BIOPROSPECTING PLANT GROWTH PROMOTING RHIZOBACTERIA ISOLATED FROM *TRIFOLIUM ALEXANDRINUM*

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ABSTRACT

Numerous studies have reported enhanced health and productivity in various plant species through the use of plant growth-promoting rhizobacteria (PGPRs), both under standard conditions and in response to stress. In the present study, 10 PGPRs were isolated from *Trifolium alexandrinum* (Berseem). The isolates were identified through morphology and biochemical testing, and their plant growth-promoting abilities were evaluated. Subsequently, functional analysis was performed on all isolates. Among the 10 isolates, 5 tested positive for Phosphate solubilization, while all were positive for Indole-3-acetic acid (IAA) and Rhamnolipid production. Following the functional evaluation, the selected isolates and consortia were inoculated into pots containing tomato seeds. By the pot culture, isolate S2B2 (*Azospirillum sp.*) showed the highest bioprospecting potential under normal and drought conditions by significantly enhancing ($p<0.05$) shoot length and leaf number. Under saline conditions, *Azospirillum sp.* demonstrated the best performance at the lowest salt concentration (50 mM) with a shoot length of 8.1 cm. This study provides insights into the mechanism of action of PGPRs. It highlights the potential of *Azospirillum sp.* as a promising bio-inoculant for improving tomato plant growth and stress resilience, particularly under normal and drought conditions.

Keywords: Bioprospecting Ability, Indole-3-acetic acid (IAA), Plant Growth-Promoting Rhizobacteria (PGPR), Phosphate Solubilization, Rhamnolipid Synthesis

INTRODUCTION

Plant Growth-Promoting Rhizobacteria (PGPRs) are beneficial microbes that inhabit the rhizosphere. PGPRs stimulates plant growth and health through nutrient acquisition, hormone production, biocontrol of pathogens, and soil improvement[1]. The dominant species found in rhizospheres are microbes from the genus *Azotobacter, Alcaligenes, Arthrobacter, Azospirillum, Rhizobium, Pseudomonas, Bacillus, Serratia, Flavobacterium, Acinetobacter*[2][3]. Genera like *Azotobacter*, *Alcaligenes*, *Arthrobacter*, *Azospirillum*, *Rhizobium*, *Pseudomonas*, *Bacillus*, *Serratia*, *Flavobacterium*, and *Acinetobacter* each offer unique benefits in agriculture. *Azotobacter* and *Azospirillum* fix nitrogen, improving soil fertility and supporting plant growth. *Rhizobium* enhances nitrogen fixation in legumes, while *Pseudomonas* and *Bacillus* provide biocontrol against plant pathogens and promote growth. *Arthrobacter* and *Flavobacterium* help in phosphorus solubilization and organic matter breakdown, improving soil health. *Serratia* and *Acinetobacter* contribute to pathogen suppression and bioremediation, aiding in the cleanup of soil contaminants. Together, these PGPR species enhance plant health, increase yields, and support sustainable farming practices. The mechanism of action of Plant Growth-Promoting Rhizobacteria (PGPRs) involves several processes that promote plant growth and health. PGPRs can solubilize phosphate, an important nutrient for plant growth[4]. PGPRs can produce plant growth hormones such as auxins and cytokinins, which stimulate plant growth and development. PGPRs can protect plants against harmful pathogens by producing antibiotics and competing for resources with pathogenic microorganisms[5]. PGPRs can help plants tolerate drought by increasing the water-use efficiency and reducing water loss through transpiration. Some PGPRs can fix atmospheric nitrogen into a form that can be used by plants, which helps to reduce the need for synthetic fertilizers. PGPRs can produce enzymes that break down organic matter in the soil, making nutrients available for plant uptake. PGPRs stimulate the plant's defense system, inducing a state of systemic resistance. This makes the plant more resistant to disease and stress. Some PGPRs can produce exopolysaccharides (EPS), which improve soil structure and stability. This can enhance soil water-holding capacity, nutrient availability, and aeration, all of which promote plant growth[6][7][8][9]. PGPRs have gained worldwide importance and acceptance for sustainable agricultural practices. The continuous use of chemicals or synthetic fertilizers reduces soil fertility in the long run and the use of intensive agricultural practices to increase crop yield decreases soil fertility and

also has a negative impact on the environment. The use of PGPRs has risen globally over the past few decades[1]. Continued research on PGPRs is necessary to fully realize their potential as a tool for sustainable agriculture and to identify the most effective strains for specific crops and environmental conditions, leading to more targeted and efficient use of PGPRs in agriculture.

