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Multifaceted Role of Rhizospheric Bacteria in Enhancing Sugar Beet Growth through Biofilm Formation, Salt Stress Mitigation and Biocontrol Potential

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Abstract

Plant growth promoting rhizobacteria (PGPR) are beneficial microbes that improve plant health through several mechanisms, including nutrient uptake, stress mitigation and pathogen suppression. This study was carried out for isolation and characterization of PGPR strains from the rhizosphere of salt-treated sugar beet (*Beta vulgaris* L.). Functional assays were performed to evaluate their ability to tolerate NaCl stress, produce ACC deaminase, secrete siderophores, form biofilms and solubilize phosphate. All 35 isolates survived in 1 *M* NaCl but only 7 were resistant to 2 *M* NaCl. ACC deaminase activity was observed in 91% of the isolates, while 57% produced siderophores. None of them showed the ability to solubilize phosphate. Biofilm formation showed strain-specific variability. Using 16S rRNA sequencing, different genera were identified including *Pseudomonas*, *Bacillus* and *Achromobacter*. In antagonism assays against *Rhizoctonia solani* and *Pseudomonas* sp. strain 31 showed the strongest inhibition. The results underline the multifunctional role of PGPR in supporting sugar beet growth under salt stress and indicate its potential for sustainable agriculture.

Keywords: *Beta vulgaris*; PGPR; Salt stress; ACC deaminase; Siderophores; *Pseudomonas*; 16S rRNA

Introduction

Sugar beet (*Beta vulgaris* L.) is an economically important crop that is widespread in temperate regions and makes an important contribution to global sugar production (Youssef and Rslan 2018). In addition to its industrial importance, sugar beet also serves as a model for studying the response of plants to environmental stress due to its moderate salt tolerance (Masri *et al.* 2019). However, excessive or prolonged salt stress can impair physiological processes, reduce root efficiency, limit nutrient uptake and ultimately lead to yield and quality losses (Rslan 2018; Sagar *et al*. 2021). In many regions, the expansion of irrigated agriculture and poor drainage has led to an accumulation of salts in the soil, posing a serious threat to crop productivity and soil health.

The use of plant growth promoting rhizobacteria (PGPR) has emerged as an environmentally friendly and sustainable alternative to conventional agrochemicals (Sharf *et al.* 2021; Bashir *et al*. 2024). PGPR are a diverse group of bacteria that colonize the rhizosphere and promote plant growth through both direct and indirect mechanisms (Gupta and Pandey 2019). Their benefits include improved nutrient availability, modulation of phytohormones, abiotic stress tolerance and suppression of phytopathogens (Zohaib *et al.* 2024). The rhizospheric microbiome plays a crucial role in plant development and health, especially in salt-stressed soils, by facilitating the cycling of essential nutrients and improving plant resilience (Berendsen *et al.* 2012).

Concerning salt stress, PGPR-mediated mechanisms are particularly valuable (Nadeem *et al.* 2014). For example, halotolerant PGPR can resist osmotic pressure by accumulating osmoprotectants and modifying their membrane composition, thus maintaining cellular integrity under high salt conditions (Etesami and Beattie 2018). 1-aminocyclopropane-1- carboxylate (ACC) deaminase-producing bacteria play an important role in reducing ethylene levels in stressed plants by degrading ACC, a precursor of ethylene that accumulates in response to salinity and inhibits root elongation (Glick *et al.* 2007; Singh *et al.* 2022). Siderophore producing bacteria improve iron solubility in alkaline and saline soils, where iron is often poorly available. As a result, they improve

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plants absorption of micronutrients and at the same time act as antagonists for pathogens in the soil (Johnstone and Nolan 2015).

Another important property of PGPR is their ability to form biofilms structured microbial communities embedded in extracellular polymeric substances. Biofilm formation increases bacterial survival and persistence in the rhizosphere as well as it improves root colonization and contributes to stress resistance and competitive exclusion of pathogens (Zhu *et al.* 2018; Ansari and Ahmad 2019). Taken together, these properties form a synergistic network of microbial functions that can significantly improve plant performance under challenging environmental conditions.

Despite the extensive individual characterization of these traits, comprehensive assessments of multiple traits and correlations between PGPR functions in the rhizosphere of crops such as sugar beet remain limited. Understanding the coexistence, independence or potential trade-offs between traits such as salt tolerance, ACC deaminase activity, siderophore production, phosphate solubilization and biofilm formation is essential for the development of efficient microbial consortia tailored to specific agro-ecosystems. In addition, the integration of functional assays with molecular identification (*e.g.*, 16S rRNA sequencing) provides deeper insights into the taxonomic affiliation and evolutionary adaptations of these beneficial microbes (Saha *et al.* 2024). The aim of this study was to isolate and characterize PGPR from the rhizosphere of sugar beet grown under salt stress. The work aimed to evaluate their functional traits relevant for promoting plant growth and mitigating stress, to assess their potential for biocontrol against *Rhizoctonia solani* and to identify promising strains using molecular techniques.

