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HALOTOLERANT PLANT GROWTH-PROMOTING RHIZOBACTERIA FROM SALINE SOILS: CHARACTERIZATION AND PLANT GROWTH PROMOTION UNDER SALT STRESS CONDITIONS

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ABSTRACT

Soil salinity poses a significant challenge to global agricultural productivity, necessitating sustainable approaches to improve crop performance under salt stress. This study was aimed to isolate and characterize halotolerant plant growth-promoting rhizobacteria from saline soils of Maharashtra and Gujrat (India), evaluate their salt tolerance and plant growth promoting traits, and assess their effects on maize and wheat growth and seed germination. Soil samples collected from Lonar lake, Mehrun lake, and coastal regions were analysed for physicochemical properties, and bacterial isolates were screened for tolerance to 2-18% NaCl and production of indole-3-acetic acid (IAA), gibberellic acid (GA), siderophores, and exopolysaccharide (EPS). The morpho-biochemical characterization and 16S rRNA sequence analyses identified the selected isolates as *Bacillus haynesii* (B_1, B_2) and *Shouchella clausii* (B_3). All isolates tolerated NaCl up to 14%, while temperature tolerance ranged from 15 to 45°C, with optimal growth at 30-35°C. *S. clausii* B_3 produced highest IAA (7.5 µg mL^{-1}), while *B. haynesii* B_2 showed highest GA production (91.3%). Siderophore production peaked at 72 h, with B_1 producing 68%, while EPS was highest in B_1. In pot trials, B_1 significantly enhanced wheat growth, and B_3 improved maize biomass. Seed germination assays showed 100% germination in treated seeds, with isolates B_1 and B_3 outperforming the controls in root/shoot elongation. The study demonstrated the potential of halotolerant PGPR as bioinoculants in salinity-stress situations.

Keywords: *Bacillus haynesii*, halotolerant PGPR, microbial bioinoculants, plant growth promotion, *Shouchella clausii*

INTRODUCTION

Soil salinity is a major constraint to the agricultural productivity around the globe, particularly in the regions where irrigation, coastal processes, and other environmental factors contribute to salt accumulation in agricultural soils. Approximately 20% of irrigated lands are affected by salinity, making the development of sustainable strategies for crop production under saline conditions increasingly important (FAO, 2021). Excessive salt accumulation imposes osmotic stress, ionic toxicity, and oxidative stress on plants, disrupting water uptake, nutrient acquisition, photosynthesis, and overall growth (Isayenkov and Maathuis, 2019; Zörb *et al*., 2019). These physiological issues induce stunted plant growth, yellowing of leaves, and ultimate yield drop. Therefore, identifying sustainable biological approaches including the use of plant growth-promoting rhizobacteria (PGPR),

capable of supporting plant growth under saline conditions, is a promising research priority.

PGPRs have attracted considerable attention as potential microbial inoculants for improving the plant performance under abiotic stress. Their beneficial effects are attributed to the production of phytohormones and other metabolites (like auxin especially IAA, cytokinins, and gibberellins, which play a key role in shaping root structure), nutrient mobilization, exopolysaccharide (EPS) production, and modulation of plant stress responses (Glick, 2014; Etesami and Beattie, 2018). The halotolerant PGPR are particularly promising due to their ability to survive and remain metabolically active at elevated salt concentrations enabling them to function effectively under saline conditions. These PGPR's produce EPS that form protective biofilms around roots, which not only limit sodium absorption but also improve soil structure and help in retaining moisture (Khan *et al.,* 2020a.b; Kumari *et al*., 2022). Studies have demonstrated that salt-tolerant PGPR can improve plant growth and stress tolerance through mechanisms including phytohormone production, EPS-mediated protection, and enhancement of plant antioxidant responses (Upadhyay and Singh, 2015; Egamberdieva *et al*., 2019; Kumari *et al*., 2022). However, the effectiveness of PGPR is dependent on the physiological adaption of individual isolates to the environmental conditions in which they are intended to be applied.

