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SALINITY INDUCED CHANGES IN PLANT GROWTH PROMOTING RHIZOBACTERIA(PGPR) ACTIVITIES

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ABSTRACT

This work was carried out at department of Microbiology, Rajaram college, Kolhapur, Maharashtra, India during year 2022-2024. This study investigated the impact of varying salinity levels (0 mM, 50 mM, 100 mM, and 150 mM) of NaCl on plant growth-promoting rhizobacteria (PGPR) activities, focusing on phosphate solubilization, potassium solubilization, indole acetic acid (IAA) production, ammonia production, and cellulase activity. Soil samples were collected from 11 saline and sodic regions in Maharashtra, India, and analysed for physical and chemical properties. Out of 11 collected soil samples only four samples (Ganeshwadi-11, Shedshal- 5.2, Shirol- 15) showed EC value higher than 4. The pH of the collected soil samples ranged from 5.16 to 8.12, indicating predominantly neutral to slightly alkaline conditions, except for Shirol, which exhibited acidic characteristics (pH 5.16). Most of the samples consisted of clayey soil, while Udgaon and Majrewadi had loamy soil, and Akiwat featured sandy soil, highlighting variations in texture, water retention, and drainage capacity. Out of these 11 samples, total 87 PGPR strains were isolated. Among 87 isolated PGPR strains, 23 demonstrated high salt tolerance. This study highlights the potential of salt-tolerant plant growth-promoting rhizobacteria (PGPR) for enhancing agricultural productivity in saline soils. The isolation of 23 salt-tolerant PGPR strains from diverse saline environments demonstrated the capacity of these bacteria to perform essential functions such as phosphate and potassium solubilization, IAA production, ammonia production, and cellulase activity under saline stress.Phosphate solubilization by PGPR varied across isolates and salt concentrations, with the highest solubilization observed at 50 mM and 100 mM for most isolates, while 0 mM showed the lowest activity overall. The highest indole acetic acid (IAA) production was observed in isolate SR16-1 at 150 mM salt concentration, while other isolates exhibited relatively stable but lower IAA production across varying salt levels. The highest cellulolytic index was observed in isolates G10-6 and SR10-3 at 150 mM NaCl, while SR16-1 showed the lowest cellulolytic activity across all salt concentrations. The highest ammonia production was observed in isolate G8-4 at 150mM NaCl, while other isolates showed moderate to low ammonia production across different salt concentrations. Strains like G121, SD102, and SR16-1 showed consistent high performance across various functional assays, represent promising candidates for further development as bioinoculants in saline and sodic soils.

These PGPR can help mitigate the negative impacts of salinity on crop growth by improving nutrient availability and promoting plant resilience. Future research should focus on understanding the molecular mechanisms underlying the salt tolerance of these isolates and evaluating their field performance to optimize their use in sustainable agriculture. These results highlight the potential of salt-tolerant PGPR in promoting sustainable agriculture in salt-affected regions. The study identified promising candidates for further development as bioinoculants for improving crop resilience and soil fertility in saline ecosystems.

(Key words: Soil salinity, plant growth-promoting rhizobacteria (PGPR), salt tolerance, sustainable agriculture)

INTRODUCTION

The current century has witnessed significant technological advancements, but it is also facing challenges such as rapid population growth and major disruptions in global agro ecosystems. One of the most pressing concerns is the decline in productivity and the degradation of sustainable agricultural practices. To address these challenges and ensure environmental health, eco-friendly strategies have become a focal point for researchers, with an increasing emphasis on sustainable agriculture. Microbial populations, particularly plant growth-promoting rhizobacteria (PGPR), have emerged as key players in these strategies. PGPR, which colonize the rhizosphere, play a vital role in enhancing plant growth through both direct and indirect mechanisms. Direct mechanisms include the