Material and Methods

Plant growth conditions and salt treatment

Sugar beet seeds of Pleno variety were obtained from Agriculture Research Center, Giza, Egypt and used to simulate salt stress in a controlled greenhouse environment. The seeds were planted in plastic pots (20 cm diameter) filled with a soil mixture consisting of equal parts of agricultural soil, sand and peat moss. The plants were irrigated with tap water until they reached to four leaf stage after one month. Under salt stress, each pot was watered twice a week with a 0.2 *M* NaCl solution (equivalent to 200 m*M*) at a rate of 100 mL pot^{-1}. The control plants were watered with distilled water. The salt treatment was continued for two months and the growth of the plants was monitored. At the end of the treatment period, soil samples were taken from the rhizosphere for microbial analysis.

Microbial isolation from rhizosphere soil

Microbial isolation was performed using the serial dilution plate technique as described by Johnson and Curl (1972). One gram of rhizospheric soil was taken from the root zone of treated sugar beet plants and homogenized in 9 mL of sterile distilled water. The suspension was vortexed for 30 min to ensure uniform dispersion of soil particles and microorganisms. Serial dilutions (10^{-1} to 10^{-6}) were prepared and 100 μL aliquots of each dilution were dispensed onto nutrient agar (NA) plates adjusted to pH 7.2. The plates were incubated at 30ºC for 48 h. Thereafter, morphologically distinguishable colonies were selected, purified by streaking and maintained on the NA plates at 4ºC and stored in a 50% glycerol stock at -80ºC for further use. A total of 35 bacterial isolates were obtained and subjected to functional assays. These assays evaluated properties such as NaCl tolerance, ACC deaminase activity, siderophore production, biofilm formation and phosphate solubilization assays.

Screening for salt resistance in rhizobacteria

The salt tolerance of the isolated rhizobacteria was assessed to evaluate their potential adaptability and survival under saline conditions. Isolates were cultured on NA medium supplemented with different concentrations of NaCl to simulate salt stress. In particular, NA plates were amended with 1 *M* and 2 *M* NaCl to test moderate and high salt tolerance, respectively. For comparison, a control plate containing no NaCl, reflecting non-saline conditions, was maintained. Each isolate was spot inoculated onto the surface of the NA plates using a sterile inoculation loop. The plates were incubated for 48 h at 28 ± 2ºC under constant ambient conditions. After incubation, bacterial growth was assessed visually and qualitatively based on colony size, density and morphology compared to the control treatment. This screening allowed the classification of isolates into halotolerant and salt-sensitive groups, which is essential for the selection of candidate strains for the plant growth promotion under saline field conditions.

ACC deaminase activity assay

The ability of rhizobacterial isolates to produce ACC deaminase was assessed using the method described by Penrose and Glick (2003) with modifications. This assay determines whether the isolates can utilize ACC as the sole source of nitrogen, indicating their potential to mitigate ethylene-induced stress in plants. The assay was performed using Dworkin and Foster (DF) minimal salt medium, which lacks a nitrogen source. The medium was supplemented with 3 m*M* ACC (Sigma-Aldrich, USA) as the sole nitrogen source, following the methods reported by Dworkin and Foster (1958) and Penrose *et al*. (2001). The inoculated plates were incubated for 72 h at 28ºC under static conditions, and daily measurements of bacterial growth were recorded. Colonies that showed growth on these plates after sub-cultivation were identified as ACC deaminase producers according to the criteria described by (Gupta and Pandey 2019).

Siderophore production assay

The ability of bacterial isolates to produce siderophores, which are iron chelating compounds that increase iron availability under limiting conditions was investigated using the Chrome Azurol S (CAS) agar plate assay described by Schwyn and Neilands (1987) with slight modifications. The CAS medium was prepared by incorporating the CAS dye complex, forming a blue-colored iron-dye complex. Siderophores secreted by the bacteria bind to the iron, releasing the dye and causing a visible color change from blue to orange or yellow. This assay provides a qualitative screening method for siderophore-producing bacteria. Each bacterial isolate was grown overnight in LB broth at 30ºC and 5 μL of the culture was spot inoculated onto the center of sterile CAS agar plates. The plates were incubated at 30ºC for 48–72 h under static conditions.

Phosphate solubilization assay

The ability of rhizobacterial isolates to solubilize insoluble inorganic phosphate was investigated using Pikovskaya's agar medium (Pikovskaia 1948), contains tricalcium phosphate [$Ca_3 (PO_4)_2$] as the sole phosphorus source. This medium is often used to evaluate the phosphate-solubilizing efficiency of PGPR. Overnight grown bacterial cultures were spot inoculated (5−10 μL) onto freshly prepared Pikovskaya's agar plates. The plates were incubated at 30ºC for 5 to 7 days under static conditions. Phosphate solubilization was indicated by the development of a clear halo (solubilization zone) around the bacterial colony, which was due to the localized dissolution of tri-calcium phosphate.

