct morphological features were selected, purified by repeated streaking, and maintained on agar slants for further study.

2.3. Morphological Characterization of Isolates

The selected bacterial isolates were characterized based on colony morphology (color, shape, elevation, margin), Gram staining, and cell shape. Morphological features were identified using Bergey's Manual of Systematic Bacteriology (Garrityet al., 2005).

2.4. Salt Tolerance Assay

Each bacterial isolate was grown in nutrient broth amended with different NaCl concentrations: 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, and 5%. Cultures were incubated, and growth was measured spectrophotometrically at 600 nm (OD600) to determine cell density (**Ventosa***et al.*, 1998).

2.5. pH Tolerance Test

To assess pH tolerance under saline conditions, isolates were cultured in nutrient broth containing 5% NaCl, with the medium adjusted to pH 5, 6, 7, and 8. After incubation, bacterial growth was evaluated spectrophotometrically at 600 nm. (**Ventosa***et al.*, **1998**).

2.6. Temperature Tolerance Test

Isolates were inoculated into nutrient broth with 5% NaCl and incubated at different temperatures: 28 °C, 37 °C, 42 °C, and 50 °C. Growth was measured at 600 nm to evaluate temperature tolerance (**Madigan***et al.*, **2021**).

2.7. Assessment of Plant Growth-Promoting (PGP) Traits

2.7.1. Phosphate Solubilization under Salt Stress

Phosphate solubilization was tested using liquid NBRIP medium containing tricalcium phosphate (**Pikovskaya**, 1948), supplemented with 5% NaCl. Soluble phosphorus was quantified spectrophotometrically at 640 nm using the method described by **Watanabe and Olsen** (1965).

2.7.2. Indole-3-Acetic Acid (IAA) Production

Isolates were cultured in Luria–Bertani (LB) broth amended with 5% NaCl to assess IAA production under salt stress (**Bakker andSchippers**, **1987**). After incubation, Salkowski reagent was added, and the development of a pink color indicated IAA synthesis. The concentration of IAA was quantified at 530 nm using a standard curve and expressed in μ g/mL (**Brick et al., 1991**). All tests were performed in duplicate.

2.7.3. Additional PGP Traits

The following additional PGP traits were evaluated in the presence of 5% NaCl:

- Hydrogen cyanide (HCN) production (Bakker and Schippers, 1987)
- Siderophore production (SchwynandNeilands, 1987)
- Nitrogen fixation and ammonium production (Bakker andSchippers, 1987)

Each trait was assessed using appropriate selective media supplemented with salt.

2.8. Molecular Identification of Halophilic Isolates

2.8.1. DNA Extraction and PCR Amplification

Selected isolates were grown in nutrient broth containing the same NaCl concentration used during isolation (**Dowson, 1957**). Genomic DNA was extracted using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Cat. No. K0721) and quantified using a NanoDropOneC spectrophotometer to adjust DNA concentration to $100 \text{ ng/}\mu\text{L}$.

PCR amplification of the 16S rRNA gene was performed in a 25 µL reaction containing:

- 100 ng genomic DNA
- 20 pmol each of universal primers
- 2× EmeraldAmp Max PCR Master Mix

Primers used:

- 8F: 5'-AGTTGATCCTGGCTCAG-3' (Attallahet al., 2014)
- 1492R: 5'-ACCTTGTTACGACTT-3' (Lane, 1991)

PCR conditions:

- Initial denaturation: 95 °C for 5 min
- 35 cycles of:

o Denaturation: 94 °C for 30 sec

o Annealing: 55 °C for 30 sec

o Extension: 72 °C for 2 min

• Final extension: 72 °C for 5 min

PCR products (~1500 bp) were confirmed via 2% agarose gel electrophoresis, purified using the Qiagen PCR Purification Kit, and sequenced at the Institute of Biochemistry and Biology, Potsdam, Germany, using an ABI sequencer.

2.8.2. Sequence Analysis and Submission

Sequences were analyzed using the NCBI BLASTN tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine homology and taxonomic identity (Altschul et al., 1990). Nucleotide sequences were submitted to the NCBI GenBank, and accession numbers were obtained for each isolate.