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production of phytohormones (Bakker *et al*., 2013), nitrogen fixation, and the release of essential nutrients such as phosphorus, potassium, and zinc (Kour *et al*., 2023; Upadhyay *et al*., 2022). Indirect mechanisms involve enhancing resistance to abiotic stresses, such as salinity, and managing plant pathogens through the production of antibiotics (Khoso *et al*., 2024; Gupta and Pandey, 2023). Several bacterial genera, including *Acinetobacter, Azospirillum*, *Bacillus, Pseudomonas*, and *Rhizobium*, are known for their PGPR potential. Agriculture and microbial growth are closely linked, influenced by the soil's physical, chemical, and biological properties. Among the various challenges faced by agriculture, soil salinity is one of the most significant. Salinity stress affects a substantial portion of the world's cultivated land, reducing crop productivity and soil health. Globally, approximately 20% of cultivated land and 33% of irrigated agricultural land are affected by high salinity, with salinized areas increasing by 10% annually (Shahbaz and Ashraf, 2013). As a result, the salinization of arable land could reach over 50% by 2050 (Shrivastava and Kumar, 2013). The impact of salinity extends beyond plants to the microbial communities in the soil, particularly PGPR. As salinity levels increase, PGPR activity and survival can be adversely affected, this may hinder their potential benefits to plants or the activity and survival of PGPR can be positively influenced, which may enhance their potential benefits to plants. Salt-tolerant PGPR has therefore garnered significant attention for their role in saline soils, where they can continue to promote plant growth despite the stress (Sunita *et al*., 2020; He *et al*., 2019). This study aimed to explore the precise impact of varying salt concentrations on the growth and activities of PGPR, with the goal of identifying salt-tolerant strains that could be utilized for improving plant health in saline soils.

MATERIALS AND METHODS

Collection of samples and isolation of PGPR

Collection of soil samples

Soil samples were collected from coastal, saline, and sodic areas across eleven different locations in Kolhapur district, Maharashtra, India. The selected locations included Udgaon, Chinchwad, Arjunwadi, Ganeshwadi, Ghalwad, Shirol, Shirti, Shedshal, Akiwat, Majrewadi, and Shirdhon. Soil was collected from a depth of 0–20 cm using a hand auger. The root systems, along with the surrounding bulk soil, were carefully extracted. Rhizospheric soil was separated using the shaking method, followed by brushing off the remaining soil from the root system (Yanai *et al*., 2003; Tiwari *et al*., 2016). The collected soil samples were placed in sterile plastic bags, labelled, and transported to the research laboratory at Rajaram College, Kolhapur, Maharashtra, India, for bacterial isolation. All soil samples were kept at 4°C during transport to minimize any microbial degradation.

Study of collected soil samples

The collected samples were analysed for various characteristics, including texture, color, pH, and electrical conductivity. Electrical conductivity of soil sample was determined by making a saturated paste of soil and deionized water. The water was extracted and the measuring the EC of the extracted solution by inserting a probe in it.The conductivity meter was used to measure the electric conductivity of soil sample. The probe displays the EC value on its digital screen and that value was recorded.

Isolation of PGPR from collected soil samples

For the isolation of PGPR from the collected rhizospheric soil samples, enrichment was carried out in nutrient broth (NB). The soil samples were serially diluted in sterile saline, and a 100 µl aliquot from each dilution was spread on sterile nutrient agar (NA) plates. The plates were incubated at 28±2°C for 72 hours, or until bacterial colonies appeared. Control plates (without soil sample) were included to ensure the sterility of the medium. Individual bacterial colonies were picked from the NA plates and streaked onto fresh nutrient broth (NB) plates for further purification. This process was repeated until isolated colonies were obtained. All steps were performed in triplicates to ensure reproducibility and minimize error.

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.

