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Isolation and Characterization of Salt-Tolerant PGPR for Enhancing Moong (*Vigna radiata*) Growth

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ABSTRACT: Soil salinity is a major abiotic stress that severely limits plant growth and agricultural productivity by inducing osmotic imbalance, nutrient deficiency, and oxidative stress. Plant Growth-Promoting Rhizobacteria (PGPR) have emerged as a sustainable approach to enhance plant tolerance against salinity stress. This study aimed to isolate and characterize salt-tolerant PGPR from saline soils and assess their plant growth-promoting potential in *Vigna radiata* (moong) under controlled pot conditions. Soil samples were collected from the Shirti region, Maharashtra, India, and analyzed for physicochemical properties. PGPR were isolated using nutrient agar enrichment techniques and screened for halotolerance at varying NaCl concentrations (1%-18%). The selected isolates were identified based on morphological, biochemical, and molecular characterization, including 16S rRNA gene sequencing. Three highly salt-tolerant isolates were identified: *Bacillus thuringiensis* SR-121, *Halomonas meridiana* SR-161, and *Alkalibacillus* sp. SR-103. The pot experiment was conducted to evaluate their impact on plant growth parameters such as seed germination, plant height, root and shoot length, fresh and dry biomass. Seeds were inoculated with bacterial suspensions and sown in sterilized soil. The results demonstrated that bacterial inoculation significantly improved plant growth under saline conditions. *Bacillus thuringiensis* SR-121 exhibited the highest plant height (16.5 cm), shoot length (12.3 cm), and biomass accumulation, followed by *Alkalibacillus* sp. SR-103 and *Halomonas meridiana* SR-161. This study highlights the potential of salt-tolerant PGPR as biofertilizers for improving crop productivity in saline soils. The findings suggest that these isolates can be utilized for sustainable agriculture by enhancing plant growth and mitigating salt-induced stress. Further field trials and formulation development are recommended to establish their large-scale application in saline agricultural environments.

Keywords: PGPR, Soil Salinity, *Vigna radiata*, Halotolerant Bacteria, Sustainable Agriculture, Biostimulants, Salt Stress Tolerance.

INTRODUCTION

Soil salinity is a major abiotic stress that significantly hampers plant growth and agricultural productivity. Salinity induces hyperosmotic and hyperionic conditions, which disrupt water uptake, nutrient acquisition, and root architecture, ultimately leading to oxidative stress and programmed cell death in plants (Meloni *et al*., 2003; Kim *et al*., 2014). The global need for sustainable agricultural practices has shifted focus toward eco-friendly biological approaches, such as the exploitation of plant growth-promoting rhizobacteria (PGPR), to mitigate the adverse effects of salinity and enhance crop productivity in salt-affected soils.

PGPR, a diverse group of beneficial bacteria residing in the rhizosphere, establish symbiotic relationships with plants and employ various direct and indirect mechanisms to enhance plant growth under stressful conditions. These mechanisms include the synthesis of phytohormones (such as indole-3-acetic acid [IAA], gibberellins, and cytokinins) (Vacheron *et al*., 2013), production of exopolysaccharides (Ashraf *et al*., 2019), siderophore-mediated iron sequestration (Rajkumar *et al*., 2010), nitrogen fixation (Santoyo *et al*., 2016), phosphate solubilization (Khan *et al*., 2014), and the induction of antioxidant defence systems to counter oxidative damage (Ahmad *et al*., 2016; Talaat and Shawky 2013; Shabani and Sabzalian 2016). Furthermore, PGPR maintain ionic homeostasis by promoting potassium retention and mitigating the toxic effects of sodium and chloride ions (Munns and Tester 2008).