Plant Growth-Promoting Rhizobacteria (PGPR) are gaining popularity in agriculture for their ability to enhance crop growth and reduce the need for chemical inputs. They act as biofertilizers by improving soil health, promoting nitrogen fixation, and making phosphorus more available to plants. PGPR also help protect plants from soilborne diseases by outcompeting harmful pathogens. In addition, they boost plant tolerance to environmental stresses like drought and high salinity, which is increasingly important in regions affected by climate change. These beneficial bacteria are used in seed treatments and inoculants for a variety of crops, such as corn, soybeans, and vegetables. PGPR are becoming a key component of both conventional and organic farming practices, as well as in urban farming and greenhouse production, supporting more sustainable agricultural methods. Berseem (*Trifolium alexandrinum*) an important forage crop in regions with nutrient-deficient soils, was selected for this study due to its natural association with diverse rhizospheric microorganisms, its high nitrogen-fixing potential, and its role in improving soil fertility. Its natural partnership with soil microorganisms makes it an excellent model for studying the effects of Plant Growth-Promoting Rhizobacteria (PGPR) on growth, nutrient absorption, and stress resilience. This research aims to explore how PGPR can improve the efficiency and sustainability of agricultural practices, offering an eco-friendlier approach to crop management. The present study is aimed to isolate and characterize effective PGPRs from *Trifolium alexandrinum* (Berseem). Functional characterization was done by Orcinol, Gordon & Weber, and Pikovaskya's method for rhamnolipid, IAA, and phosphate solubilization, respectively. Further, their efficacy is determined using the pot culture method.

METHODOLOGY

Collection of soil samples

Rhizospheric soil and root samples were collected aseptically in sterile plastic bags from *Trifolium alexandrinum* (Berseem) field soil (*Figure 1*).

Figure 1 : Site of Collection of Soil Samples

Physical characterization of soil

The various physical characteristics of soil viz., temperature, pH, and moisture content were studied to know the optimum growing conditions of rhizospheric bacteria. Temperature is determined to characterize whether the bacterium belongs to a psychrophilic, mesophilic, or thermophilic group, and pH is to characterize whether the bacteria are acidophilic, neutrophilic, or alkalophilic. Using a knife, the pit was dug, the thermometer was inserted and kept for 15 minutes and the temperature was recorded. The procedure was repeated at several sites. 10 gm rhizospheric soil was suspended in 90 ml distilled water. Suspension was kept in a shaker incubator for 1 hour and pH was measured. 100 gm of rhizospheric soil was dried under open shade till dry. The dried soil was measured and moisture content was calculated using the following equation:

$$Water\ (\%)\ by\ mass = \frac{Wet\ weight - Dry\ weight}{Dry\ weight}\ X\ 100$$

Equation 1 : Moisture Content by Mass

Isolation and enumeration of rhizospheric bacteria from the soil sample

The Rhizospheric soil was carefully separated from the roots of Berseem using a brush in a Petri dish. Suspension was prepared by adding 10 gm rhizospheric soil to 90 ml normal saline buffer (NSB; 0.85%) and was placed on a shaker incubator for 1 hour. The suspension (1.0 ml) was then aseptically transferred to test tube 1 containing 9 ml pre-sterilized NSB to a dilution of $1X10^{-2}$. From test tube 1, likewise, 10^{-3} to 10^{-8} were prepared. 1.0 ml suspension was transferred to labelled sterile Petri plates and

simultaneously molten nutrient agar was added and distributed uniformly. Plates were prepared in duplicates and incubated at 37±2°C for 24 to 48 hours. Morphologically distinct colonies obtained from pour plating were picked and re-streaked on fresh nutrient agar plates. The plates were then incubated at 37±2°C for 24 to 48 hours.Isolated colonies were transferred to pre-sterilized NB tubes and incubated at 37±2°C for 24 to 48 hours. 0.5 ml overnight culture and 0.5 ml sterilized glycerol was added to 2 ml cryovials and stored at -20°C.

Functional analysis of the isolates

Phosphate solubilization

Although phosphorus levels in soil are generally high, most of it is in an insoluble form, rendering it unavailable for plant growth. Soil bacteria synthesize low molecular weight organic acids like gluconic and citric acid, which help solubilize inorganic phosphorus. Meanwhile, the breakdown of organic phosphorus into usable forms occurs through various phosphatases that facilitate the hydrolysis of phosphoric esters. Isolates were spot inoculated on Pikovaskya's agar and plates were incubated at 37±2°C for 96 to 120 hours. Isolates exhibiting clear zones were considered positive[10].The Phosphate solubilization index (PSI) was calculated by the following formula:

$$PSI = \frac{Total\ Diameter\ (colony + clear\ zone)}{colony\ diameter}$$

Equation 2 : Phosphate Solubilization Index (PSI)

Indole acetic acid production

The isolates were introduced into nutrient broth and incubated at a temperature of 37±2°C for 5 to 7 days. The broth-containing cultures were transferred to ten ml Falcon tubes were used for centrifugation at 3000 rpm for 30 minutes. The pellets were removed, and to the 5 ml of supernatant, 2 drops of orthophosphoric acid and 2 ml of Salkowski's reagent were added. Absorbance was taken against blank at 540 nm[11].