Despite considerable research on PGPR-mediated salinity tolerance, important gaps remain. Many previous studies have focused on the individual bacterial isolates or a limited number of plant growth-promoting characteristics (Acharya *et al.,* 2024; Valenzuela *et al.,* 2025), while comparatively fewer studies have combined assessment of salt tolerance, multiple growth-promoting traits, taxonomic identification, and direct validation of selected indigenous isolates on crop plants (Awan *et al.,* 2020; Alonazi *et al.,* 2025; Ebadi *et al.,* 2025). Furthermore, saline environments represent valuable reservoirs of microorganisms that are naturally adapted to high salt concentrations, but the functional potential of locally occurring halotolerant rhizobacteria remains insufficiently explored in several regions (Yan *et al.,* 2024). There is urgent need to identify the indigenous isolates that can tolerate relatively high salt concentrations while simultaneously expressing multiple plant growth-promoting characteristics and demonstrating beneficial effects on agriculturally important crops. The present study attempted to address this gap by systematically isolating and characterizing halotolerant bacterial isolates from distinct saline environments in Maharashtra and Gujarat (India), thus providing an opportunity to recover bacteria adapted to different saline conditions. The selected isolates were evaluated for salt tolerance and multiple plant growth-promoting attributes, including indole-3-acetic acid (IAA), gibberellic acid, siderophore, and exopolysaccharide production. Morphological and biochemical characterization followed by 16S rRNA gene sequencing was used to establish their taxonomic identity. The plant growth-promoting potential of the selected isolates was subsequently validated using seed germination and pot experiments with maize and wheat.

This study focuses on the integrated evaluation of indigenous halotolerant bacterial isolates from geographically distinct saline environments, combining their salt tolerance, multiple plant growth-promoting characteristics, molecular identification, and plant-level validation. The approach enables to identify the bacterial strains expressing both environmental adaptability and functional potential, rather than selecting them solely on a single plant growth-promoting trait. This study was aimed to isolate halotolerant bacterial isolates from saline soils, evaluate their tolerance to varying NaCl concentrations and characterize their morpho-biochemical properties; as well as assess their selected plant growth-promoting traits like IAA, gibberellic acid, siderophore, and exopolysaccharide production. The promising isolates were identified using 16S rRNA gene sequencing; and evaluated for their impact on seed germination and early plant growth promotion in maize and wheat.

MATERIALS AND METHODS

Collection of samples and isolation of PGPR

Study area and collection of soil samples: The study was conducted during 2023 in saline environments

of Maharashtra and Gujarat states of India. Rhizospheric soil samples were collected from four locations *viz*., Lonar lake, Buldhana district, Maharashtra (19.9765°N, 76.5080°E); Mehrun lake, Jalgaon, Maharashtra (20.9796°N, 75.5643°E); Girgaon coastal region, Mumbai, Maharashtra (18.953°N, 72.813°E); and Surat Coastal region, Gujarat India (21.112°N, 72.814°E). Soil samples were carefully extracted from 0-20 cm depth using a sterile hand auger without disturbing the root systems. The bulk soil surrounding roots was removed, and rhizospheric soil separated by gently shaking and brushing the remaining soil from the root surface (Vessey *et al.,* 2003; Tiwari *et al.,* 2016). The samples were transferred to sterile polyethylene bags, transported to the laboratory at 4°C, and processed for bacterial isolation.

Physicochemical analysis of soil: The soil samples were examined for colour, texture, pH, and electrical conductivity (EC). Soil pH and EC were determined as per the standard soil analytical procedures (Jackson, 1973), using a digital pH meter (Eutech pH 700, Thermo Fisher Scientific, USA) and a conductivity meter (Eutech CON 700, Thermo Fisher Scientific, USA), respectively.

Isolation of rhizobacterial isolates: Rhizobacteria were isolated following serial dilution and spread-plate method using enriched nutrient broth (NB) [Lihan *et al.,* 2021]. Briefly, 1 g rhizospheric soil was suspended in sterile saline and serially diluted. An aliquot (100 µL) of an appropriate dilution was spread onto nutrient agar (NA) plates and incubated at 37 ± 2°C for 48-72 h. Morphologically distinct colonies were selected and purified by repeated streaking on NA plates. Pure isolates were maintained on NA slants at 4°C until use.