Biofilm formation assay

The bacterial isolates were first grown overnight under aerobic conditions in Luria-Bertani broth (LB). The resulting cultures were then diluted with fresh LB to obtain a standardized optical density of OD_{600} = 0.02. For the assay, 160 μL aliquots of the diluted cultures were transferred to individual wells of a 96-well polystyrene microplate and the plates were incubated at 28ºC for 48 h under static conditions to promote biofilm formation. After incubation, the development of the biofilm was examined using the crystal violet staining method. The wells were carefully emptied and washed at least five times with distilled water to remove planktonic and loosely attached cells. The plates were then air-dried at room temperature for 30 min. The biofilms were stained by adding 200 μL of a 0.1% crystal violet solution to each well and incubating at 28ºC for 20 min. After removal of excess stain and thorough washing, the wells were air-dried again and 70% ethanol was added to solubilize the bound dye. To quantify biofilm biomass, absorbance was measured at 590 nm using a microplate reader after 20 min ethanol incubation. The assay was performed with five biological replicates per isolate to ensure reproducibility and statistical robustness.

Antagonism assay against *Rhizoctonia solani*

Three PGPR strains were selected based on their varying degrees of NaCl tolerance, ACC deaminase activity, siderophore production and biofilm formation. The antagonistic activity of selected PGPR isolates against *Rhizoctonia solani* was evaluated using a dual culture plate confrontation assay according to Calvo *et al*. (2010). The bacterial isolates were first cultured in LB broth at 30ºC for 24 h and the cell suspensions were adjusted to OD_{600} = 1 with sterile saline. Potato dextrose agar (PDA) plates were prepared and inoculated by streaking the bacterial suspension in a straight line 2 cm from the edge of the plate. A 5 mm diameter mycelial plug of *R. solani*, taken from a 5-day-old PDA culture, was then placed on the opposite side of the plate at a distance of 5 cm from the center of the bacterial streak. All plates were incubated at 25−28ºC for 5 to 10 days under static conditions. Inhibition of fungal growth was monitored visually.

16S rRNA gene analysis of bacterial isolates

To determine the taxonomic identity of the three selected bacterial isolates which used in antagonism assay, 16S rRNA gene sequencing was conducted. Genomic DNA was extracted using the boiling lysis method as described by Cheng and Jiang (2006) with slight modifications. In brief, a small amount of a bacterial colony from an overnight culture was suspended in 100 μL of sterile distilled water in a sterile micro-centrifuge tube. The suspension was mixed thoroughly and then incubated in a boiling water bath at 100ºC for 10 min to lyse the bacterial cells and release genomic DNA. After boiling, the samples were immediately placed on ice for 5 min to stabilize the DNA. They were then centrifuged for 5 min at 12,000 rpm to pellet the cell debris. The resulting supernatant, which contained crude genomic DNA, was carefully transferred to a new sterile tube and further purified using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, USA) according to the manufacturer's protocol.

The extracted DNA served as template for PCR amplification of the 16S rRNA region (V4-V9) using universal primers, 515F (5`- GTGCCAGCMGCCGCGGTAA-3`) and 1492R (5`- GGTTACCTTGTTACGACTT-3`). PCR reactions were prepared in a volume of (25 μL with 30 ng genomic DNA, 5 μL of 5× PCR buffer, 2.5 μL of each primer), 1.5 μL $MgCl_2$, 0.5 μL Taq DNA polymerase (50 U/μL), 0.5 μL 10 m*M* dNTPs and nuclease-free water to reach the final volume. Amplification was performed in a BIO-RAD C1000^{TM} Thermal Cycler, CA, USA, using the following PCR conditions: 94°C for 5 min, followed by 30 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1.5 min, 72°C for 7 min. The amplified amplicons were purified using the GeneJET PCR Purification Kit (Thermo Scientific, USA) and analyzed by agarose gel electrophoresis. The purified amplicons were submitted to Colors Medical Lab (Cairo, Egypt) for Sanger sequencing.

The resulting sequences were subjected to a similarity search using the NCBI BLASTn (Altschul *et al.* 1990) to identify closely related taxa in the GenBank database. The 16S rDNA sequences were deposited in GenBank, and accession numbers were assigned for each isolate. To determine phylogenetic relationships, sequences were aligned with the NCBI 16S ribosomal RNA sequences database using BLASTn. Clustal Omega (Sievers *et al.* 2011) was used for multiple sequence alignment and the phylogenetic tree was visualized using the iTOL tool (Letunic and Bork 2021).

Statistical analysis

All experiments were performed in triplicate. Data analysis was conducted using Microsoft Excel and Python programming (Pandas and Seaborn libraries). Descriptive statistics including mean, standard deviation, minimum and maximum values, were calculated for the biofilm absorbance data. Boxplots, bar charts and histograms were used to visualize the data to assess the distribution and variation between strains. For correlation analysis, Pearson's correlation coefficient was used to determine the relationships between biofilm formation and other PGPR traits (*e.g.*, 2 *M* NaCl tolerance, siderophore production, ACC deaminase activity) according to Steel and Tori (1996). Strains were ranked based on average biofilm absorption to identify high and low biofilm formers.