Particularly noteworthy are the halotolerant and halophilic PGPR, which have evolved mechanisms to survive and thrive in saline environments while supporting plant health. These bacteria combat salinity-induced stress by synthesizing osmolytes for cellular osmotic adjustment, enhancing nutrient availability, and reducing the effects of high ethylene levels that cause chlorosis and senescence in plants (Subramanian *et al*., 2016). Moreover, PGPR-mediated biosynthesis of IAA through tryptophan metabolism has been well-

documented, with key pathways including the indole-3-pyruvic acid (IPA), indole-3-acetamide (IAM), and indole-3-acetonitrile (IAN) pathways (Talaat, 2015). Phosphate-solubilizing bacteria (PSB), a subset of PGPR, play a vital role in improving plant phosphorus uptake by releasing soluble phosphate through the secretion of organic acids such as gluconic, citric, and oxalic acids. These bacteria not only enhance nutrient availability but also reduce the pH of the rhizosphere, facilitating the solubilization of rock phosphate and other insoluble forms of phosphorus (Bharti *et al*., 2014).

Given the potential of PGPR to alleviate salinity stress and improve plant growth, this study aims to isolate and identify salt-tolerant PGPR from saline soils and evaluate their efficacy in promoting plant growth under controlled pot experiments. By exploring the physiological and biochemical traits of these isolates, the research seeks to uncover promising candidates for sustainable agriculture in salinity-affected regions.

MATERIALS AND METHODS

A. Collection of samples and isolation of PGPR

(i) Collection of Soil Samples. Samples of soil was taken from the barren lands of Shirti, Kolhapur district of Maharashtra, India. Using a hand auger, soil was extracted from a depth of 0 to 20 cm, carefully from the root systems and the bulk soil surrounding them was removed. After separating the rhizospheric soil by shaking, the leftover soil was brushed away from the root system (Yanai *et al*., 2003; Tiwari *et al*., 2016). After being labeled and placed in sterile plastic bags, the gathered soil samples were taken to Rajaram College's research facility in Kolhapur, Maharashtra, India, for bacterial isolation. To reduce microbial deterioration, all soil samples were transported at 4°C.

(ii) Study of collected soil samples- A variety of properties, such as texture, color, pH, and electrical conductivity, were examined in the gathered samples. By creating a saturated paste of soil and deionized water, the electrical conductivity of a soil sample was ascertained. After extracting the water, a probe was inserted into the extracted solution to measure its EC. The electric conductivity of a soil sample was measured using a conductivity meter. The EC value was captured and is shown on the probe's digital screen.

(iii) Isolation of PGPR from Collected Soil Samples- Enrichment in nutrient broth (NB) was used to isolate PGPR from the rhizospheric soil samples that were collected. A 100 µL aliquot of each dilution of the soil samples was spread out on sterile nutrient agar (NA) plates after they had been serially diluted in sterile saline. For 72 hours, or until bacterial colonies formed, the plates were incubated at 28±2°C. To make sure the medium was sterile, control plates—which did not contain a soil sample—were included. For additional purification, individual bacterial colonies were selected from the NA plates and streaked onto new nutrition agar (NA) plates. Until isolated colonies were obtained, this process was repeated.

(iv) Screenings for halophilic potential in isolated PGPR strains-Salt-tolerant bacteria were screened from the isolated plant growth-promoting rhizobacteria (PGPR) by growing them on nutrient agar plates containing varying concentrations of NaCl. Nutrient agar was prepared with NaCl concentrations of 2%, 4%, 6%, 8%, 10%, 12%, 14%, 16%, and 18%. Control plates without NaCl were included to serve as a reference for normal growth conditions and to distinguish salt tolerance from general growth ability. The bacterial inoculum was standardized to an optical density (OD600) of 0.1 to ensure uniformity across all samples. The isolated PGPR strains were spot-inoculated onto the prepared nutrient agar plates with different NaCl concentrations. All experiments were performed in triplicates to ensure reproducibility and reliability of the results. The plates were incubated at 30°C for 48–72 hours to allow adequate bacterial growth. Salt tolerance was assessed based on the visible growth of the bacteria, with tolerance determined by colony formation and size. PGPR isolates that exhibited substantial growth at NaCl concentrations greater than 6% were considered salt-tolerant and selected for further studies.

B. Identification of isolated PGPR strains

The selected bacterial isolates were initially identified based on their morphological, cultural, and biochemical characteristics. Morphological traits, such as colony size, color, shape, margin, opacity, elevation, and consistency, were studied macroscopically on nutrient agar plates, while cellular characteristics, including cell shape, size, Gram reaction, and motility, were analyzed microscopically (Sneath, 1958). Following the Gram staining results, a series of biochemical tests were performed in accordance with Bergey's Manual of Systematic Bacteriology (Bergey & Breed 1957).