Rhamnolipid production

Rhamnolipids are a class of glycolipids, best characterized as bacterial surfactants. Acid hydrolysis of rhamnolipid releases rhamnose moiety which in the presence of a strong acid, undergoes dehydration, resulting in the formation of furfural. Orcinol reacts with furfural in the presence of ferric chloride catalyst, producing a green compound that exhibits an absorbance peak at 665 nm. Recovered isolates were inoculated in nutrient

broth and incubated at 37±2ºC for 5-7 days. The broth-containing cultures were placed into 10 ml Falcon tubes and centrifuged at 3000 rpm for 30 minutes. Pellets were discarded, and to 9 ml supernatant, 1 ml Orcinol reagent was added. The samples were then kept in a boiling water bath for 30 minutes. Tubes were cooled and absorbance was taken against blank at 665 nm[12].

Characterization of isolates

The morphological features of the isolates were examined, including colony characteristics such as colour, shape, margin, elevation, and surface, as well as cell morphology, which encompasses shape, Gram reaction, and arrangement. The isolates were biochemically characterized using various tests viz., triple sugar iron test, IMVIC test, nitrate reduction test, oxidase test, and catalase test.

Pot culture

The pot culture method is useful for studying the efficacy of PGPRs on a small scale. The growth-promoting activities of recovered isolates were tested for seed germination and seedlings by treating soil with overnight cultures of recovered isolates. In the procedure, for normal conditions, 100 grams of soil were placed in a pot with 5 tomato seeds sown as a control, while in saline conditions; salt was added in concentrations of 50mM, 100mM, 150mM, and 200mM to the same setup. For drought conditions, the same soil and seed setup was used, with watering adjusted to every 3rd day. Broth cultures of inoculum S1B2, S2B2, and consortia were added to respective setups. All setups were prepared in triplicates. Shoot length and leaf count were measured every 5th day across all conditions. The pots were maintained in the open shade at 27-30ºC and facilitated with a water supply. Growth was observed and shoot lengths were measured on the 5th, 10th, 15th, and 20th days.

STATISTICAL ANALYSIS

All experiments were performed in triplicate, and results are expressed as Mean ± SD. A one-way Analysis of Variance (ANOVA) was used to determine the statistical significance ($P < 0.05$) of differences between treated and control groups. Data from different experimental conditions (normal, drought, and saline) were separately analyzed to assess the efficacy of PGPR treatments. Functional elucidation of recovered isolates (Table 2), Indicates statistically significant values by ANOVA ($p < 0.05$) and Bioprospecting ability of the selected isolates by measuring shoot length (cm) and number of leaves (Table 3), Indicates statistically significant values by ANOVA ($p < 0.05$)

RESULTS AND DISCUSSION

Determination of physical characteristics of soil

As documented by Dastogeer et al.[13] a gradual increase in temperature, pH, and decline in moisture content may selectively influence the diversity of rhizospheric bacteria.The physical characteristics of soil are reported in Table 1. Mesophilic temperatures support the metabolic activities of PGPR, enabling efficient nutrient solubilization, production of plant growth regulators, and biocontrol against phytopathogens. Neutral pH conditions foster the proliferation of diverse PGPR populations, enhancing their functional diversity and plant growth-promoting capabilities. Adequate moisture content ensures the viability and activity of PGPR in the rhizosphere, facilitating root colonization, nutrient acquisition, and plant-microbe signaling. Understanding the physicochemical parameters of the environment is crucial for elucidating the interactions between PGPR and their surrounding ecosystem. Mesophilic temperatures, neutralophilic pH, and optimal moisture levels create a conducive rhizospheric environment for PGPR colonization, growth promotion, and nutrient cycling.

Parameters	Value	Classification
Temperature	25.0±1.7°C	Mesophilic
pH	7.50±0.92	Neutralophilic
Moisture (%)	21.5%	-

Values expressed are mean ± SD values

Table 1 : Physical characteristics of soil

Functional elucidation of recovered isolates

It is important to understand how rhizobacteria exert their beneficial effect on plants. To determine the potentiality of recovered rhizobacteria to be used as bioinoculants, various functional characteristics viz., production of IAA, rhamnolipid, and phosphorus solubilization were studied. The results of functional elucidation of the isolates are presented in Table 2. Phosphate solubilization was documented to be a major characteristic of PGPRs[1]. Isolates S1B2, S2B2, S2B3, and S3B3 exhibited phosphate solubilization capabilities, as evidenced by the formation of clear zones around the colonies on the agar plates, with PSI 4.07±0.29, 4.94±0.21, 2.73±0.19, and 2.82±0.37, respectively. Isolates S1B1, S1B3, S1B4, S3B1, and S3B2, on the other hand, did not display phosphate solubilization activity. Phosphate solubilization is a crucial trait for enhancing plant nutrient uptake and isolates exhibiting this ability, such as S1B2, S2B2, S2B3, and S3B3, hold the potential for improving soil fertility and plant growth. Several