Screening of bacterial isolates for halotolerance

The salt tolerance of bacterial isolates was assessed by spot inoculation on nutrient agar containing 2, 4, 6, 8, 10, 12, 14, 16, and 18% (w/v) NaCl. NA without NaCl served as control. Bacterial suspensions, standardized to an OD_{600} of 0.1 and 10 µL of each suspension, were spot-inoculated onto the respective media. All experiments were performed in completely randomized design with each treatment replicated thrice to ensure reproducibility and reliability of results. Plates were incubated at 30°C for 48-72 h, and growth was recorded based on visible colony development. Isolates showing substantial growth at NaCl concentrations >6% were considered halotolerant and selected for further characterization. The experiment was conducted in CRD with each treatment triplicated three times.

Identification of PGPR strains

The selected halotolerant bacterial isolates were tentatively identified based on their morphocultural, physiological and biochemical characteristics. Morphological traits such as colony size, colour, shape, margin, opacity, elevation, and consistency were examined macroscopically on nutrient agar plates, while cellular characteristics like cell shape, size, Gram reaction, and motility were assessed by using light microscope [Olympus CX23 biological microscope, Tokyo, Japan] (Sneath, 1958). A series of biochemical tests like catalase, oxidase, amylase, lipase, caseinase, nitrate reductase, gelatinase, and urease tests, were performed as per standard procedures (Holt *et al.,* 1994). To assess their salt tolerance, the isolates was grown under varying NaCl concentrations (2-16%) and temperatures (15-50°C) conditions. For molecular identification, genomic DNA was extracted from the selected isolates, and the extracted 16S rRNA genes were amplified using universal primers 27F and 1492R under standard PCR conditions (Weisburg *et al.* (1991). The amplified products were purified and subject to Sanger's sequencing. The sequences obtained were compared with reference sequences in the NCBI GenBank database using BLASTn to identify the closest matches (Altschul *et al*., 1990). The taxonomic identity was assigned based on sequence similarity with reference sequences. The selected isolates were identified as *Bacillus haynesii* (B_1 and B_2) and *Shouchella clausii* (B_3).

Indole-3-acetic acid production (IAA)

IAA production by test bacterial isolates was quantified by colorimetric method of Gordon and Weber (1951), as modified by Sarker and Al-Rashid (2013). Briefly, each isolate was cultured in 5 mL LB

broth supplemented with 0.1% tryptophan and incubated at 28°C for 72 h. A control was maintained with only growth medium (without any inoculation). The cultures were centrifuged at 3,000 rpm for 10 min, and 1 mL cell-free supernatant was mixed with 2 mL Salkowski reagent and incubated at room temperature for 25 min during which a pink colour development indicated the presence of IAA. Absorbance was measured at 530 nm using a SpectraMax microplate reader**.** IAA concentration was calculated from a standard curve prepared using varying IAA concentrations (0-100 µg mL^{-1}). Each sample was assayed in triplicate, and the entire procedure was repeated twice to verify the accuracy and consistency of the results.

Gibberellic acid production

Gibberellic acid (GA) production was quantified as per Berrios *et al.* (2004) with slight modification. The isolates were cultured in LB broth at 37°C for 72 h, then 1 mL culture supernatant was acidified with 3.75 M HCl and extracted with 30 mL ethyl acetate. The organic phase was analyzed spectrophotometrically at 254 nm using a Milton Roy Spectronic 21D spectrophotometer. GA concentration was determined from a calibration curve prepared using GA standards ranging from 50 to 600 µg mL^{-1}.

Exopolysaccharide (EPS) production

EPS production was determined by phenol-sulfuric acid method of Dubois *et al.* (1956). The isolates were cultured in LB broth supplemented with 4% glucose, and then centrifuged at 10000 rpm for 15 min at 4°C. EPS in supernatant was precipitated with three volumes of cold absolute ethanol at 4°C for 24 h, recovered by centrifugation, washed with 70% ethanol, and dissolved in distilled water. The EPS solution was reacted with 5% phenol and conc. H_2SO_4, and kept as such at room temperature for 30 min for colour development. Then absorbance measured at 490 nm using a Shimadzu UV-1800 UV-Vis spectrophotometer. EPS concentration was calculated from a glucose standard curve.