Studies on effect of varying salt concentrations on PGPR functionality

Phosphate solubilisation

A qualitative assay for phosphate solubilisation was conducted using Pikovskaya medium supplemented with tricalcium phosphate (Pikovskaya, 1948). Pikovskaya medium was prepared with varying salt concentrations: 0 mM, 50 mM, 100 mM, and 150 mM. All experiments were conducted in triplicates to ensure reproducibility and reliability of the results, and the average values of the measurements were considered for analysis.

Pottasium solubilisation

Pottasium solubilization bacteria (KSB) were isolated using solid Aleksandrov medium (Meena *et al*., 2015). The K solubilization efficiency (KE) of each isolate was calculated according to Khanghahi *et al*. (2018) using the following formula:

KE = Diameter of solubilization halo / Diameter of the colony

Indole acetic acid production (IAA)

To test IAA production, the isolates were evaluated using a colorimetric method adapted from Sarker and Al-Rashid (2013). A control group was prepared with only the growth medium, without any inoculation. IAA concentration in the culture supernatant was estimated using Salkowski reagent (Gordon and Weber, 1951). Each sample was tested in triplicate, and the entire procedure was repeated twice to verify the accuracy and consistency of the results.

Ammonia production

Ammonia production was assessed in a test tube containing 20.0 ml of sterile peptone water at pH 7.2. The amount of NH_3 produced was measured at intervals from 24 to 144 hours. Ammonia estimation was performed using a spectrophotometric method as outlined by Cappucino and Sherman (1992).

Cellulase production

Cellulase activity was assessed using carboxymethyl cellulose (CMC) agar plates following the method described by Ghose (1987). The formation of clear zones around the colonies indicated cellulase production. The diameter of these zones was measured using a calliper to calculate the cellulolytic index (CI), which reflects the cellulase activity of the bacteria. Cellulase activity was classified based on the CI value: low (CI < 1), medium (CI = 1–2), and high (CI > 2). The formula for calculating the cellulolytic index (Choi *et al*., 2005) is: Cellulolytic Index= (Clear Zone Diameter - Colony Diameter) / Colony diameter

RESULTS AND DISCUSSION

Collection and characterization of soil samples-

Soil samples were collected from a total of eleven locations. The Table 1 provides an overview of the characteristics of the collected soil samples.The analyzed soil samples varied in color, texture, pH, and electrical conductivity (EC) across different locations. Most soils were clayey and black, except for some loamy and sandy samples. The pH ranged from 5.16 (acidic) in Shirol to 8.12 (alkaline) in Shirdhon, with most soils being neutral to slightly alkaline. EC values varied significantly, with Shirol (15 dS/m) and Ganeshwadi (11 dS/m) showing the highest salinity, while Ghalwad (0.5 dS/m) had the lowest conductivity, indicating minimal salt presence.A total of 87 isolates were obtained from 11 soil samples, among which 23 exhibited salts tolerant. All 23 isolates tolerated salt concentrations above 6% and were therefore selected. From these, the top 12 isolates *viz.,* G8-4 (8%), G8-5 (8.5%), G10-6 (10%), SD10-2 (10%), SD10-3 (10.5%), SD12-2 (12%), SD16-2 (16%), SR10-3 (10%), SR12-1 (12%) and SR16-1 (16%) were chosen for further studies. The values in parentheses indicate the highest salt concentration tolerated by each isolate.

Studies on effect of varying salt concentrations on PGPR functionality

Phosphate solubilisation

It is evident from the graph (Figure 1) that phosphate solubilization was more effective when salt was present in the media compared to when it was absent. Each isolate exhibited a different pattern of phosphate solubilization, reflecting its ability to tolerate and function under different salt conditions. Some isolates (G12-1, SD10-2) showed consistently higher phosphate solubilization across all salt concentrations, while others (SR16-1, SR12-1) displayed significantly lower phosphate solubilization at higher salt concentrations. Most isolates had moderate to high solubilization zones, suggesting a baseline ability to solubilize phosphate in a non-saline environment. Isolates G12-1 and SD10-2 consistently exhibited the largest phosphate solubilization zones across all salt concentrations, highlighting them as potentially salt-tolerant PGPR candidates. Isolates SR12-1 and SR16-1 showed the least solubilization under all conditions, suggesting poor adaptability to salt stress. The results highlighted the variability in phosphate solubilization among PGPR isolates under salt stress. Isolates such as G12-1 and SD10-2 were promising candidates for saline soil applications, whereas others, such as SR12-1 and SR16-1, might not be suitable for high-salinity environments. This information was critical for selecting PGPR strains for agricultural use in salt-affected areas.