researches suggest the production of phytohormone-like acting compounds involved in the phytostimulatory action exerted by the plant-beneficial rhizobacterium species like *Bacillus*, and *Pseudomonas*[14]. In the study, 5-day-old cultures of recovered isolates were tested using the colorimetric method of Gordon and Weber[11]. Almost all the recovered isolates tested positive for indole-3-acetic acid (IAA), as indicated by the absorbance values at 540 nm, similar to the results documented by Glick[8]. Among them, S2B2 exhibited the highest IAA production with an average absorbance of 0.562. Indole-3-acetic acid (IAA) is a key phytohormone involved in plant growth and development, and its production by certain rhizobacteria can promote root growth and nutrient uptake. Isolates S1B2, S2B1, S2B2, S3B2, and S3B3 demonstrated significant IAA production, suggesting their potential to enhance plant growth and Vigor. Rhamnolipid, a rhamnose-containing glycolipids biosurfactant has a detergent-like structure and is believed to solubilize the phospholipids and thus help in the survival of bacterium especially against zoosporic fungi. In the present study, recovered isolates were analyzed using the Orcinol method and 100 % of isolates displayed rhamnolipid production, with varying degrees of activity. Isolate S2B2 exhibited the highest rhamnolipid production with an average absorbance of 0.366, followed by S1B2 (0.320) and S2B1 (0.313). Rhamnolipids are biosurfactants produced by various microorganisms, including some plant growth-promoting bacteria. These compounds play important roles in enhancing soil aggregation, nutrient availability, and plant-microbe interactions. Isolate S2B2 exhibited the highest rhamnolipid production among the tested isolates, indicating its potential for improving soil health and plant performance. Overall, the findings highlight the diverse functional traits present among the tested rhizobacterial isolates, which can be exploited for sustainable agriculture practices aimed at enhancing crop productivity and soil fertility. Further investigations into the molecular mechanisms underlying these traits and their interactions with host plants are warranted to fully harness their potential in agricultural applications.

Isolates	Phosphate Solubilization		IAA (540 nm)	Rhamnolipid (665 nm)
		PSI (Phosphate Solubilization Index)		
S1B1	-	-	0.109±0.030	0.132±0.080
S1B2	+	4.07±0.29*	0.378±0.010	0.320±0.050
S1B3	-	-	0.069±0.060	0.223±0.030
S1B4	-	-	0.080±0.020	0.310±0.010
S2B1	+	1.46±0.15	0.224±0.020	0.313±0.020
S2B2	+	4.94±0.21*	0.562±0.020*	0.366±0.010*
S2B3	+	2.73±0.19	0.092±0.040	0.80±0.08*

Isolates	Phosphate Solubilization		IAA (540 nm)	Rhamnolipid (665 nm)
		PSI (Phosphate Solubilization Index)		
S3B1	-	-	0.133±0.070	0.72±0.02*
S3B2	-	-	0.257±0.090	0.80±0.03*
S3B3	+	2.82±0.37	0.272±0.010	0.161±0.020

** Indicates statistically significant values by ANOVA ($p < 0.05$)*

Table 2 : Functional elucidation of recovered isolates

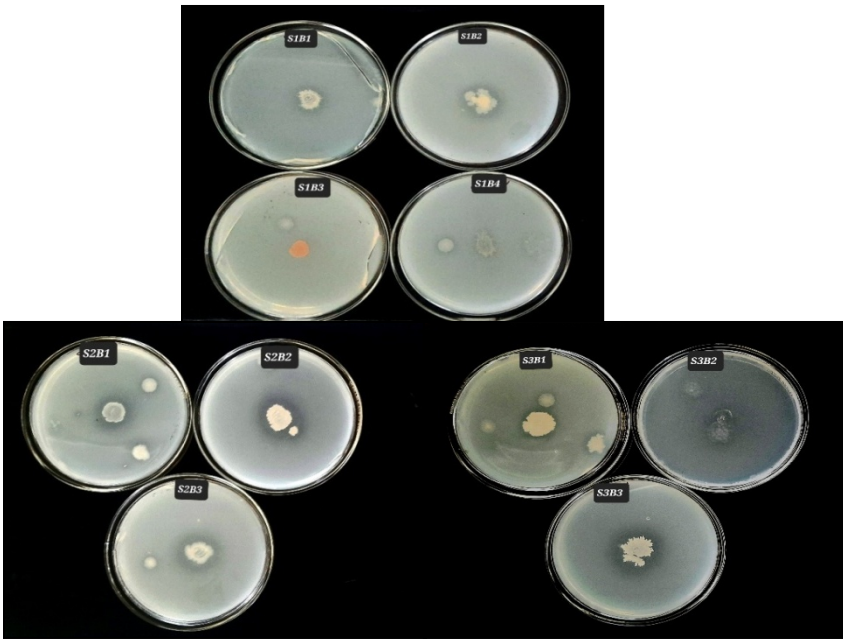


Figure 2 :Phosphate solubilization test

Characterization of isolates

Isolates were characterized based on morphological and biochemical tests[15]. The morphological characterization of the isolates involved examining both colony and cell morphology. For colony morphology, the isolates were assessed based on colour, shape, margin, elevation, and surface characteristics. Isolate S1B2 presented with a creamy white colony colour, circular shape, entire margin, convex elevation, and a smooth surface. Isolate S2B2 displayed a pale-yellow colony colour, irregular shape, undulate margin, flat elevation, and a rough surface. For cell morphology, the isolates were studied under a microscope. Isolate S1B2 was rod-shaped, Gram-negative, and typically arranged in single or paired cells. Isolate S2B2 exhibited a spiral shape, and Gram-negative reaction, and cells were observed in scattered arrangements. Various biochemical tests were conducted to characterize the isolates. Isolate S1B2 tested positive in the triple sugar iron (TSI) test, indicating its ability to ferment glucose, lactose,