The physiological characterization of isolates was conducted to assess their growth under varying NaCl concentrations (1%–18%) and temperatures (20°C–50°C), confirming their salt tolerance. For precise species-level identification, the 16S rRNA gene was amplified and sequenced. The obtained sequences were analyzed using the NCBI GenBank database through BLAST to identify the closest matches, confirming the bacterial isolates as salt-tolerant PGPR species.

C. Plant Growth Promotion Study–Pot Assay

(i) Inoculum Preparation. The inoculums for all three isolates were prepared by using following method. A purified single colony of each bacterial strain (*Bacillus thuringiensis* SR-121, *Halomonas meridiana* SR-161, and *Alkalibacillus* sp. SR-103) was inoculated into a sterile 100 mL Erlenmeyer flask containing 25 mL of an appropriate growth medium specific to each organism. For *B. thuringiensis* SR-121, nutrient broth was used; for *H. meridiana* SR-161, nutrient broth was prepared; and for *Alkalibacillus* sp. SR-103, nutrient broth adjusted to pH 9.0 using sterile NaOH solution was used. The flasks were incubated at 30°C for 24 hours with constant shaking at 150 rpm to ensure adequate aeration and uniform growth. This standardized procedure ensures the optimal growth of each organism while maintaining consistent incubation conditions.

(ii) Standardization of Inoculum Concentration. After 24 hours of incubation, the optical density (OD) of the bacterial cultures was measured at 600 nm (OD_{600}) using a spectrophotometer. The cultures were diluted with sterile distilled water to achieve a standardized concentration of 10^8 CFU/mL (colony-forming units per milliliter).

(iii) Pot Experiment. The pot experiment was conducted to evaluate the plant growth-promoting effects of three bacterial isolates: *Bacillus thuringiensis* (SR-121), *Halomonas meridiana* (SR-161), and *Alkalibacillus* species (SR-103). Seeds of *Vigna radiate* (Moong) were used for this experiment. Moong seeds were surface-sterilized using 0.2% $HgCl_2$ solution for 2 minutes, followed by thorough rinsing with sterile distilled water three times to remove any residual disinfectant. The sterilized seeds were then soaked in the bacterial suspensions for 5 hours at room temperature to allow proper inoculation.

The experiment included four treatments: (1) control, where seeds were treated with sterilized distilled water (no bacterial inoculation); (2) seeds inoculated with the bacterial suspension of *Bacillus thuringiensis* (SR-121); (3) seeds inoculated with the bacterial suspension of *Halomonas meridiana* (SR-161); and (4) seeds inoculated with the bacterial suspension of *Alkalibacillus* species (SR-103). After inoculation, the seeds were sown in 20 cm diameter × 20 cm depth pots filled with 3.5 kg of autoclaved, sterilized soil (pH 6.0). The experiment was laid out in a compltely block design (CBD), with each treatment consisting of three replicates and five seeds planted per pot. The pots were maintained in a polyhouse at a temperature of 20–25°C, which was monitored daily throughout the experiment. Sterile distilled water was used for irrigation at seven-day intervals to maintain proper moisture levels. To prevent contamination and maintain soil moisture, pots were covered with sterile plastic sheets or films throughout the experiment. The pots were examined daily, but observations were recorded on the 15th, 25th, 35th, and 45th days after sowing. Forty-five days after inoculation, a total of 20 plants (5 plants from each replicate in triplicate) were randomly selected from each treatment for growth evaluation. Plant growth parameters, including plant length (cm), fresh weight of roots and shoots (g), and dry weight of roots and shoots (g), were recorded. For dry weight determination, root and shoot samples were separated, placed into small paper bags, and dried in an oven at 80°C for 72 hours. This experiment allowed for a comparative analysis of the plant growth-promoting effects of the three bacterial isolates under controlled conditions.

RESULTS AND DISCUSSION

A. Collection and Characterization of Soil Samples

Three soil samples were collected from various locations of Shirti, Kolhapur, Maharashtra. The following table (1) provides an overview of the characteristics of the collected soil samples.

Table 1: Characteristics of collected soil samples.