Potassium solubilisation

All isolates exhibited reduced efficiency at 0 mM, reinforcing the idea that a certain level of salinity was necessary for optimal solubilization activity. Isolates SD12-2 and SD10-2 demonstrated the highest KE at 150 mM (Figure 2), indicating their strong potential for potassium solubilization in saline conditions. The isolates showed varying degrees of salt dependency. Most isolates exhibited minimal KE at 0 mM, suggesting that salt was essential for maximum solubilization efficiency. Isolates G8-4, G8-5, and G10-6 showed a significant increase in KE as the salt concentration rose, with peak values at 150 mM. Isolate SD10-2 maintained a high KE across all salt concentrations but performed best at 150 mM. Isolate SR10-3 and SR8-4 displayed moderate increase in KE with rising salt concentration, following similar peak patterns at 150 mM. In contrast, isolates SD16-2 and SR12-1 exhibited lower KE across all concentrations compared to other isolates.

Indole acetic acid production

The bar graph (Figure 3) illustrated the production of indole acetic acid (IAA) in various isolates at different salt concentrations. The tested concentrations were 0 mM,

50 mM, 100 mM, and 150 mM NaCl. Data indicated IAA production in micrograms per milliliter (µg ml^{-1}). IAA production across the isolates generally increased with higher salt concentrations, peaking at 150 mM. The 0 mM salt concentration typically resulted in lower IAA production, implying that salt concentration had a significant effect on the metabolic activity of the isolates. Isolates such as SD10-3 and SD12-2 exhibited moderate IAA production at 150 mM, while others, like G8-4 and G8-5, maintained relatively consistent levels across different concentrations, with less variation. The isolate SR16-1 displayed the highest IAA production at 150 mM salt concentration, reaching approximately 170 µg ml^{-1}. This suggested that isolate SR16-1 was highly responsive to higher salt levels and could be a candidate for optimal IAA production. Isolate SR16-1 stood out due to its high IAA production at this concentration, making it a potential candidate for further research or practical applications in plant growth-promoting scenarios.

Ammonia production

As shown in Figure 5, at 150 mMNaCl concentration (represented by purple bars), ammonia production tended to be the highest, with several isolates exhibiting elevated ammonia production, particularly isolate G8-4. Isolate G8-4 produced the highest ammonia levels (approximately 14 ppm) at 150 mM NaCl and consistently outperformed other isolates across different salt concentrations. Isolate G8-5 also produced a considerable amount of ammonia at 150 m M NaCl, though at a much lower level than G8-4. At 0 mM NaCl concentration (represented by red bars), ammonia production was the lowest across most isolates, suggesting that higher salt levels were potentially beneficial for ammonia production in certain isolates. For isolates G12-1, SD12-2, and SR10-3, ammonia production significantly increased as NaCl concentration rose, particularly at 100 mM and 150 mM. Isolate G8-4 was the most efficient ammonia producer, especially at 150 mMNaCl, followed by G8-5. The results indicated that NaCl concentration had a noticeable effect on ammonia production, with some isolates benefiting from higher salt levels, while others showed no significant increase.

Cellulase production

For most isolates, the cellulolytic index increased as the NaCl concentration rose, with the highest activity generally observed at 150 mM NaCl. This trend suggested that several isolates could tolerate and maintain high cellulase production in saline environments. Isolates G10-6, G12-1, SR10-3, and SR12-1 exhibited the highest cellulolytic indices, with CI values close to or exceeding 10 at 150 mM. These isolates were highly effective cellulase producers under saline conditions, making them suitable candidates for applications in salt-affected environments.