and sucrose with gas production. It was also positive in the IMViC test (Indole, Methyl Red, Voges-Proskauer, and Citrate utilization tests), suggesting the presence of tryptophanase, stable acid production, acetoin production, and citrate utilization. Isolate S1B2 was positive for nitrate reduction, oxidase, and catalase tests. Based on these morphological and biochemical characteristics, Isolate S1B2 was identified as *Rhizobium sp*. Isolate S2B2 tested positive for the TSI test, indicating its ability to ferment the same sugars without gas production. It was positive for the Indole test but negative for Methyl Red, Voges-Proskauer, and Citrate tests, suggesting different metabolic capabilities compared to isolate S1B2. Isolate S2B2 was also positive for nitrate reduction, oxidase, and catalase tests. Based on these results, Isolate S2B2 was identified as *Azospirillum* sp. The identification of *Rhizobium* and *Azospirillum* through detailed morphological and biochemical characterization aligns with their known plant growth-promoting traits. Rhizobium is well-documented for its nitrogen-fixing capabilities in leguminous plants[16], while *Azospirillum* is known for its associative nitrogen fixation and production of growth hormones such as Indole-3-acetic acid (IAA)[17].

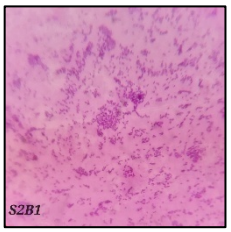
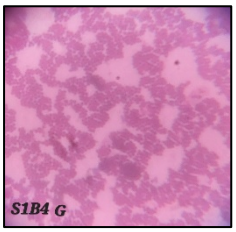
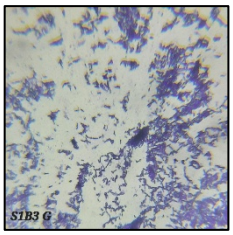


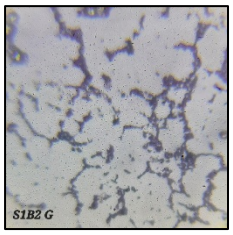
Figure a : - Microscopic View of Isolate S1B1, Gram +ve Rods



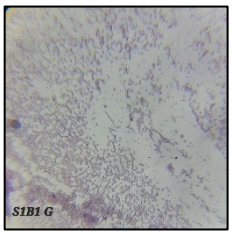
(b) : Microscopic View of Isolate S1B1, Gram +ve Rods

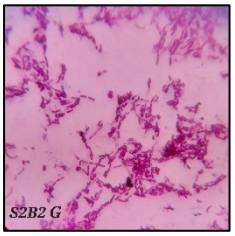


(c): Microscopic View of Isolate S1B3, Gram +ve Cocci

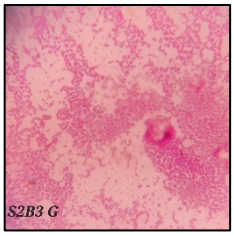


(d) : Microscopic View of Isolate S1B4, Gram -ve Cocci

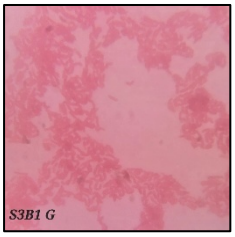




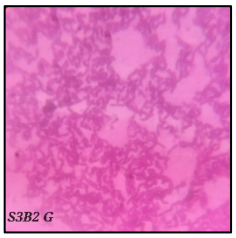
(e) Microscopic View of Isolate S2B1, Gram -ve Cocci



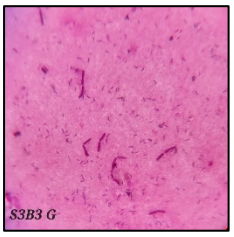
(f) : Microscopic View of Isolate S2B2, Gram -ve Rods



