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Isolation and identification of plant growth promoting bacteria associated with soil collected from Bikaner, Rajasthan.

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Abstract
The present study aimed to isolate and characterize plant growth promoting bacteria (PGPB) from rhizospheric soil collected from the arid region of Bikaner, Rajasthan, India. A total of fifteen morphologically distinct bacterial isolates (BIK1a–BIK3e) were successfully isolated using serial dilution and nutrient agar culture techniques. Preliminary Gram staining revealed that 73.3% of the isolates were Gram-positive, while 26.7% were Gram-negative. Biochemical characterization showed high enzymatic diversity: all isolates (100%) were catalase positive, 86.7% exhibited urease activity, and 80% demonstrated amylase (starch hydrolysis) capability. H_2S production was detected in 46.7% of isolates, whereas 93.3% and 66.7% tested positive for Methyl Red (MR) and Voges–Proskauer (VP) tests respectively, indicating varied fermentative pathways.
Plant growth promoting traits were prominent among the isolates. Siderophore production was recorded in 60% of the isolates, while 73.3% exhibited phosphate solubilization activity on Pikovskaya's agar medium. Indole acetic acid (IAA) production was positive in 80% of isolates, reflecting their potential to enhance root development. The nitrate reduction test was positive in 73.3% of strains, confirming their role in nitrogen cycling. These results demonstrate a substantial presence of physiologically and biochemically diverse PGPB in the desert soil of Bikaner. Such isolates, particularly BIK1e, BIK2a, and BIK3d which showed multiple positive traits, represent promising candidates for formulation of biofertilizers aimed at sustainable agriculture in arid ecosystems.

Keywords: Desert soil microbiota, plant growth promoting bacteria, Bikaner, isolation and identification

Introduction
Soil microorganisms play a pivotal role in maintaining soil health, fertility, and overall ecosystem productivity. Among them, plant growth promoting bacteria (PGPB) represent an important group of beneficial rhizospheric microbes that directly or indirectly stimulate plant growth through a variety of mechanisms such as biological nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and enhancement of nutrient uptake efficiency (Glick, 2012; Bhattacharyya and Jha, 2012) [2, 5]. In addition to promoting plant growth, PGPB can improve plant tolerance to abiotic stresses, suppress phytopathogens, and contribute to sustainable agriculture by reducing dependency on chemical fertilizers (Backer *et al.*, 2018) [1].
The arid and semi-arid soils of Rajasthan, especially those of the Bikaner region, are characterized by low organic matter content, high salinity, limited water availability, and extreme temperature fluctuations (Choudhary *et al.*, 2019) [4]. These harsh conditions exert strong selective pressure on microbial communities, leading to the evolution of stress-tolerant and metabolically versatile bacteria. Such native microbial strains can serve as valuable bioresources for developing bioinoculants tailored for crop production under arid and degraded land conditions. However, despite the ecological significance of desert microbiota, studies focusing on the isolation and characterization of indigenous PGPB from the desert soils of northwestern India remain limited.
Plant growth promoting bacteria isolated from arid regions have shown remarkable adaptive capabilities and have been successfully employed to enhance crop yield and soil fertility in water-deficient environments (Sandhya *et al.*, 2017) [9]. Their ability to produce indole-3-acetic acid (IAA), siderophores, and extracellular enzymes further supports nutrient availability and plant resilience under stress. Exploring such bacteria from the rhizospheric soils of Bikaner not only enriches our understanding of microbial diversity in desert ecosystems but also provides a foundation for the development of eco-friendly biofertilizers suitable for arid agriculture.
Therefore, the present study was undertaken to isolate, identify, and biochemically characterize plant growth promoting bacteria from rhizospheric soil samples collected from the desert region of Bikaner, Rajasthan. The primary objectives were to evaluate their morphological and biochemical properties, and to assess key plant growth promoting attributes such as phosphate solubilization, siderophore production, urease and catalase activity, and indole acetic acid synthesis. This work aims to identify promising native bacterial isolates with potential application in enhancing soil fertility and crop productivity in arid and semi-arid ecosystems.