Isolates such as G8-4, G8-5, and SR8-4 showed moderate cellulolytic activity, with CI values peaking between 6 and 8 at higher NaCl concentrations. While not as efficient as the top performers, these isolates still exhibited resilience in saline conditions. Isolate SR16-1 consistently demonstrated the lowest CI values, barely exceeding 1, regardless of the NaCl concentration. This indicated weak cellulase activity and limited adaptability to salt stress. The high CI values at 150 mM NaCl for most of the isolates suggested that many PGPR strains were capable of thriving and producing cellulase in saline conditions, which was beneficial for agricultural applications in salt-affected soils. Isolates G10-6, G12-1, SR10-3, and SR12-1 were strong candidates for biotechnological applications due to their robust cellulase production under high NaCl concentrations. Overall, the study highlighted the potential of certain PGPR isolates for use in saline environments, with clear differences in their cellulase production capabilities

The results demonstrated that several PGPR strains exhibited salt tolerance, which was particularly evident in their ability to perform key functions like phosphate and potassium solubilization, IAA production, ammonia production, and cellulase activity under saline conditions. This aligns with the findings of Sunita *et al*. (2020), who reported that halotolerant PGPR isolates, when exposed to saline conditions, showed enhanced solubilization of phosphorus and potassium, further promoting plant growth under osmotic stress. The performance of these isolates varied across different salt concentrations, with certain strains, such as G12-1 and SD10-2, showing high functionality at elevated salt levels. This suggests that these isolates can thrive in saline environments, making them suitable candidates for agricultural applications in salt-affected soils.Phosphate solubilization activity increased in most isolates under saline conditions, which is consistent with previous studies that have reported enhanced solubilization in response to osmotic stress. Isolates like G12-1 and SD10-2 consistently demonstrated high solubilization across all salt concentrations, suggesting their robustness in promoting nutrient availability in saline soils. Similar results were observed by Khoso *et al*. (2024), who found that salt-tolerant PGPR strains exhibited enhanced phosphate solubilization under saline stress, improving nutrient cycling in soils with high salt content. Similarly, potassium solubilization was more efficient in the presence of salt, and strains like SD12-2 and SD10-2 were highly effective at higher NaCl concentrations. This highlights the essential role of these PGPR in improving soil fertility and plant nutrient uptake under saline stress, as reported by Meena *et al*. (2015), who identified several PGPR capable of solubilizing potassium from mineral sources, enhancing plant growth under saline conditions.The production of IAA, a key plant growth regulator, was enhanced at higher salt concentrations, which suggests that PGPR may help plants cope with salinity stress by promoting root development and improving nutrient uptake. This pattern aligns with the idea that PGPR could alleviate salinity-induced growth inhibition in plants by stimulating root elongation through IAA production. Glick (1995) also found that PGPR strains could promote plant growth by enhancing IAA production in saline environments, which helps plants tolerate osmotic stress and facilitates better root system development. Strains