3. Results and Discussion

3.1. Morphological Characterization of Isolates

The two isolates (SprA and SprB) demonstrated distinct morphological traits consistent with members of the genus *Stenotrophomonas*, as described in Bergey's Manual of Systematic Bacteriology (Garrityet al., 2005). Colonies appeared light yellow, flat, translucent, mucous, and with smooth edges. Microscopic analysis showed Gram-negative, non-sporulated, rod-shaped or coccoid bacilli, aligning with typical *Stenotrophomonas* spp. morphology.

3.2. Growth Performance Under Salt Stress

The isolates' ability to tolerate varying NaCl concentrations (2–5%) is shown in **Figure 1**. Both isolates demonstrated growth at all concentrations tested. However, SprA exhibited the highest overall growth, with OD_{600} values ranging from 0.112 to 0.900 across the different salinity levels.

These findings confirm the halotolerant nature of both isolates, with substantial NaCl resistance up to 5%. Previous studies reported that while *Stenotrophomonas* spp. typically tolerate up to 1% NaCl (**Suzina***et al.*, **2018**), *S. rhizophila* strains can grow well at 5% (**Wolf** *et al.*, **2002**). This tolerance is attributed to the accumulation of compatible solutes such as amino acids, carbohydrates, and polyols, which help maintain osmotic balance (**ShivanandandMugeraya**, **2011** and **Gupta** *et al.*, **2015**).

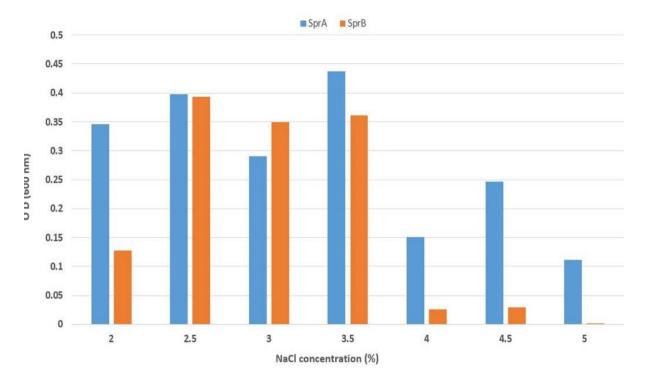


Figure (1). Growth performance of isolates under different NaCl concentrations.

3.3. pH Tolerance

The effect of pH on bacterial growth is illustrated in **Figure 2**. Both isolates grew well between pH 6 and 9, with no growth at pH 5. SprA showed optimal growth at pH 7–9, with maximum OD_{600} values of 0.181, 0.192, and 0.172, respectively.

These results align with **Wang** *et al.* (2022), who reported that *Stenotrophomonas* strains tolerate a pH range of 5–10, with optimal growth near pH 9. This adaptability supports their application in variable rhizosphere conditions.

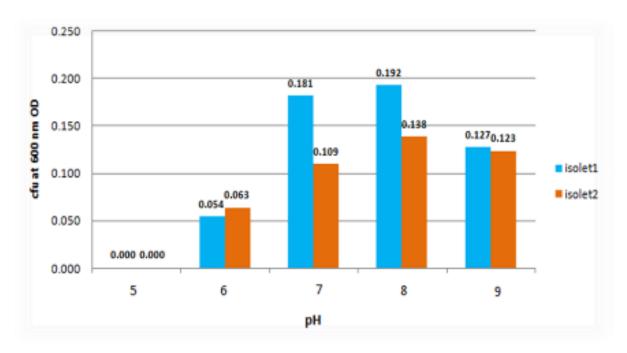


Figure (2). Growth performance of isolates at various pH

3.4. Temperature Tolerance

As shown in **Figure 3**, both isolates grew at all tested temperatures ($28 \,^{\circ}\text{C}$ to $50 \,^{\circ}\text{C}$), with optimum growth observed at $37 \,^{\circ}\text{C}$. Growth at elevated temperatures ($42 \,^{\circ}\text{C}$ and $50 \,^{\circ}\text{C}$) supports the thermotolerant nature of the isolates.

These findings agree with those of **Suzina***et al.* (2018) and **Wolf** *et al.* (2002), who reported that *Stenotrophomonas* species thrive in the 15–45 °C range, with optimal growth at 30–37 °C.