(g) : Microscopic View of Isolate S2B3, Gram -ve Cocci



(h) : Microscopic View of Isolate S3B1, Gram -ve Rods



(i) : Microscopic View of Isolate S3B2, Gram -ve Rods

(j) : Microscopic View of Isolate S3B3, Gram -ve Rods

Figure 3 : Microscopic Views

Bioprospecting ability of the isolates on tomato plant growth

The bioprospecting ability of the two rhizobacteria on tomato plant growth under various conditions was assessed by measuring shoot length and the number of leaves over 20 days. The results are reported in Table 3. Under normal conditions, control showed moderate growth, with a maximum shoot length of 6.3 cm and one leaf by Day 20. Inoculum S1B2 demonstrated comparable growth with control, achieving a shoot length of 5.4 cm and maintaining three leaves. Inoculum S2B2 outperformed all, reaching 8.06 cm in shoot length and three leaves. The consortia exhibited the least growth under normal conditions, with a final shoot length of 4.3 cm and three leaves. Under drought conditions, the control demonstrated significant growth, reaching a shoot length of 7.8 cm and five leaves by Day 20. Inoculum S1B2 showed reduced growth compared to the control, with a shoot length of 7.1 cm and three leaves. Inoculum S2B2 achieved a higher shoot length of 8.4 cm and maintained seven leaves, indicating strong drought resilience. The consortia also performed well, with a shoot length of 7.3 cm and three leaves. In saline conditions, growth varied with salt concentration. At 50 mM, the control had a shoot length of 7.9 cm and two leaves. Inoculum S1B2 showed a shoot length of 6.4 cm and three leaves. Inoculum S2B2 demonstrated good growth with a shoot length of 8.1 cm and three leaves. The consortia had moderate growth with a shoot length of 5.1 cm and

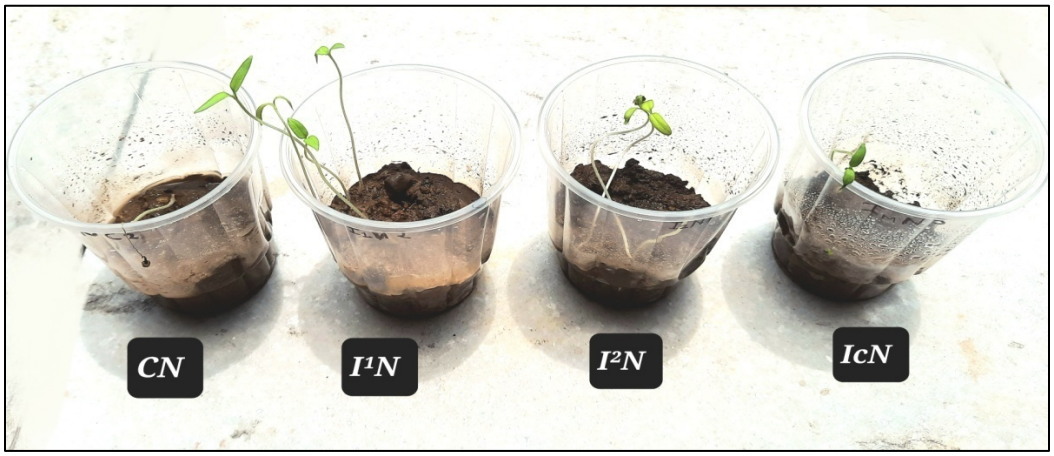
three leaves. At 100 mM, the control had a shoot length of 7.4 cm and two leaves. Inoculum S1B2 showed reduced growth with a shoot length of 1.6 cm and one leaf. Inoculum S2B2 showed better performance compared to S1B2, with a shoot length of 5.3 cm and one leaf. Higher salt concentrations (150 and 200 mM) significantly inhibited growth across all isolates, with minimal to no shoot length and leaf development observed, indicating severe stress impact. In conclusion, inoculum S2B2 showed the highest bioprospecting potential under normal and drought conditions by significantly enhancing shoot length and leaf number. Under saline conditions, S2B2 demonstrated the best performance at the lowest salt concentration with a shoot length of 8.1 cm. The superior performance of S2B2 (*Azospirillum sp*.) in promoting tomato plant growth, especially under saline and drought conditions, highlights its potential as a robust bio-inoculant. Its ability to enhance shoot length and leaf number suggests that *Azospirillum sp*. can be particularly effective in improving crop resilience to abiotic stress. Overall, this study demonstrates the significant bioprospecting potential of these rhizobacteria in sustainable agriculture, particularly in enhancing the growth and stress tolerance of tomato plants. Future research should focus on field trials and the molecular mechanisms underlying these beneficial interactions to fully harness their agricultural potential.

Isolates	Day 5		Day 10		Day 15		Day 20	
	L^S	L^N	L^S	L^N	L^S	L^N	L^S	L^N
C N	3.5±1.0	0	5.1±2.8	1.00±0.94	5.6±2.7	1.00±0.94	6.3±2.8	1.00±0.94
I^1 N	1.83±2.50	1.00±0.94	5.16±3.60	3.0±2.4	5.2±3.6	3.0±2.4	5.4±3.8	3.00±2.4
I^2 N	1.6±2.3	2±0	7.5±0.74*	3.0±0.94	7.5±0.7*	3.00±0.94	8.06±0.90*	3.00±0.94
I^c N	NG	0	3.3±2.4	3.0±2.4	3.4±2.5	3.0±2.4	4.3±3.0	3.0±2.4
C D	4.0±1.8	1.00±0.94	9.1±1.4*	5.0±0.94	9.2±1.3*	5.0±0.94	7.8±1.2*	5.00±0.94
I^1D	0.16±0.20	2.00±1.63	6.7±1.1	3.0±1.8	7.00±1.27	3.0±1.8	7.10±1.26*	3.0±1.8
I^2 D	4.5±0.4	3.0±0.8	6.7±0.9	7.0±0.9	6.9±0.9	7.0±0.9	8.40±0.94*	7.0±0.9
I^c D	1.50±2.12	1.00±0.94	6.9±1.5	3.0±0.9	7.1±1.3*	3.0±0.9	7.30±1.45*	3.0±0.9
C S50	NG	0	7.5*	2	7.8*	2	7.9*	2
C S100	NG	0	6.8	2	7.2*	2	7.4*	2
C S150	NG	0	NG	0	NG	0	NG	0