like SR16-1 showed high IAA production at 150 mM NaCl, could be further explored for their potential in improving plant growth in salt-stressed environments. This finding mirrors Kour *et al*. (2023), who observed that certain PGPR isolates produced higher IAA levels in saline conditions, contributing to improved plant health and growth under stress.Ammonia production by PGPR isolates plays an important role in soil nitrogen cycling, enhancing nutrient availability for plants. The results showed that ammonia production was consistent among several isolates, indicating their potential as biofertilizers. This is consistent with Vessey (2003), who reported that PGPR strains capable of ammonia production contribute significantly to nitrogen fixation in soil, thereby improving plant nutrition. Cellulase production was another key feature observed in the study, with many isolates demonstrating high cellulolytic activity under saline conditions. These isolates, such as G10-6 and SR10-3, could be particularly useful in improving soil structure and promoting plant growth by decomposing organic matter in saline soils. The cellulase activity in these isolates is supported by the findings of Choi *et al*. (2005), who showed that cellulase-producing PGPR could break down cellulose in the rhizosphere, thus improving soil texture and enhancing nutrient availability.The ability of PGPR to thrive under high salt concentrations is crucial for their application in saline soils. Our findings identified several promising PGPR strains, such as G12-1, SD10-2, and SR16-1 exhibited superior salt tolerance across multiple functional assays. These isolates could be used as bioinoculants for improving crop productivity and soil health in salt-affected regions. This is supported by Shrivastava and Kumar (2014), who emphasized the potential of using halotolerant PGPR strains to mitigate the negative effects of salinity on crop production. Additionally, Kour *et al*. (2023) highlighted that PGPR capable of surviving in saline environments could be effectively used to promote the growth of crops in regions where salinity is a major limiting factor.

This study highlights the potential of salt-tolerant plant growth-promoting rhizobacteria (PGPR) for enhancing agricultural productivity in saline soils. The isolation of 23 salt-tolerant PGPR strains from diverse saline environments demonstrated the capacity of these bacteria to perform essential functions such as phosphate and potassium solubilization, IAA production, ammonia production, and cellulase activity under saline stress. Strains like G121, SD102, and SR16-1 showed consistent high performance across various functional assays, represent promising candidates for further development as bioinoculants in saline and sodic soils. These PGPR can help mitigate the negative impacts of salinity on crop growth by improving nutrient availability and promoting plant resilience (Cordero *et al*., 2023). The findings indicate that salinity stress influences PGPR activities, with varying responses observed among different isolates. Future research should focus on understanding the molecular mechanisms underlying the salt tolerance of these isolates and evaluating their field performance to optimize their use in sustainable agriculture.

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.	Udgaon	Brown	Loamy	7.30	2.7
2.	Chinchwad	Black	Clayey	7.22	3.1
3.	Arjunwadi,	Black	Clayey	7.30	1.8
4.	Ganeshwadi	Red	Clayey	7.00	11.0
5.	Ghalwad	Black	Clayey	7.20	0.5
6.	Shirti	Black	Clayey	7.65	0.9
7.	Shedshal	Black	Clayey	7.18	5.2
8.	Shirol	Brown	Loamy	5.16	15.0
9.	Akiwat	Brown	Sandy	7.30	1.3
10.	Majrewadi	Red	Loamy	7.00	2.0
11.	Shirdhon	Brown	Clayey	8.12	2.7