Results

NaCl tolerance of bacterial isolates

Bacteria isolated from the rhizosphere of sugar beet were tested for their ability to tolerate 1 *M* and 2 *M* NaCl concentrations. The results showed that all tested strains (35 out of 35) were able to survive in 1 *M* NaCl, demonstrating a moderate level of halotolerance. However, at 2 *M* NaCl, the survival rate decreased significantly, with only 7 strains (14, 15, 16, 17, 18, 23 and 47) being resistant at this higher concentration. This indicates that although many bacterial isolates from the rhizosphere can withstand saline conditions but their tolerance has an upper limit beyond which growth is inhibited. After isolation, the bacterial strains were subjected to functional assays to determine their plant growth-promoting properties under stress conditions. These assays evaluated properties such as ACC deaminase activity, siderophore production, biofilm formation and phosphate solubilization assays (Fig. 1).

ACC, siderophore production and phosphate solubilization among isolates

Analysis of bacterial isolates for the production of ACC deaminase revealed that 32 out of 35 strains (91%) exhibited ACC deaminase activity (Fig. 2), while only 3 strains (8.6%) lacked this ability. Similarly, analysis of siderophore production among bacterial isolates revealed that 20 out of 35 strains (57%) were positive for siderophore production (Fig. 3), while 14 strains (40%) did not exhibit this trait. In contrast, analysis of the phosphate-solubilizing ability among the bacterial isolates from the sugar beet rhizosphere revealed that none of the 35 strains exhibited phosphate solubilization activity (Fig. 4).

Biofilm formation analysis

Biofilm formation ability of isolates showed substantial variability, with strains 50 and 20 exhibiting the highest biofilm-forming capacities (OD570 values of 3.26 and 2.50, respectively), whereas strain 37 exhibited the lowest capacity (OD570 value of 0.55). Histogram analysis suggested a bimodal distribution of biofilm-forming capabilities among the tested strains (Fig. 5). Correlation heatmap of plant growth promoting rhizobacterial assays showed that a slight positive correlation (0.15) between 2 *M* NaCl tolerance and ACC deaminase activity (Fig. 6).

16S rRNA amplification, sequencing and functional insights

For further characterization of the bacterial isolates from the sugar beet rhizosphere, three selected strains (16, 23 and 31) were subjected to 16S rRNA gene amplification and sequencing. These strains were selected based on their varying degrees of NaCl tolerance, ACC deaminase activity, siderophore production and biofilm formation. In particular, strains 16 and 23 showed high halotolerance (2 *M* NaCl) and were positive for ACC deaminase activity, while strain 31 showed moderate halotolerance (1 *M* NaCl) and could not survive at 2 *M* NaCl. The selection of these strains ensured a broad representation of both moderately and highly salt tolerant bacteria with different functional properties relevant for promoting plant growth. A 950 bp segment of the 16S rRNA gene was successfully amplified and sequenced for these isolates using V4-V9 universal primers. Taxonomic classification based on sequence analysis revealed that two of the bacterial strains belonged to the phylum *Pseudomonadota*, while one strain belonged to the phylum *Bacillota*. Further identification at the genus level assigned strain 16 to the genus *Achromobacter* (*Achromobacter* sp.), strain 23 to the genus *Bacillus* (*Bacillus* sp.) and strain 31 to the genus *Pseudomonas* (*Pseudomonas* sp.) (Fig. 7).

Antagonistic activity against *R. solani*

Due to its destructive effect, *R. solani* was selected as a preliminary target indicator for control in the current study. To evaluate the biocontrol potential of the selected bacterial strains (16, 23 and 31), a plate confrontation assay was performed according to the method described by Laslo *et al.* (2012). For the antagonism assay, the selected bacterial isolates were cultured in LB liquid medium at 30°C on a shak-

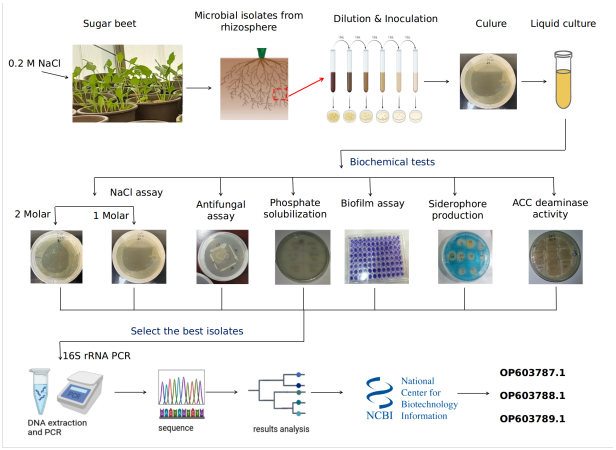

Fig. 1: The workflow for the isolation and characterization of PGPR from the rhizosphere of sugar beet grown under salt stress