Characteristics of soil	Location of soil collection	Colour of soil	Texture of soil	pH of soil	EC of soil (dS/m)
1.	Shirti	Black	Clayey	7.6	4.1
2.	Shirti	Brown	Clayey	8.2	5.0
3.	Shirti	Black	Clayey	7.5	4.9

B. Isolation and screening of halophilic PGPR

A total of 27 bacterial isolates were obtained from the three samples. Among them, three highly salt-tolerant isolates—SR 121, SR 161, and SR 103—were selected for further study. The salt tolerance levels of these three isolates are presented in Table 2.

Table 2: Salt tolerance of isolated PGPR.

NaCl(%) Isolate	2	4	6	8	10	12	14	16	18	20
SR121	+	+	+	+	+	+	-	-	-	-
SR161	+	+	+	+	+	+	+	+	-	-
SR103	+	+	+	+	+	-	-	-	-	-

Identification of isolates. SR121 and SR161 exhibited similar temperature tolerance, growing between 20°C and 50°C but not at 15°C. SR103, however, had a broader temperature range, growing even at 15°C but not at 50°C. Regarding NaCl tolerance, all three isolates could grow in NaCl concentrations ranging from 1% to 9%, but none can tolerate 17% NaCl. Isolate SR161 was the only isolate that could tolerate 16% NaCl concentrations. This suggests that SR121 and SR161 are more thermotolerant, while SR103 is more psychrotolerant. Additionally, all isolates display halotolerance but are not extreme halophiles. Considering the outcomes of the colonial characteristics (table 3), biochemical tests (Table 5), cell characteristics (Table 3), it was concluded that the bacterium likely belongs to the genus *Bacillus, Halomonas and alkalibacillus*.

Table 3: Morphological characters of PGPR isolates.

Sr. No.		Variable	SR121	SR161	SR103
1.	Colonial characteristics	Size	3mm	2 mm	2 mm
2.		Shape	Round	Round	Round
3.		Color	White	White	White
4.		Margin	Entire	Entire	Entire
5.		Elevation	Convex	Flat	Convex
6.		Opacity	Opaque	Opaque	Transparent
7.		Consistency	Sticky	Sticky	Creamy
8.	Cell characteristics	Shape	Rods	Rods	Rods
10.		Gram nature	Positive	Negative	Positive
11.		Motility	Non-motile	Motile	Motile
12.		Spore	Yes	Yes	No

Table 4: Physiological characters of PGPR isolates (+ indicates growth and – indicates no growth).

Isolate	Temperature range °C							
	15	20	25	30	35	40	45	50
SR121	-	+	+	+	+	+	+	+
SR161	-	+	+	+	+	+	+	+
SR103	+	+	+	+	+	+	+	-

Table 5: Biochemical characters of PGPR isolates (+ indicates positive test and – indicates negative test growth).

Sr. No.	Test	Result			Sr. No.	Test	Result		
		SR121	SR161	SR103			SR121	SR161	SR103
1.	Catalase	+	+	+	5.	Caseinase	+	-	+
2.	Oxidase	-	+	+	6.	Nitrate reductase	+	+	+
3.	Amylase	+	-	+	7.	Gelatinase	+	-	+
4.	Lipase	+	+	+	8.	Urease	-	+	-

Table 6: Carbohydrate fermentation results of PGPR isolates (+ indicates the production of acid and/or gas during fermentation; - Indicates no acid or gas production).

Sugar	Glucose		Fructose		Lactose		Mannose		mannitol		Sucrose		Sorbitol		Galactose	
	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
SR121	+	-	+	-	+	-	+	-	+	-	+	-	-	-	-	-
SR161	+	-	+	-	-	-	+	-	+	-	+	-	+	-	-	-
SR103	+	-	+	-	-	-	+	-	-	-	+	-	-	-	-	-

ATAGTGTGTCTAGTGCGAGCAGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTG
AGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATA
ACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTC
GCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGAT
CGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAA
TGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTG
TTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAA
GCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAA
ACTGGGAGACTTGAGTGCAGAAAAGGAAAGTGGA

Fig. 1. The partial nucleotide sequence (828 bp) of the amplicon of 16S rRNA gene obtained from isolate SR121

Fig. 2. Phylogenetic tree of bacterial isolate SR121 based on 16S r RNA gene sequences analyzed based on neighbor-joining method.