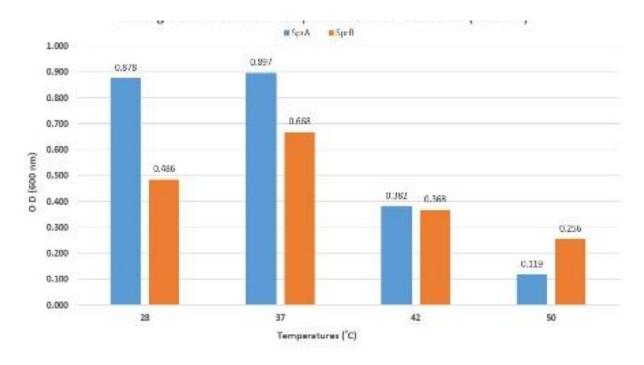


Figure (3). Growth performance of isolates at different temperatures

3.5. Plant Growth-Promoting (PGP) Traits Under Salt Stress

The PGP traits of the isolates under 5% NaCl stress are summarized in **Table 2**.

3.5.1. Indole-3-Acetic Acid (IAA) Production

Both isolates produced IAA under salt stress. SprB produced significantly higher levels $(27.50 \,\mu\text{g/mL})$ than SprA $(13.64 \,\mu\text{g/mL})$. These findings are consistent with previous studies reporting IAA production by *Stenotrophomonas* spp. under abiotic stress conditions (**Silambarasan***et al.*, 2020 and Patel and Saraf, 2017).

3.5.2. Phosphate Solubilization

Both isolates solubilized inorganic phosphate, with SprB again showing greater efficiency (81.21 ppm) than SprA (75.50 ppm). This supports previous findings from **Xiao** *et al.* (2009) and **Singh** and **Jha** (2017).

3.5.3. Additional PGP Traits

Qualitative assessments confirmed the ability of both isolates to produce hydrogen cyanide (HCN), siderophores, and ammonia, as well as to fix nitrogen. SprB showed higher activity in most categories.

These traits underscore the potential of both isolates—particularly SprB—as effective plant growth-promoting rhizobacteria (PGPR) in saline environments. Similar observations have been made for *Stenotrophomonas maltophilia* by **Singh andJha** (2017), **Huda** *et al.* (2022), and **Fittonet** *al.* (2019), validating the genus's role in stress mitigation and plant enhancement.

Table ((2).	Plant	growth-	promoting	traits	of isolates	under salt stress
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PGP Trait	SprA	SprB
HCN production	+	++
Siderophore production	+	++
Ammonia production	++	+++
Nitrogen fixation	++	++
Phosphate solubilization (ppm)	75.50	81.21
IAA production (μg/mL)	13.64	27.50

3.6. Molecular Identification via 16S rRNA Sequencing

The 16S rRNA gene sequences of both isolates showed over 98% similarity to reference sequences of *Stenotrophomonas rhizophila* in the NCBI GenBank database. Accession numbers for the sequences are listed in **Table 3**.

These results confirm the identity of both isolates as *S. rhizophila*, a species known for its remarkable abiotic stress tolerance, including salinity and drought (**Egamberdieva***et al.*, **2019**). *S. rhizophila* produces compatible solutes like trehalose and betaine, and exopolysaccharides (EPS) that enhance soil structure and moisture retention—traits that make it ideal for use as a bioinoculant in salt-affected soils.

GenBank Accession Sequence Length Strain NCBI GenBank database No. (bp) *Stenotrophomonasrhizophila*strain SprA PO438080 1081 A 16S ribosomal RNA gene, partial s - Nucleotide - NCBI Stenotrophomonasrhizophilastrain 969 B 16S ribosomal RNA gene, SprB PQ438113

Table (3). Molecular identification of bacterial isolates via 16S rRNA sequencing

4. Conclusion

The isolates identified as *Stenotrophomonas rhizophila* exhibited strong tolerance to salt, temperature, and pH stresses, alongside key plant growth-promoting traits under saline conditions. Their ability to produce IAA, solubilize phosphate, and fix nitrogen under salt stress highlights their potential as microbial biofertilizers. These findings support the application of *S. rhizophila* strains in sustainable agriculture, particularly for enhancing crop productivity in salt-affected environments.

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