Siderophore production

For siderophore production, the test isolates were inoculated into Luria-Bertani (LB) broth and incubated at 37°C for 3-4 days with continuous shaking at 120 rpm. Then cultures were centrifuged at 10000 rpm for 10 min to separate the cell biomass and the supernatants were collected for analysis. The siderophore production was estimated using Chrome Azurol S (CAS) assay (Senthilkumar *et al.*, 2021). For this 1 mL culture supernatant was mixed with 1 mL CAS reagent and the mixture allowed to react for colour development. Then absorbance was measured at 630 nm using a Shimadzu UV-1800 UV–Vis spectrophotometer. The percentage of siderophore units was calculated using the formula:

$$\text{Percent siderophore (\%)} = \frac{\text{Ar} - \text{As)}}{\text{Ar}} \times 100$$

wherein A_r is the absorbance of reference/control (CAS reagent without culture supernatant) and A_s is the absorbance of sample containing cell-free culture supernatant. All assays were performed in triplicate.

Assessment of plant growth promotion effect

A pot experiment was conducted to evaluate the plant growth-promoting effects of 3 bacterial isolates, selected on the basis of their high salt tolerance (growth up to 14% NaCl) and expression of multiple plant growth-promoting traits, including IAA, GA, siderophore, and EPS production during initial screening. Maize and wheat seeds were surface-sterilized for 2 min using 0.2% $HgCl_2$ solution, followed by thorough rinsing with sterile distilled water to remove residual disinfectant. The sterilized seeds were soaked in bacterial suspension, as per treatment, for 5 h at room temperature to allow proper inoculation. The experiment comprised of four treatments: uninoculated control, and bacterial treatments using three most effective isolates (B_1, B_2 and B_3). Five seeds were sown in 20 cm dia. × 20 cm deep pots filled with 3.5 kg autoclaved, sterilized soil (pH 6.0). The experiment was conducted in a randomized block design, with each treatment replicated three times using five seeds pot^{-1}. The experiment was conducted in a polyhouse at 20-25°C and maintained under same irrigation

conditions. Sterile distilled water was applied at 7-day intervals to maintain adequate 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, and the plant growth parameters *viz*., plant length, fresh and dry weights of roots and shoots were assessed on 15^{th}, 25^{th}, 35^{th}, and 45^{th} days after sowing. For dry weight, root and shoot samples were oven dried at 80^{o}C for 72 h.

Seed germination assay

Seed germination assay was conducted to assess the influence of three promising bacterial isolates on seed germination and early seedling growth of maize (*Zea mays* L., cv. Hybrid 9444) and wheat (*Triticum aestivum* L. cv. Godrej). The seeds were first surface-sterilized with 0.2% $HgCl_2$ solution for 2 min, then thoroughly rinsed three times with sterile distilled water. The sterilized seeds were soaked in individual bacterial suspensions for 5 h at room temperature while control seeds were soaked in sterile distilled water under identical conditions. Ten seeds per treatment replicate were placed on sterile moistened Whatman filter paper and incubated in a growth chamber at 20-25^{o}C. The experiment was carried out in triplicate, for each crop and treatments, with moisture maintained by adding sterile distilled water as and when desired. Seed germination was observed daily, while root length, shoot length, and seedling vigour index were noted on 5^{th} and 7^{th} day. The seedling vigour index was calculated as per the following standard formula:

$$\text{Seedling vigour index} = \text{Germination percentage} \times (\text{root length} + \text{shoot length})$$

Statistical analysis

All experiments were performed with three independent replicates**,** and the results expressed as mean ± standard deviation (SD)**.** The seed germination and pot experiments were conducted in a completely randomized design. The data obtained from plant growth parameters, seed germination, IAA, gibberellic acid, siderophore, and EPS assays were subjected to one-way analysis of variance (ANOVA) to determine the significance of differences ($p \leq 0.05$) among treatment means. Wherever the ANOVA indicated a significant treatment effect, means were separated using Tukey's honestly significant difference (HSD) multiple-comparison test. The statistical analyses were performed using GraphPad Prism version 10.0 (GraphPad Software, San Diego, CA, USA)**.**