The phylogenetic analysis of the salt-tolerant bacterial isolate SR121 (Fig. 1 and 2) was performed using 16S rRNA gene sequencing, and the results revealed its evolutionary relationships with closely related species. The phylogram illustrates that SR121 clusters closely with *Paracoccus* sp. (MH558368.1), indicating significant genetic similarity and suggesting a potential association with the genus *Paracoccus*, which is known for its metabolic versatility and salt-tolerance traits (Liu *et al*., 2017; Barnawal *et al*., 2012). Additionally,

SR121 shows phylogenetic proximity to *Bacillus thuringiensis* strains (KY622894.1 and HQ670547.1) and *Bacillus sp.* (AB299732.1), which are renowned for their plant growth-promoting and biocontrol properties, including the production of bioactive compounds and tolerance to abiotic stresses (Zhang *et al*., 2018; Kumar *et al*., 2022). Interestingly, *Acetobacter pasteurianus* (FM178868.1) appears as a more distantly related outgroup, emphasizing the distinctiveness of SR121 and its close cluster with salt-tolerant and agriculturally beneficial bacteria. The association with *Paracoccus sp.* and *Bacillus thuringiensis* highlights SR121's potential for applications in saline environments, particularly as a plant growth-promoting rhizobacterium (PGPR). These genera are well-documented for their roles in enhancing nutrient uptake, producing phytohormones, and mitigating salt-induced oxidative stress in plants (Qin *et al*., 2019; Joshi *et al*., 2020). The presence of SR121 within this cluster underscores its suitability for biotechnological applications, such as the development of biofertilizers for saline soils. Future studies focusing on its functional characterization and secondary metabolite production could unlock its potential for sustainable agriculture under salt-stress conditions (Sharma *et al*., 2021). This phylogenetic analysis provides a strong foundation for further research on SR121's beneficial traits and ecological roles.

CGTAAAGCGGTCTACTATGCAGTCGAGCGGTCTTTATCCAGCTTGCTGTTTGCTGATAAGCGGCG
GACGGGTGAGTAATGCATAGGAATCTGCCCCATAGTGTGGGAAAACCTGGGGAAACCCAGGCT
AATACCCCAAACATCCTATCGTTCTCCGGGGGCTTCGGCTCCCGCTATCGGATGATCCTATTTCGG
ATTAGCTCCTTGGCGAAGTAAATGCTCACCAAGGCAACGATCCATAACTGGTCTGATACGATGAT
CACCCACATCGGACTGAGACCGGCCCGAACTCCTCACGGAGGCAACTCCTACGAATATTGGACAA
TGGGAATCTTCCTGATCGGGCCATGCCCTGTGTGAGAAAAACGCCCTGAGTTTGTAAAGTTTTTT
CATCGAAGAACACTGCCTAAAGGTTAAAACCCGCTCGGAAATACCTCACTCTCCCATGACGCACC
CTTATTATCCATGCCCGCCTACCTGCTTGTAAGCAGGCTGCAAGCTATAATCGTAATTACTGTTGT
TAAAAATTACTGAGGTGGCTTGATAAGCCGGTTGTCCTTTCCCCCTGATCAACCTGGCCACGGCTT
CCCGAAGTGTCGTCCTTGAGTGCTGGAGAGGATGGTAGCATTCCCAGGTGTAGTGGAATTACTG
CTGTAAGATCGAAATGAATACCAGATGGCGAAGGAACGCCTTCTGGACTGACACTGACTCTGAC
GTGCAAATGACATGGGAGGCCCGAAAGGATTGAGATACCATGGTAGTTCACATACCCCTGGGAA
TGGTCACGCCAGCCGTTGGGGGAGCCTCTCAGCTGGTTTGGGGGTATACC

Fig. 3. The partial nucleotide sequence (828 bp) of the amplicon of 16S rRNA gene obtained from isolate SR161.