Materials and methods
Collection and processing of soil samples
Soil was collected from desert area of Bikaner, Rajasthan in sterilized conditions. The collected samples were kept in sterilized packaging. The collected samples were subjected to bacterial strain isolation.
After soaking in 90% (v/v) ethanol for one minute and 1% (v/v) NaOCl for ten minutes, the healthy soil was surface sterilized and then rinsed six times with sterile distilled water. The sterilized samples were plated on Nutrient Agar (NA) medium and cultivated at 30°C for 24 to 48 hours. The colonies were streaked on NA agar plates after being collected and diluted using dilution series (De Boer, 1972).

The final wash water was incubated in NA to perform a sterility check; the absence of microbiological growth confirmed the sterility check.

Fig 1: Collection of soil from soil

Morphological identification by Gram's staining
A smear of the bacterial isolates' suspension was made on a glass slide, let to air dry, and then stained using Hucker's modified Gram's staining procedure in order to examine their morphological characteristics (Coico, 2006). Compound microscopes with high power and oil immersion lenses were used to examine the air-dried stained slides.

Identification of by biochemical tests
Catalase test
The isolate was inoculated and incubated in LB broths for 24 to 48 hours at 370C in order to perform the enzyme catalase test. A few drops of 3% H2O2 were added to a loop of bacterial culture that had been spread out on a slide. Effervescence was a sign of a successful outcome (Cappuccino and Sherman, 1992) [3].

H2S production test
Lead acetate paper strips from Hi Media are used to demonstrate an organism's capacity to generate H2S from sulfur-containing amino acids or inorganic sulfur compounds. Peptone water was used to inoculate bacteria. A strip of lead acetate paper was placed between the culture tube's inner wall and plug, and it was cultured for 18 to 24 hours at 30°C. When the entire strip or just the tip turns black, H2S is being produced (Cappuccino and Sherman, 1992) [3].

Methyl Red and Voges Proskauer Test
The endophytic isolates were added to two sets of Methyl Red and Voges-Proskauer (MR VP) broth, which was then incubated for 48 hours at 30°C. A few drops of an alcoholic methyl red solution were added to the first pair of tubes. A positive MR test result was indicated by the development of a pronounced red color. The second set of tubes was filled with 5% naphthol solution in 70% ethyl alcohol, and it was gently shaken for fifteen minutes. The appearance of a red color suggested a good reaction in the formation of acetyl methyl carbinol. This shows that the VP test was successful.

Amylase production
The amylase activity was investigated using a starch hydrolysis test.

After streaking the endophytic isolates on nutrient agar plates with 2% insoluble starch, they were allowed to incubate at room temperature. By flooding the plates with iodine solution, the hydrolysis of starch was examined. The presence of clear zones surrounding the colonies was noted, and a positive reaction was taken into consideration (Neha *et al.,* 2021) [7]

Identification of plant growth promoting bacteria
Siderophore production test
The iron chelator known as a siderophore complexes with iron and makes it easily accessible to plant root surfaces. Microorganisms in soil compete with one another for the uptake of iron. The more organic substrates are added to the soil, the more siderophores are produced. Schwyn and Neilands' 1987 Chrome Azurol S agar medium was used to measure siderophore production. On the CAS plate, a bacterial culture was visible. The emergence of an orange halo over a dark blue background was used to measure the siderophore production following three days of incubation at 28°C.

Urease test
Phenol red, a pH indicator, was added to urea broth containing inoculated bacteria. The ammonia-producing organisms cause the broth's pH to rise. The broth's dramatic color shift from yellow to deep pink during four to five days of development was noted as a favourable outcome (Neha *et al.,* 2021) [7]

IAA production
By using Kovac's reagent and looking for the formation of a red circle, the development of indole was determined. 0.2 ml of Kovac's reagent (conc. HCl, 25 ml; amyl alcohol, 75 ml; p-dimethyl amino benzaldehyde, 5g) was added to tryptone broth to inoculate the new isolates, and everything was well mixed (Cappuccino and Sherman, 1992) [3].

Phosphate solubilization
After nitrogen, phosphate is one of the most important nutrients for microorganisms. By secreting certain organic acids, a number of bacterial and fungal species break down and dissolve insoluble phosphates into soluble forms. Phosphate solubility in bacteria was assessed using Pikovskaya's agar medium (Gour, 1990). On Pikovskaya's agar medium, spot inoculation was carried out. A distinct zone forming around the colonies after 48–72 hours of incubation was deemed positive.

Results
A total of fifteen morphologically distinct bacterial isolates (designated as BIK1a–BIK3e) were successfully obtained from the rhizospheric soil collected from the desert region of Bikaner, Rajasthan. The isolates displayed diverse colony morphologies varying in color, size, and elevation, suggesting high microbial diversity within the desert soil microflora.