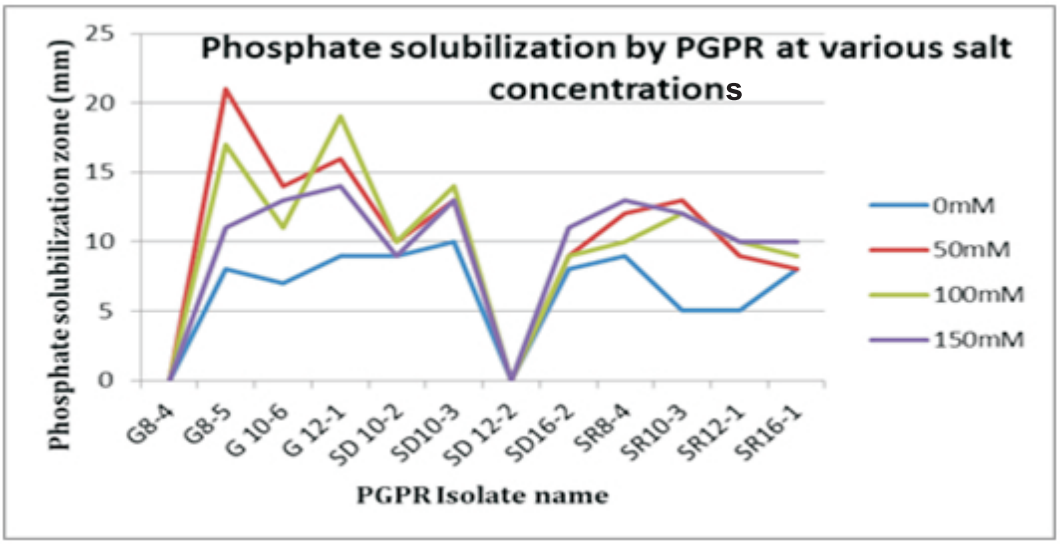

Figure 1. Phosphate solubilisation by PGPR at various NaC1 concentrations

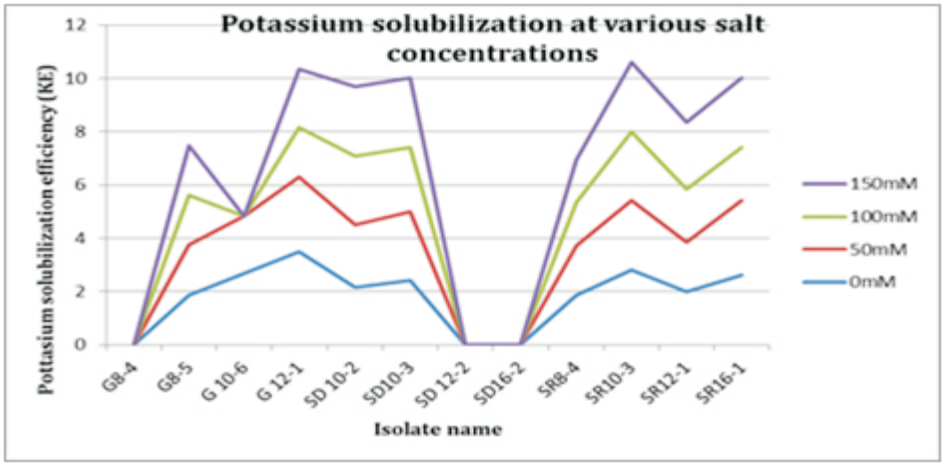

Figure 2. Potassium solubilisation by PGPR at various NaC1 concentrations

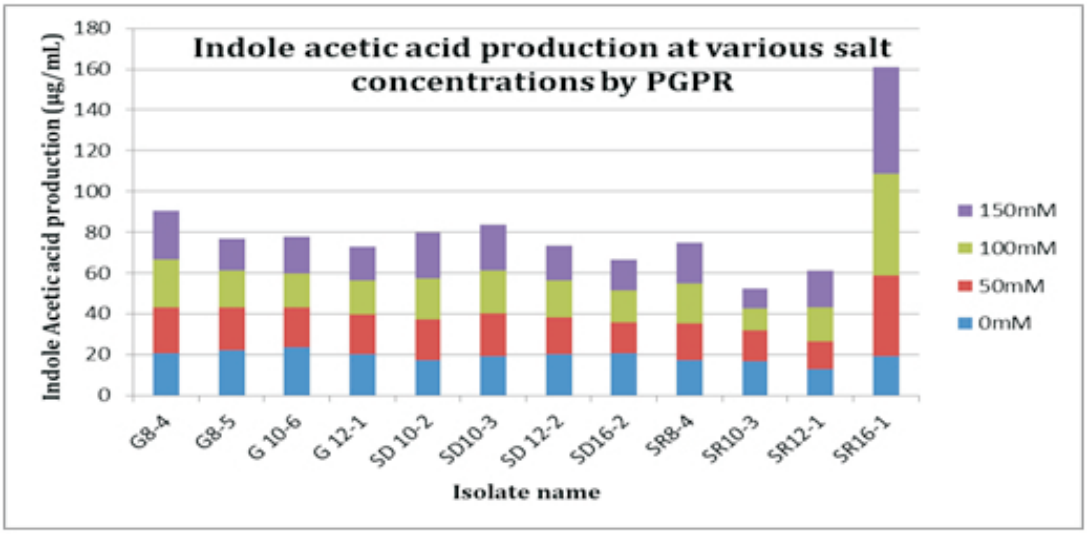