RESULTS AND DISCUSSION

Isolation and selection of halotolerant bacteria

One hundred and twenty isolates, comprising of one hundred bacterial and twenty fungal isolates, were recovered from the four saline sampling sites. Among these, thirty three isolates exhibited strong halotolerance, with growth at 12% NaCl, and several isolates tolerated the concentrations up to 14%. Based on the initial screening for salt tolerance and plant growth-promoting characteristics, including IAA, gibberellic acid, siderophore and exopolysaccharide (EPS) production, three bacterial isolates, designated B_1, B_2 and B_3, were selected for further characterization and plant assays. The selection was based on their consistent high salt tolerance together with multiple plant growth-promoting traits, rather than based on a single activity. This multifactorial selection was intended to identify the isolates possessing both environmental adaptability and functional potential for use under saline conditions, for choosing effective halotolerant PGPR candidates (Etesami and Beattie, 2018; Egamberdieva *et al.*, 2019).

Culturo-morphological and physio-biochemical characteristics

The selected isolates were Gram-positive rods (Fig. 1-3). Isolate B_1 and B_2 formed white, round,

Fig. 1: Microscopic view of Gram-stained cells of the halotolerant bacteria; isolate B_1 (left hand side), isolate B_2 (middle one) and isolate B_3 (right hand side)

opaque colonies with entire margins and were motile and spore-former, whereas isolate B_3 produced white, round, relatively transparent colonies and was non-motile and non-spore-former. The cells were approximately 1.5-3.0 µm in length. All these isolates tolerated NaCl up to 14% concentration, confirming their strong halotolerant phenotype. Isolate B_2 showed broadest temperature tolerance (20-45^{o}C), whereas isolate B_3 grew from 15-40^{o}C and isolate B_1 from 20-40^{o}C. The biochemical profiling further differentiated the isolates. All the three isolates were positive for catalase, amylase, caseinase, nitrate reductase and gelatinase activities. Oxidase and lipase activities were detected in isolates B_1 and B_2 only, whereas urease activity was observed in only isolate B_3. The combination of Gram-positive rod morphology, endospore formation in isolates B_1 and B_2, and the observed biochemical profiles were observed to be consistent with their subsequent molecular identification. These characteristics revealed their physiological adaptability, key feature of PGPR intended for saline environments.