Isolates	Day 5		Day 10		Day 15		Day 20	
	L^S	L^N	L^S	L^N	L^S	L^N	L^S	L^N
C S200	NG	0	NG	0	NG	0	NG	0
I^1S50	NG	0	6.00±1.08	3.0±0.9	6.2±0.9	3.0±0.9	6.4±1.2	3.0±0.9
I^1S100	NG	0	1.5±2.1	1.00±0.94	1.5±2.1	1.00±0.94	1.60±2.35	1.00±0.94
I^1S150	NG	0	NG	0	NG	0	NG	0
I^1S200	NG	0	NG	0	NG	0	NG	0
I^2S50	NG	0	4.6±0.8	3.00±0.94	4.9±0.8	3.00±0.94	8.10±0.81*	3.00±0.94
I^2S100	NG	0	4.7±3.3	1.00±0.94	5.0±3.5	1.00±0.94	7.3±3.7*	1.00±0.94
I^2S150	NG	0	NG	0	NG	0	NG	0
I^2S200	NG	0	NG	0	NG	0	NG	0
I^c S50	1.83±1.40	0	3.6±3.5	3.00±0.94	4.5±3.5	3.00±0.94	5.1±3.0	3.00±0.94
I^c S100	NG	0	3.2±2.6	1.0±0.9	5.6±0.9	1.0±0.9	5.80±0.97	1.0±0.9
I^c S150	NG	0	NG	0	NG	0	NG	0
I^c S200	NG	0	NG	0	NG	0	NG	0

L^S : Length of Shoot, **L^N:** Number of Leaves, NG: No Growth, C: Control, N: Normal Conditions, S: Saline Conditions, D: Drought Conditions, S1B2: Inoculum 1, S2B2: Inoculum 2, I^c: Consortia Inoculum, S50, S100, S150, S200: Different concentrations of salt in mM.

Table 3 : Bioprospecting ability of the selected isolates by measuring shoot length (cm) and number of leaves



C: Control, N: Normal Conditions, I: Inoculum ie. 1 and 2, Ic: Consortium

Figure 4(a): Pots in normal conditions

C: Control, D: Drought Conditions, I: Inoculum ie. 1 and 2, Ic: Consortium

Figure 4(b): Pots in drought condition

Figure 4(c): Saline condition control pots

Figure 4(d): Inoculum 1 in different saline concentration

Figure 4(e): Inoculum 2 in different saline concentrations

Figure 4(f): Consortia in different saline conditions

C: Control, S: Saline Conditions in different concentrations ie. 50, 100, 150 and 200, I: Inoculum ie. 1 and 2, Ic: Consortium.

Figure 4 : These images are from day 20.

CONCLUSION

The present study has shed light on the mechanisms underlying the action of Plant Growth-Promoting Rhizobacteria (PGPR) and their potential as potent bioinoculants for enhancing tomato production. A number of studies have documented the increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions. In the present study, 10 isolates were recovered from rhizospheric soil from Trifolium Alexandrinum. Isolates were characterized based on Staining and biochemical tests. Out of 10 isolates, 1 was gram-Positive rod,2 were gram-positive cocci, 3 were gram-negative rods and 4 were gram-negative cocci. It is very important to understand how rhizobacteria exert their beneficial effect on plants. In order to determine the potentiality of recovered rhizobacters so that they can be used as bioinoculants, various functional characteristics viz., production of IAA, rhamnolipid and solubilization of phosphorus were studied. IAA and

rhamnolipid production were found to be the most prominent characteristic among the recovered isolates.

Phosphate solubilization was a major characteristic of PGPRs. However, in the present study out of 10 isolates, 5 isolates (S1B2, S2B1, S2B2, S2B3 and S3B3) were found positive. In the present study, 5 days old cultures of recovered isolates were tested using colorimetric method. Almost all the recovered isolates were tested positive for the presence of indole-3-acetic acid (IAA). It's absorbance at 540 nm was recorded between

0.069(S1B3) to 0.562 (S1B1). Rhamnolipid, a rhamnose- containing glycolipids biosurfactant, has a detergent like structure and is believed to solubilize the phospholipids and thus helps in survival of bacterium especially against zoosporic fungi. In the present study, recovered isolates were analysed using Orcinol method and 100 % of isolates were found positive for the secretion of rhamnolipid.

In the study of pot culture, Isolate 2 (S2B2) exhibited enhanced shoot growth under normal conditions and drought conditions until the 20^{th} day. Isolate 2(S2B2) also demonstrated improved shoot growth in saline conditions up to a concentration of 100, accompanied by an increased number of leaves. On the other hand, the consortium exhibited a growth of 4.3 ± 3.0 until the 20th day under normal conditions but did not show growth enhancement surpassing that of Isolate 2(S2B2).