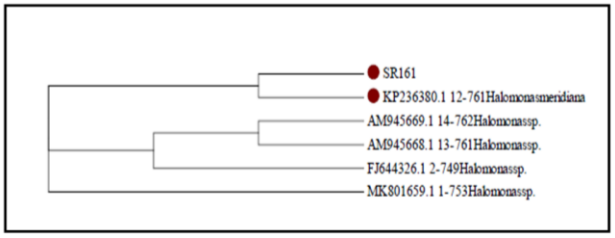

Fig. 4. Phylogenetic tree of bacterial isolate SR161 based on 16S r RNA gene sequences analyzed based on neighbor-joining method.

The phylogenetic tree shows the clustering of a bacterial isolate (labeled as SR161) with other related species (Fig. 3 and 4), including *Halomonas meridiana* (KP236380.1) and other *Halomonas* species. The isolate SR161 is closely related to *Halomonas meridiana*, as indicated by their close grouping, suggesting that SR161 belongs to or is highly similar to the *Halomonas* genus. Other sequences in the tree, such as those labeled AM945669.1, AM945668.1, FJ644326.1, and MK801659.1, represent additional *Halomonas* species that are more distantly related to SR161. This phylogenetic analysis provides evidence that SR161 is a strain of *Halomonas*, likely adapted to saline environments, consistent with the genus's known ability to thrive under high salt conditions. The phylogenetic placement of SR161 within the *Halomonas* genus highlights its potential role as a salt-tolerant PGPR. The *Halomonas* genus is widely recognized for its halophilic nature and ability to support plant growth under saline conditions by employing mechanisms such as exopolysaccharide production, osmotic adjustment, and efficient ion transport (Mishra *et al*., 2018; Kumar *et al*., 2020). Its close relationship with *Halomonas meridiana*, a species known for its salt tolerance and biotechnological applications, further validates SR161 as a promising candidate for developing biofertilizers for saline soils. The ability of *Halomonas* strains to mitigate salt stress in plants has been extensively reported. For example, studies have demonstrated that *Halomonas* species enhance plant growth by producing phytohormones such as indole-3-acetic acid (IAA), solubilizing phosphate, and scavenging reactive oxygen species (ROS) (Bashan *et al*., 2016). Additionally, their capacity to synthesize exopolysaccharides helps in maintaining soil structure and alleviating salt-induced osmotic stress (Qurashi & Sabri, 2012).

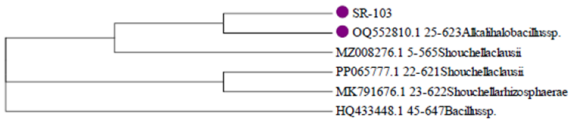

Fig. 5. Phylogenetic tree of bacterial isolate SR121 based on 16S r RNA gene sequences analyzed based on neighbor-joining method.

CAAAGACAGCAGTTAATATCGTGTCTAGTCGAGCGGACAGATGGGGAACTTGGTCCCTTACATTT
TCGGCGGACAGGTGAGTAACACGTGGGCAACCTGCCCCTTATACTGGGATAACTCCCGGAAACC
GGAGCTAATACCGGATAATCCCTTTCTCCCCCTGGGGAGAGGGTGAAAGATGGGTTCTCCTATCT
CTATAGGATGGGCCCCCGCCGCACTATCTCTTTGTGAAGGTAACGGCTTACCCCGGCGACAATAC
GTATACCACATGAGAGGGGGAGCGCCCCCACTGTGACTGAGACACGCCCCCCACTCCTATAGGA
GGCGCCACTATGGAATCTTCTCCACTGTGCAAAAGTCTGAGAGAGAACCGCCCCGTGAGTGAGG
AAAGGGTTCTGGTCGCAAAGCTCTGTTGTGAGGGAAAAAACGCTACCGTTCTAAAATGGGGGTG
TCTTGTCAGGGCCTCTCCCCAAAACCCCGCGTAAATATATGTGCCCACCCCCGGTAATATATATGT
GGGGCACGTTTTCCGGAAATATTGGGGGCATAACGCGCGCAGGCGCCTTTTCATATCTGAGATG
TAATCTCTCGGCTCACCCCCCCGCGGGCTTGTGAATTGGGGAGCTCTAGAGGGCAA

Fig. 6. The partial nucleotide sequence (828 bp) of the amplicon of 16S rRNA gene obtained from isolate SR103.