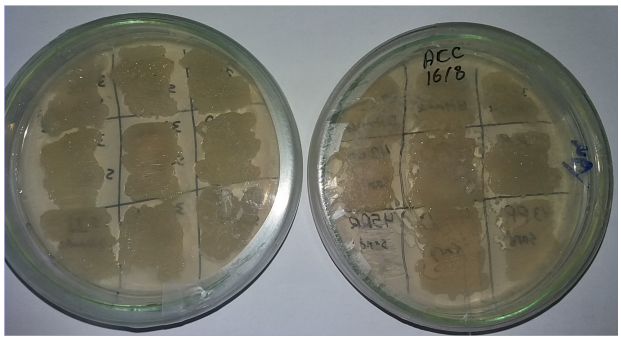

Fig. 2: A sample of ACC production across bacterial strains

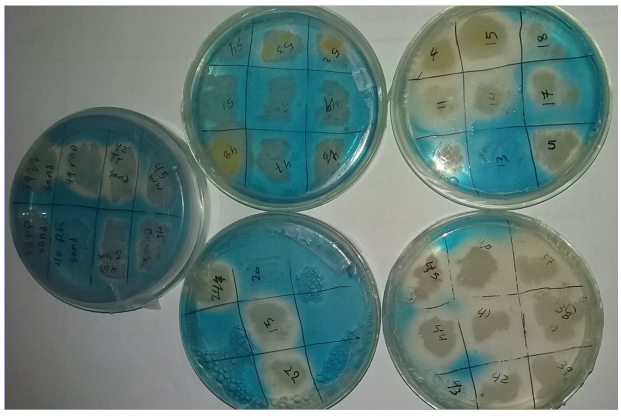

Fig. 3: Siderophore production across bacterial strains

er at 200 rpm for 24 h, after which their concentration was adjusted to an OD600 value of 1.0. The bacterial suspension was streaked on the left side of a PDA plate, 2 cm from the edge. 5 mm diameter of *R. solani* mycelial plug was taken from fungal culture (5-day old) and placed 5 cm from the center of the bacterial inoculation site. The plates were incubated under controlled conditions and the inhibition of fungal growth was observed. The results showed differences

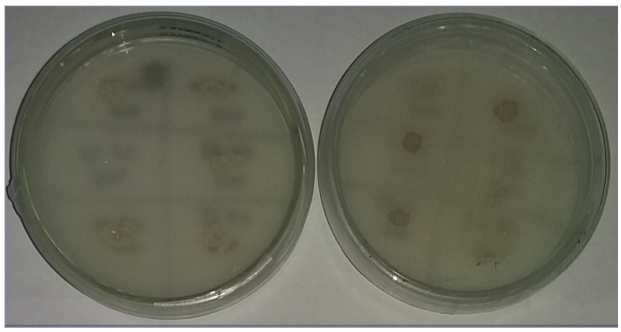

Fig. 4: A sample of phosphate solubilization across bacterial strains

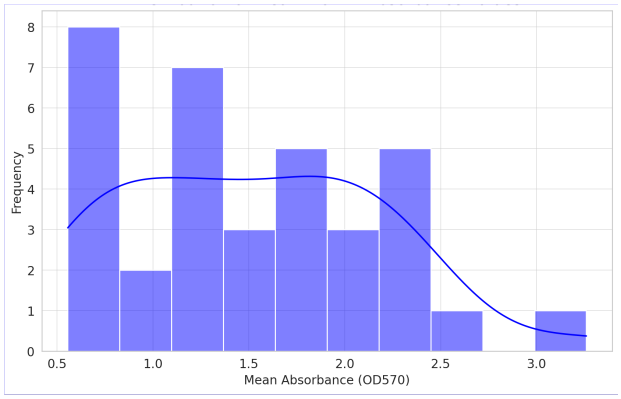

Fig. 5: A histogram analysis to visualize the overall distribution of biofilm formation

in antagonistic activity between the bacterial isolates. Contrary to initial expectations, strain 31 showed the strongest anti-fungal activity and formed a large zone of inhibition around *R. solani*, indicating its potential as an effective biocontrol agent. In contrast, strains 16 and 23 showed weaker inhibitory activity as indicated by the smaller zones of inhibition in the confrontation assay.

Discussion

Saline stress conditions were successfully simulated in sugar beet plants through the salt treatment experiment. The application of 200 m*M* NaCl for two months led to the selection of salt-tolerant microbial communities in the rhizosphere. The ability of bacteria to survive under high salinity is often associated with mechanisms such as osmoprotectant production, ion homeostasis, biofilm formation and ACC deaminase activity (Etesami and Beattie 2018). These salt-adapted bacteria could play an important role in improving the resilience of sugar beet by improving root function and nutrient uptake and reducing salt-induced plant stress.

ACC deaminase-producing bacteria play a crucial role in plant growth promotion (PGP) by reducing ethylene levels under stress conditions such as salinity, drought and heavy metal toxicity (Singh *et al*. 2022).