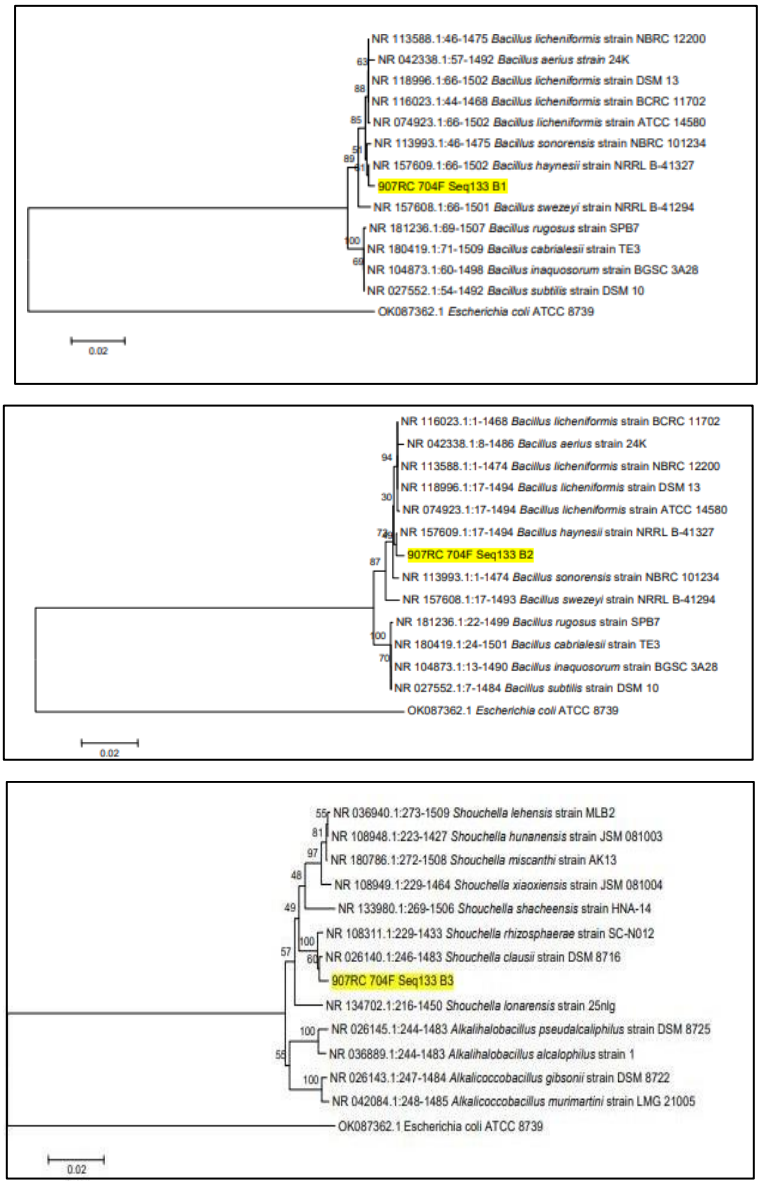

Fig. 2: Phylogenetic tree of three isolated halotolerant PGPRs; *Bacillus haynesii* B_1 (upper one); *B. haynesii* isolate B_2 (middle), and *Shouchella clausii* isolate B_3 (bottom one)

Molecular identification

16S rRNA gene sequencing identified the isolate B_1 and B_2 as *Bacillus haynesii* and the isolate B_3 as *Shouchella clausii*. Phylogenetic analysis confirmed the isolates B_1 and B_2 belonging to *B. haynesii* lineage, with bootstrap values of 91 and 72, respectively; while isolate B_3 clustered with *S. clausii* with 100% bootstrap support (Fig. 2). Molecular results, therefore, corroborated the phenotypic characteristics and established the taxonomic identity of the three halotolerant isolates.

Plant growth-promoting traits

IAA production increased progressively up to 72 h in all isolates and declined slightly thereafter. *S. clausii* B_3 produced highest IAA (7.5 µg mL^{-1} at 72 h), followed by *B. haynesii* isolate B_2 (6.2 µg mL^{-1}) and isolate B_1 (5.8 µg mL^{-1}). At 96 h, IAA production decreased to 6.0, 5.0 and 4.5 µg mL^{-1} in B_3, B_2 and B_1, respectively (Fig. 3). The decline after 72 h may be attributed to the precursor depletion or subsequent IAA degradation, as reported for bacterial auxin production (Spaepen *et al.*, 2007). The ability of IAA-production of these isolates reveal their potential to stimulate root development and improve plant establishment.