The phylogenetic tree (Fig. 5 and 6) highlights the evolutionary relationship of the salt-tolerant bacterial isolate SR-103 with closely related species based on 16S rRNA gene sequence analysis. SR-103 is closely clustered with *Alkalihalobacillus* spp. (OQ552810.1), indicating a high genetic similarity and suggesting its potential affiliation with the *Alkalihalobacillus* genus, which is known for its ability to thrive in high-salinity and alkaline environments (Patel *et al*., 2022). Additionally, the tree shows SR-103's evolutionary proximity to species like *Shouchella clausii* and *Shouchella rhizosphaerae*, which are associated with beneficial traits such as phosphate solubilization, nitrogen fixation, and stress-resistance mechanisms (Zhang *et al*., 2018). The outgroup, *Bacillus* spp.,

represents a more distantly related lineage, emphasizing the distinctiveness of SR-103 and its related cluster.

The association of SR-103 with *Alkalihalobacillus* spp. underscores its potential as a halotolerant plant growth-promoting rhizobacterium (PGPR). Members of this genus are well-documented for producing osmolytes, exopolysaccharides, and antioxidant enzymes, which mitigate salinity-induced oxidative stress in plants (Qin *et al*., 2019; Joshi *et al*., 2020). These traits position SR-103 as a promising candidate for developing biofertilizers aimed at improving plant growth under salt-stress conditions. Furthermore, its phylogenetic relationship with *Shouchella* spp. suggests additional functional capabilities relevant to agricultural applications, such as the production of plant growth regulators and enhanced nutrient solubilization (Sharma *et al*., 2021; Kumar *et al*., 2022). This analysis establishes a foundation for further characterization and application of SR-103 in managing salinity-stressed soils and enhancing crop productivity.

POT EXPERIMENT

A plant-based bioassay conducted under poly-house conditions demonstrated the growth-promoting effects of *Bacillus thuringiensis* SR-121, *Halomonas meridiana* SR-161, and *Alkalibacillus* sp. SR-103 on Moong plants. Inoculation with these bacterial strains led to a notable improvement in overall plant growth (Fig. 7-9). After 45 days of growth, a statistically significant increase in both root and shoot biomass was observed in the treated moong plants compared to the control (Table 7).

Table 7: Moong plant growth promotion study by inoculation with *Bacillus thuringiensis* SR-121, *Halomonas meridiana* SR-161, and *Alkalibacillus* sp. SR-103.

Isolates	Seed germination (%)	Plant height (cm)	Root length (cm)	Shoot length (cm)	Plant fresh weight (mg)	Plant dry weight (mg)	Root fresh weight (g)	Root dry weight (g)	Number of leaves
SR-121	100± 0	16.5±0.5	4.2±0.2	12.3±0.4	810±1	420±1	220±0.5	115±1	9±0
SR-161	80±0.2	11.5±0.3	2.5±0.2	9±0.5	540±0.5	390±1	180±0.5	90±0.5	8±0
SR-103	100±0	14±0.5	4±0.1	12±0.3	810±2	330±0.5	210±0.5	100±0	6±0
Control	100±0	13.8±0.5	4±0.5	9.8±0.1	620±1	220±1	200±0.5	100±0	6±0

Among the tested bacterial isolates, *Bacillus thuringiensis SR-121* showed the most pronounced effect, with 100% seed germination, the highest plant height (16.5 cm), shoot length (12.3 cm), and root length (4.2 cm). Additionally, SR-121-treated plants exhibited the greatest plant fresh weight (810 mg) and plant dry weight (420 mg), indicating enhanced biomass accumulation. *Alkalibacillus* sp**.** SR-103 also demonstrated a positive effect on plant growth, with a significant increase in shoot length (12 cm) and root length (4.0 cm) compared to the control. *Halomonas meridiana* SR-161 had a moderate effect on growth promotion but still outperformed the control in most parameters.

Fig. 7. Pots with grown seedlings in Moong plant growth promotion study experiment.

Fig. 8. Seedlings of Moong after 45 days.

Fig. 9. Moong seedlings after 45 days.