Figure 3. IAA production by PGPR at various NaC1 concentrations

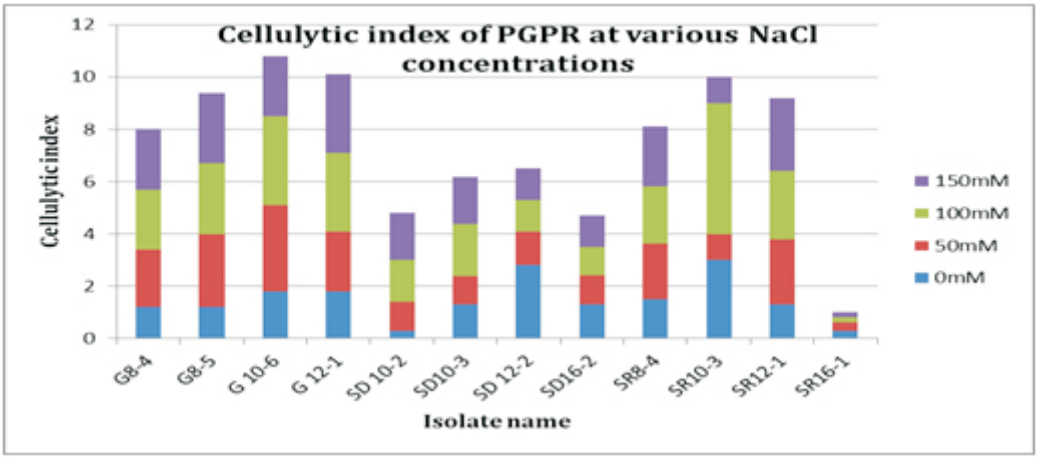

Figure 3. Cellulytic index of PGPR at various NaC1 concentrations

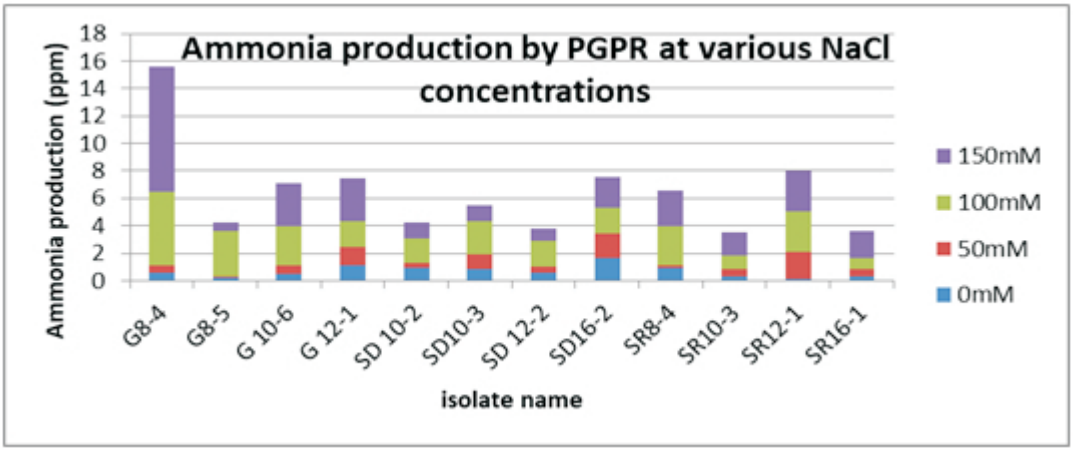

Figure 5. Ammonia production by PGPR at various NaC1 concentrations

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