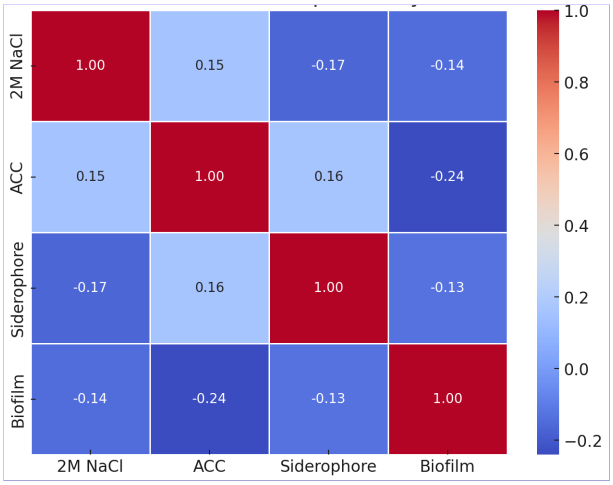

Fig. 6: Correlation heatmap of plant growth promoting rhizobacteria assays

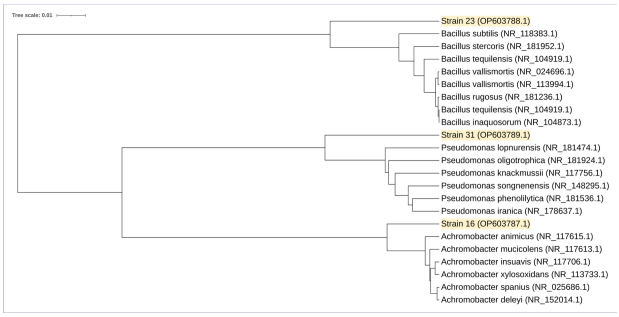

Fig. 7: Phylogenetic tree of the selected isolates and other closely related bacterial species

The ACC deaminase results indicate that the majority of the bacterial community associated with the sugar beet rhizosphere has the ability to degrade ACC, a precursor of ethylene, which is a key stress hormone in plants. Siderophores are specialized molecules secreted by bacteria to scavenge iron from the environment and make it more bioavailable under iron-limiting conditions (Johnstone and Nolan 2015). The siderophores results indicates that a significant portion of the bacterial community associated with sugar beet roots is capable of producing siderophores, which are iron-chelating compounds that are critical for bacterial survival and plant growth promotion.

Phosphate solubilization results indicate that the bacterial community investigated in this study does not contribute significantly to phosphate mobilization in the rhizosphere. Phosphorus is an important macro nutrient for plant growth, but its bioavailability is often limited as it tends to form insoluble complexes in soil (Zhu *et al*. 2018). Many PGPR facilitate phosphorus uptake by converting these insoluble forms into plant-available forms through the secretion of organic acids and phosphatases (Bashir *et al*. 2024). However, the lack of phosphate-solubilizing activity in these isolates raises questions about their role in nutrient cycling in the sugar beet rhizosphere. Several factors could explain why none of the isolates showed phosphate solubilization. One possibility is that the bacterial community in the sugar beet rhizosphere is not actively involved in phosphate solubilization, implying that these bacteria may have other functional roles in promoting plant growth rather than phosphorus acquisition (Saha *et al.* 2024). Another explanation could be that the rhizosphere already contains sufficient amounts of bioavailable phosphorus, which reduces the selection pressure on the bacteria to develop phosphate-solubilizing capabilities (Castagno *et al.* 2021). In addition, these isolates may rely on alternative nutrient acquisition strategies, such as ACC deaminase activity and siderophore production, which contribute to plant health by mitigating stress and improving iron availability rather than focusing on phosphorus solubilization (Azadikhah *et al*. 2019; Sagar *et al*. 2021).

Biofilm formation is a crucial trait for PGPR, enabling them to colonize plant roots, persist in the rhizosphere and achieve beneficial effects such as pathogen suppression and nutrient mobilization (Zhou *et al*. 2018; Ansari and Ahmad 2019). The biofilm-forming ability of the tested bacterial strains was evaluated using crystal violet microtiter plate assays, with absorbance values measured at OD570. As shown in the boxplot analysis (Fig. 8), the data exhibit a wide range of biofilm production, with some strains showing high biofilm formation while others produce lower levels. In particular, outliers were observed indicating strains with exceptionally high or low biofilm formation, suggesting strain-specific differences in microbial adhesion and extracellular matrix production. As shown in Fig. 9, the bar chart displays the mean biofilm absorbance for each strain. The highest biofilm-forming strains were strain 50 (OD570 = 3.26) and strain 20 (OD570 = 2.50), indicating their strong adhesion and extracellular matrix production. In contrast, the lowest biofilm formation was observed in strain 37 (OD570 = 0.55), which could indicate a weak potential for root colonization. These differences could be due to differences in exopolysaccharide production, quorum sensing or adaptability to the environment. A histogram analysis (Fig. 5) was performed to visualize the overall distribution of biofilm formation. The data show a bimodal or skewed distribution, indicating that the strains segregate into high and low biofilm formers rather than following a normal distribution. This pattern indicates that biofilm formation may be controlled by strain-specific genetic or regulatory mechanisms and it is not evenly distributed across the microbial community.