Fig. 10. Growth of Moong pods to seedlings in a Pot experiment.

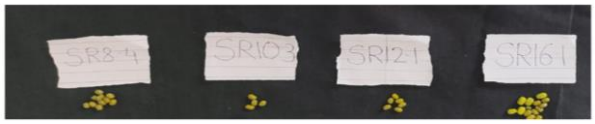

Fig. 11. Seeds of pods from respective seedlings.

The results of this study highlight the potential of plant growth-promoting bacteria (PGPB) in enhancing the growth of moong plants. The observed improvement in seed germination and biomass accumulation can be attributed to the production of phytohormones, improved nutrient uptake, and enhanced root development facilitated by bacterial inoculation (Backer *et al*., 2018). Among the tested isolates, *Bacillus thuringiensis* SR-121 demonstrated the highest plant growth-promoting effects. Species of *Bacillus* are known for their ability to produce indole-3-acetic acid (IAA), siderophores, and other bioactive compounds that enhance plant growth (Lopes *et al*., 2021). Additionally, *Bacillus* spp. are reported to promote plant stress tolerance by inducing systemic resistance and improving nutrient availability (Goswami *et al*., 2016). *Halomonas meridiana* SR-161, while showing a lower impact than SR-121, still contributed to growth promotion. Members of the *Halomonas* genus are halotolerant bacteria capable of facilitating plant growth under abiotic stress conditions, particularly by improving osmotic balance and nitrogen fixation (Mapelli *et al*., 2013). Similarly, *Alkalibacillus* sp. SR-103 enhanced growth parameters, particularly shoot length and biomass accumulation. Previous studies suggest that alkaliphilic bacteria improve soil fertility by solubilizing phosphate and enhancing nutrient uptake, leading to improved plant growth (Etesami & Maheshwari 2018). Compared to the control, all three bacterial inoculants significantly improved growth parameters, supporting their potential as biofertilizers for sustainable agriculture. These findings align with previous studies demonstrating the positive impact of bacterial inoculants on leguminous crops such as pea and chickpea (Sharma *et al*., 2020).

The images 10 and 11 illustrate the effects of different bacterial inoculants (SR8-4, SR103, SR121, and SR161) on pod formation and seed yield in moong plants. The first image presents matured seed pods collected from plants treated with these bacterial strains, showing variations in pod number, size, and coloration. Notably, the SR161-treated plants appear to have developed more pods, suggesting a positive influence on pod formation. The second image displays the seeds extracted from the respective pods, highlighting differences in seed count and size among treatments. The plants inoculated with SR121 and SR161 produced a greater number of seeds, indicating their effectiveness in enhancing seed yield compared to SR103 and SR8-4. These findings suggest that specific bacterial strains, particularly SR121 and SR161, significantly improve pod development and seed production in moong plants, making them potential biofertilizer candidates for sustainable agriculture

CONCLUSIONS

This study demonstrated the significant potential of salt-tolerant plant growth-promoting rhizobacteria (PGPR) in enhancing the growth of *Vigna radiata* under saline conditions. The three identified isolates—*Bacillus thuringiensis* SR-121, *Halomonas meridiana* SR-161, and *Alkalibacillus* sp. SR-103—exhibited varying degrees of salinity tolerance and plant growth promotion. Among them, *Bacillus thuringiensis* SR-121 showed the most pronounced positive impact on seed germination, plant height, and biomass accumulation. The results highlight the effectiveness of PGPR in mitigating salt stress by improving nutrient uptake, producing phytohormones, and enhancing root development. These findings support the potential application of salt-tolerant PGPR as biofertilizers for sustainable agriculture in saline environments.

FUTURE SCOPE

Future research should focus on large-scale field trials to validate the efficacy of these PGPR isolates under natural agricultural conditions. Additionally, studies on the molecular mechanisms underlying their stress tolerance and plant growth-promoting traits will provide deeper insights into their functionality. The development of commercial bioformulations incorporating these beneficial microbes could offer an eco-friendly alternative to chemical fertilizers for enhancing crop productivity in saline soils. Further exploration of microbial consortia combining multiple PGPR strains may optimize their synergistic effects for improved stress resilience and yield enhancement in various crops.

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