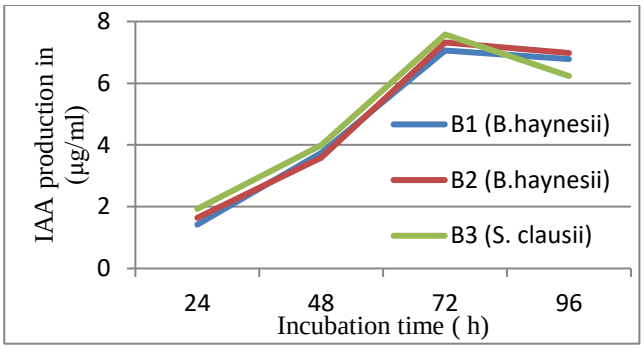

Fig. 3: IAA production by halotolerant isolates

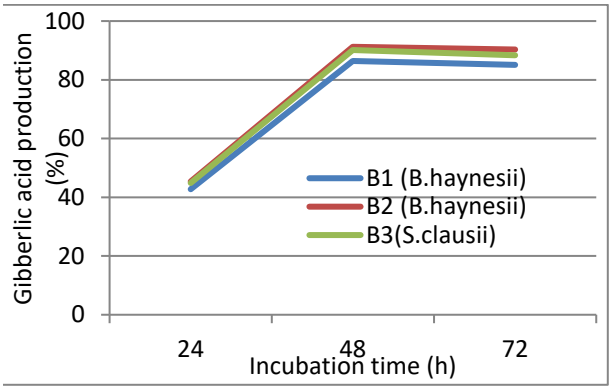

Fig. 4: Gibberellic acid production by halotolerant isolates

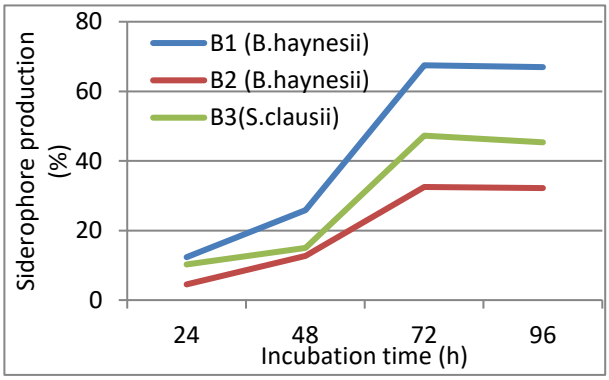

Fig. 5: Siderophore production by halotolerant isolates

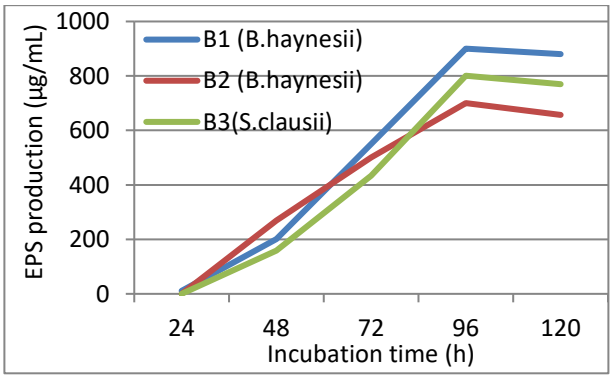

Fig. 6: EPS production by halotolerant isolates

GA production showed a different temporal pattern, reaching maximum levels at 48 h. Isolate B_2 showed highest GA production (91.3%), followed by isolate B_3 (89.5%) and isolate B_1 (87.4%), with a slight decline at 72 h (Fig. 4). The high GA-producing ability of *Bacillus* isolates is in agreement with earlier reports describing bacterial gibberellin production and its contribution to plant growth (Gutiérrez-Mañero *et al.*, 2001; Bottini *et al.*, 2004).

Siderophore production increased with incubation time and attained maxima at 72 h (Fig. 5). Isolate B_1 showed highest activity (~ 68%), followed by isolates B_3 (48%) and B_2 (32%). The higher siderophore production by isolate B_1 indicates greater capacity for iron chelation, which may contribute to the nutrient acquisition and microbial competitiveness in the rhizosphere. Siderophore-producing PGPR are especially considered valuable under nutrient-limited and saline conditions (Ahmed and Holmström, 2014; Saha *et al.*, 2016).

EPS production increased progressively and reached its highest level at 96 h (Fig. 6). Isolate B_1 produced higher amount of EPS (910 µg mL^{-1}), followed by isolate B_3 (800 µg mL^{-1}) and B_2 (710 µg mL^{-1}). EPS production is particularly relevant to salinity tolerance because extracellular polymers contribute to the biofilm formation and modification of rhizosphere microenvironment. The strong

EPS-producing ability of isolate B_1, together with its ability of salt tolerance and siderophore production, reveals its strong performance in subsequent plant assays.