The correlation heatmap of assays was calculated using Pearson's correlation coefficient. This statistical measure evaluates the linear relationship between different bacterial traits (2 *M* NaCl tolerance, ACC deaminase activity, siderophore production and biofilm formation) and provides insight into the potential relationships between these plant growth-promoting traits. The lack of differences in 1 *M* NaCl tolerance, where all isolates survived, emphasizes the

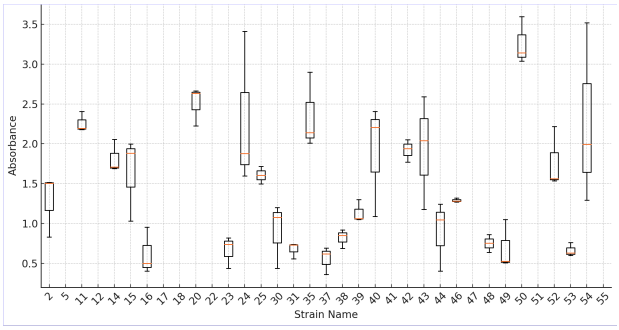

Fig. 8: Boxplot analysis illustrates the variation in biofilm formation across bacterial strains

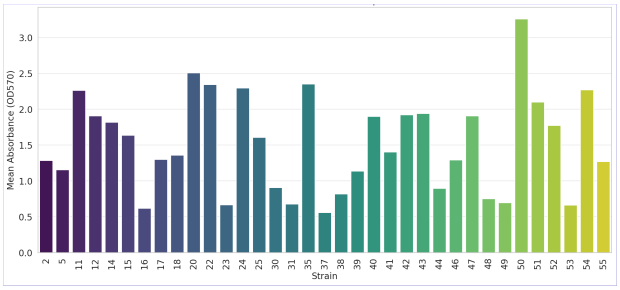

Fig. 9: Mean biofilm absorbance for each strain

intrinsic adapt- ability of rhizosphere bacteria to moderate salinity. However, tolerance to higher salt concentrations (2 *M* NaCl) was more selective, indicating that extreme salinity is a stronger environmental filter for bacterial survival. A slight positive correlation between 2 *M* NaCl tolerance and ACC deaminase activity suggests that bacteria capable of breaking down ACC may have an adaptive advantage in saline environments. This finding aligns with previous studies indicating that ACC deaminase-producing bacteria mitigate ethylene stress, allowing bacterial cells to better withstand osmotic challenges associated with high salinity (Chandwani and Amaresan 2022). However, the correlation is relatively weak; suggesting that while ACC deaminase may contribute to halotolerance, other adaptive mechanisms such as osmoprotectant production and ion homeostasis likely play a more dominant role.

A negative correlation (-0.24) between biofilm formation and ACC deaminase activity suggests that bacteria producing higher levels of ACC deaminase are more likely to form weaker biofilms. This finding could indicate that ACC deaminase-producing bacteria prioritize mitigation of ethylene stress over biofilm-mediated protection. While biofilms provide structural and chemical resistance under stress conditions, ACC deaminase activity could serve as an alternative stress adaptation mechanism, especially in environments where ethylene suppression promotes bacterial survival. The weak negative correlation (-0.14) between biofilm formation and NaCl tolerance indicates that biofilm-producing bacteria are not necessarily the most salt-tolerant. While biofilms may enhance bacterial survival under stress conditions by providing physical protection and moisture retention, halotolerant strains may rely on alternative mechanisms such as synthesis of osmoprotectants and membrane modifications rather than biofilm formation (Haroon *et al*. 2023).

A weak negative correlation (-0.16) between siderophore production and NaCl tolerance indicates that siderophore-producing bacteria do not necessarily thrive in high-salt environments. Siderophores are primarily responsible for iron acquisition, which is critical for bacterial survival in iron-deficient soils, but may not be directly involved in osmotic stress resistance. This suggests that siderophore-producing bacteria from the rhizosphere of sugar beet are probably adapted to nutrient acquisition strategies rather than salinity tolerance. In general, the weak or negative correlations between biofilm formation, siderophore production and NaCl tolerance suggest that these traits do not contribute significantly to adaptation to salt stress (Malheiro and Sim˜oes 2017; Ao *et al*. 2020; Murugan *et al.* 2024) Instead, bacterial survival in saline environments is likely determined by other metabolic and physiological mechanisms such as ion regulation, accumulation of compatible solutes and changes in membrane stability (Liang *et al.* 2020; Gaffney *et al*. 2021).