Although this study characterized isolates biochemically, future work should involve genetic characterization using molecular techniques such as 16S rRNA sequencing to validate the identity and explore the phylogenetic relationships of the isolates. To fully capitalize on this potential, it is imperative to delve deeper into several aspects. Firstly, exploring the genetic diversity of recovered isolates and investigating the kinetics of effective PGPRs would offer a more comprehensive understanding of their mechanisms. Additionally, research focusing on elucidating the key molecular and physiological processes involved in PGPR-mediated plant growth promotion could pave the way for the development of more efficacious PGPR-based products. Further, evaluating PGPR performance under real-world field conditions is essential for practical application, helping to optimize application methods, dosage rates, and timing. Moreover, assessing the associated risks and developing best practices for safe and responsible PGPR use is crucial for sustainable agriculture. Finally, investigating the integration of PGPRs with other agricultural practices, such as crop rotation and integrated pest management, could offer holistic approaches to enhance crop productivity and sustainability. In conclusion,

continued research efforts in these areas hold promise for maximizing the benefits of PGPRs in agriculture.

REFERENCES

[1] Gamalero, E. and Glick, B.R. 2011. Mechanisms used by plant growth-promoting bacteria. Bacteria in Agrobiology: Plant Nutrient Management. 17-46. https://doi.org/10.1007/978-3-642-21061-7_2

[2] Ahemad, M. and Kibret, M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. Journal of King Saud University-Science.26(1):1-20. https://doi.org/10.1016/j.jksus.2013.05.001

[3] Singh, A., Yadav, V.K., Chundawat, R.S., Soltane, R., Awwad, N.S., Ibrahium, H.A., Yadav, K.K., and Vicas, S.I. 2023. Enhancing plant growth promoting rhizobacterial activities through consortium exposure: a review. Frontiers in Bioengineering and Biotechnology. 11:1099999. https://doi.org/10.3389/fbioe.2023.1099999

[4] Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., and Singh, V. 2015. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J MicrobBiochem Technol. 7(2):96-102. https://doi.org/10.4172/1948-5948.1000188

[5] Hasan, A., Tabassum, B., Hashim, M., and Khan, N. 2024. Role of plant growth promoting rhizobacteria (PGPR) as a plant growth enhancer for sustainable agriculture: a review. Bacteria.3(2):59-75.https://doi.org/10.20944/preprints202310.1504.v1

[6] Rodríguez, H. and Fraga, R. 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnology Advances. 17(4-5):319-39. https://doi.org/10.1016/s0734-9750(99)00014-2

[7] Lugtenberg, B. and Kamilova, F. 2009. Plant-growth-promoting rhizobacteria. Annual Review of Microbiology. 63(1):541-56. https://doi.org/10.1146/annurev.micro.62.081307.162918

[8] Glick, B.R. 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica. 2012(1), 963401. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3820493/

[9] Vurukonda, S.S., Vardharajula, S., Shrivastava, M., and SkZ, A. 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological Research. 184:13-24. https://doi.org/10.1016/j.micres.2015.12.003

[10] Pikovskaya, R.I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology. 17, 362-370.

[11] Gordon, S.A. and Weber, R.P. 1951. Colorimetric estimation of indole acetic acid. Plant Physiology. 26(1):192. https://doi.org/10.1104/pp.26.1.192

[12] Koch, A.K., Käppeli, O., Fiechter, A., and Reiser, J. 1991. Hydrocarbon assimilation and biosurfactant production in Pseudomonas aeruginosa mutants. Journal of Bacteriology. 173(13):4212-9. https://doi.org/10.1128/jb.173.13.4212-4219.1991

[13] Dastogeer, K.M., Tumpa, F.H., Sultana, A., Akter, M.A., and Chakraborty, A. 2020. Plant microbiome–an account of the factors that shape community composition and diversity. Current Plant Biology. 23:100161. https://doi.org/10.1016/j.cpb.2020.100161

[14] Idris, E.E., Iglesias, D.J., Talon, M., and Borriss, R. 2007. Tryptophan-Dependent Production of Indole-3-Acetic Acid (IAA) Affects Level of Plant Growth Promotion by Bacillus amyloliquefaciens FZB42. Molecular Plant-Microbe Interactions. 20(6):619-26. https://doi.org/10.1094/mpmi-20-6-0619

[15] Sherman, N. 1986. Microbiology, a Laboratory Manual. Addison-Wesley Publishing Company.

[16] Zahran, H.H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiology and Molecular Biology Reviews.63(4):968-89.https://doi.org/10.1128/mmbr.63.4.968-989.1999

[17] Fukami, J., Cerezini, P., and Hungria, M. 2018. Azospirillum: benefits that go far beyond biological nitrogen fixation. Amb Express. 8(1):73. https://doi.org/10.1186/s13568-018-0608-1

APPENDIX

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

- First author carried out the experiments.
- Second author planned the experiments, analysed the data, and drafted the manuscript.
- All authors read and approved the final manuscript.

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