Effect of bacterial inoculation on maize and wheat growth

Bacterial inoculation improved plant biomass compared to the uninoculated control, although the response varied from isolate to isolate and crop to crop (Table 1). In maize, isolate B_3 produced the highest fresh shoot biomass (820 mg), followed by isolate B_1 (800 mg); whereas in wheat, isolate B_1 produced highest fresh shoot biomass (515 mg), followed by isolate B_2 (423 mg). The isolate B_1 recorded the highest fresh root biomass in both maize and wheat (410 and 340 mg, respectively). The number of leaves plant^{-1} was higher in inoculated treatments than in untreated control. In maize, isolates B_1 and B_3 gave 12 leaves plant^{-1}, compared to 7 in control, while in wheat, isolate B_3 recorded the highest 11 leaves plant^{-1}, followed by 10 leaves plant^{-1} by isolate B_1, compared to 7 in control. The higher response of wheat to isolate B_1 and maize to isolate B_3 indicates that PGPR effects appear dependent on bacterial strain–crop interaction. Differences in root exudates, bacterial colonization and the ability of individual strains to express growth-promoting traits influence plant responses (Etesami and Beattie, 2018). The enhanced plant growth in inoculated plants is may be attributed to the production of phytohormones and other beneficial metabolites by PGPR (Goswami *et al.*, 2016).

Table 1: Wheat and maize bioassay on growth promotion by inoculation with *Bacillus haynesii* (B_1), *B. haynesii* (B_2), and *Shouchella clausii* (B_3)

Treatments	Fresh shoot biomass (mg)		Fresh root biomass (g)		No. of leaves plant^{-1}	
	Maize	Wheat	Maize	Wheat	Maize	Wheat
Isolate B_1	800	515	410	340	12	10
Isolate B_2	520	423	380	290	10	7
Isolate B_3	820	400	335	298	12	11
Control	620	355	220	198	7	7
$CD_{0.05}$	0.77	0.35	0.45	0.35	0.78	0.23
F test (P value)	0.033	0.033	0.005	0.005	0.003	0.002

Seed germination and seedling growth

Both maize and wheat seeds inoculated with PGPR isolates depicted 100% germination compared to 95% in uninoculated control. In maize, isolate B_3 produced maximum early seedling growth, particularly with respect to the plant height, and root and shoot elongation (Table 2). In wheat, isolate B_1 showed most consistent improvement in plant height, root length and shoot length, followed by isolate B_3, whereas isolate B_2 showed a comparatively moderate response. The improved early growth observed following the bacterial inoculation is consistent with the efficiency of a PGPR to produce phytohormones and facilitate nutrient acquisition. The contrasting responses of maize and wheat further reveals that the effectiveness of individual PGPR strains may depend on the host plant. Overall, the isolate B_1 showed better performance in wheat, while isolate B_3 was more effective in maize, supporting their potential as crop-specific halotolerant inoculants.

Table 2: Effect of bacterial inoculation on maize seedling growth (values are the mean of 10 seeds)

Treatments	Plant height (cm)	Root length (cm)	Shoot length (cm)
Isolate B_1	3.09	1.08	1.89
Isolate B_2	2.74	0.88	1.86
Isolate B_3	3.24	1.31	1.83
Control	2.00	0.38	1.41
$CD_{0.05}$	0.02	0.33	0.32
F test (P value)	0.01	0.03	0.33

Fig. 7: Seed germination assay of maize and wheat after PGPR inoculation

Conclusion: This study revealed the potential of halotolerant PGPR isolated from saline soils for promoting cereal crop growth. *Bacillus haynesii* B_1 showed overall better performance, particularly

in wheat, while *Shouchella clausii* isolate B_3 exhibited notable influence on maize growth and IAA production. The selected isolates expressed multiple plant growth-promoting traits, including gibberellic acid, siderophore and exopolysaccharide production, together with tolerance to high NaCl concentrations. These characteristics support their potential as candidate bioinoculants for improving the crop establishment and productivity in salt-affected environments. Further field-scale evaluation is required to validate their efficacy and agronomic stability under natural saline conditions.

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Author contributions: SDP conducted the main experiments, collected and analysed the data, and drafted the manuscript. AHJ and NMAR supervised the research work, interpreted the results, revised the drafted manuscript, and critically evaluated the work. All authors read and approved the final manuscript.

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