The 16S rRNA results indicate a diverse taxonomic composition of the selected isolates, reflecting their adaptability to the sugar beet rhizosphere. A phylogenetic tree was constructed using the 16S rRNA amplified sequences to visually represent the evolutionary relationships between these isolates and other closely related bacterial species as shown in Fig. 6. This analysis sheds light on the evolutionary divergence of the selected isolates and their possible functional role in the rhizosphere. The 16S rDNA sequences of these isolates were deposited in the NCBI database, and the assigned accession numbers are as follows: *Bacillus* sp. strain 23 (OP603788), *Achromobacter* sp. strain 16 (OP603787) and *Pseudomonas* sp. strain 31 (OP603789). The identification of halotolerant and ACC deaminase-producing bacteria from the rhizosphere of sugar beet highlights their potential role in improving plant resistance under salt stress conditions. The presence of the genera *Bacillus*, *Pseudomonas* and *Achromobacter* among the isolates is consistent with previous studies reporting that these bacterial groups are often associated with PGPR due to their ability to produce bioactive compounds, solubilize nutrients and alleviate stress (Glick *et al*. 2007; Etesami and Beattie 2018). Among the selected isolates, *Achromobacter* sp. strain 16 and *Bacillus* sp. strain 23 showed strong salt tolerance and ACC deaminase activity, indicating their potential as bio-inoculants for salt-affected soils. In contrast, *Pseudomonas* sp. strain 31 showed only moderate halotolerance (1 *M* NaCl), indicating intra-genus variability in adaptation to salt stress.

The phylogenetic classification of these isolates supports their functional differentiation, as *Pseudomonadota* and *Bacillota* have different metabolic adaptations.

Pseudomonadota species, particularly *Pseudomonas* and *Achromobacter*, are known for their versatile metabolic capabilities, including siderophore production, biofilm formation and stress tolerance (Bhattacharyya and Jha 2012). *Bacillus* species, on the other hand, are known for their sporulation ability and robust survival strategies, making them excellent candidates for bio-fertilizers in agriculture (Saxena *et al*. 2020). The presence of strains with moderate and high halotolerance in combination with variations in siderophore production and biofilm formation suggests that these bacteria use different survival strategies to thrive in the rhizosphere. Biofilm-forming bacteria may rely on colonization of surfaces and protection from environmental stress, while siderophore-producing strains enhance iron uptake and plant nutrient availability. The variation in these traits between closely related isolates suggests functional diversity within the rhizosphere microbial community that may contribute to the overall stability and resilience of the plant-microbe system.

For antagonistic activity, *Rhizoctonia solani* was used due to its destructive effect, *Rhizoctonia solani* is a soilborne plant pathogen and belongs to *Rhizoctonia* and known for its broad host range, morphological diversity and aggressive virulence (Bolton *et al.* 2006). It is responsible for serious crop losses worldwide, making it a major economic problem in agriculture (Taheri and Tarighi 2011). As shown in results, the observed differences suggest that strain 31 produces potent antifungal compounds, while the other strains may have limited production of antifungal metabolites or rely on alternative mechanisms to promote plant growth. The strong antagonism of strain 31 may be due to its ability to produce antibiotic compounds, hydrolytic enzymes (such as chitinases or proteases) or volatile organic compounds, which are known to suppress fungal pathogens (Whipps 2001; Raaijmakers *et al*. 2009). The identification of strain 31 as *Pseudomonas* sp. is consistent with previous studies reporting that *Pseudomonas* species are effective biocontrol agents due to their ability to synthesize phenazines, lipopeptides and siderophores that inhibit fungal growth (Bhattacharyya and Jha 2012).

In contrast, the weaker inhibition observed with strains 16 and 23 suggests that these isolates may not be as effective in directly suppressing fungi, but may still provide other plant growth-promoting benefits. Their ability to produce ACC deaminase and tolerate high salinity levels suggests that they increase plant stress resistance rather than directly inhibiting fungal pathogens (Etesami and Beattie 2018). This observation highlights the multiple functional roles of PGPR, with some strains exhibiting strong biocontrol activity, while others contribute through indirect mechanisms such as stress tolerance and nutrient solubilization (Glick *et al.* 2007).

Conclusion

This study demonstrates the potential of rhizosphere-associated PGPR to improve sugar beet growth under saline conditions. Most isolates exhibited strong ACC deaminase activity and moderate to high halotolerance, while siderophore production and biofilm formation varied among strains. The absence of phosphate solubilization highlights a functional shift in nutrient acquisition strategies. Correlation analysis revealed weak associations between the traits, suggesting that the mechanisms for stress mitigation and growth promotion are largely independent of each other. Molecular identification confirmed taxonomic diversity among the selected isolates, including *Pseudomonas*, *Achromobacter* and *Bacillus* species. In particular, *Pseudomonas* sp. strain 31 showed strong antifungal activity against *Rhizoctonia solani*, making it a promising biocontrol agent. These results support the use of multi-trait PGPR consortia for sustainable crop production in saline environments and highlight the need for further validation in the field and genomic characterization of these isolates.

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Not applicable to this paper.

Author Contributions

WMR, SREAH, HMAE and MIM conceived the study, designed the experiments, writing, review and editing the paper. WMR performed the experiments and analyze the data. SREAH, HMAE and MIM did supervision.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

All data supporting the results of this study are included in the article. The 16S rRNA gene sequences of the bacterial isolates have been deposited in the NCBI GenBank database and are publicly available under the following accession numbers: OP603787.1, OP603788.1 and OP603789.1.

Ethics Approval

Not applicable to this paper.

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