Morphological and Gram Staining Characterization
Gram's staining revealed that out of fifteen isolates, eleven (73.3%) were Gram-positive and four (26.7%) were Gram-negative. The majority of the Gram-positive isolates were rod-shaped and arranged singly or in chains, while Gram-negative isolates were mostly short rods. This dominance of Gram-positive bacteria indicates their higher adaptability to the arid, nutrient-limited soil environment typical of desert ecosystems.

Biochemical Characterization

Biochemical assays demonstrated considerable variability among isolates (Table 1). All isolates (100%) tested positive for catalase activity, indicating their aerobic nature and the ability to detoxify hydrogen peroxide. Urease activity was observed in 86.7% of isolates, showing their role in nitrogen cycling and conversion of urea into ammonia. The amylase test revealed that 93.3% of isolates were capable of starch hydrolysis, signifying extracellular enzyme production for nutrient mobilization.

Hydrogen sulfide (H_2S) production was recorded in 46.7% of isolates, whereas Methyl Red (MR) and Voges–Proskauer (VP) tests were positive in 93.3% and 66.7% of isolates, respectively. This indicates the coexistence of both mixed acid and butanediol fermentation pathways among desert soil bacteria. The indole production test was positive for 73.3% isolates, confirming their potential to produce indole acetic acid (IAA) or related compounds that promote root elongation and cell differentiation.

Plant Growth Promoting Traits

Among the fifteen isolates, a high percentage exhibited plant growth promoting (PGP) activities (Figure 2). Siderophore production was detected in nine isolates (60%), visualized by the formation of distinct orange halos on Chrome Azurol S (CAS) agar plates, indicating strong iron-chelating potential. Phosphate solubilization activity was recorded in eleven isolates (73.3%) as clear halo zones on Pikovskaya's agar medium, confirming their ability to mobilize insoluble phosphates into bioavailable forms.

Nitrate reduction was observed in 73.3% of isolates, suggesting efficient nitrogen utilization capability. IAA production was recorded in 80% of isolates, highlighting their role in plant root stimulation and rhizosphere development.

Three isolates—BIK1e, BIK2a, and BIK3d—exhibited the maximum number of positive traits, including catalase, urease, amylase, MR, VP, indole, phosphate solubilization, and siderophore production. These isolates demonstrated more than eight PGP-related characteristics, indicating strong potential for development as effective bioinoculants.

Quantitative analysis of PGP attributes showed that the frequency of key traits among isolates followed the order: Catalase (100%) > Amylase (93.3%) > MR (93.3%) > Urease (86.7%) > IAA (80%) > Phosphate Solubilization (73.3%) > Nitrate Reduction (73.3%) > Siderophore (60%) > VP (66.7%) > H_2S (46.7%).

The predominance of Gram-positive, catalase- and phosphate-solubilizing bacteria suggests that the desert soil of Bikaner harbors a metabolically active and stress-tolerant microbial community with multifaceted plant growth promoting potential.

Table 1: Results of all test of the isolated bacteria

S no.	Bikaner	Gram staining	Catalase	Siderophore	Urea	H2s	Mr	Vp	Indole	Starch	Nitrate reduction	Phosphate solubilization
1	BIK1a	+	+	-	+	-	+	-	+	+	+	+
2	BIK1b	-	+	-	-	-	+	+	+	+	+	-
3	BIK1c	+	+	-	+	-	+	-	-	+	+	-
4	BIK1d	+	+	+	+	-	+	+	-	+	+	-
5	BIK1e	-	+	+	+	+	+	+	+	+	+	+
6	BIK2a	-	+	+	+	+	+	+	+	+	+	+
7	BIK2b	+	+	-	+	+	+	+	+	+	-	+
8	BIK2c	+	+	+	+	+	+	+	+	+	-	-
9	BIK2d	+	+	-	+	+	+	+	+	+	+	-
10	BIk2e	+	+	-	+	+	+	+	+	+	+	-
11	BIK3a	+	+	+	+	+	+	-	+	+	-	+
12	BIK3b	+	+	+	+	+	+	-	+	+	-	+
13	BIK3c	-	+	+	+	+	+	+	+	+	-	+
14	BIK3d	+	+	+	+	+	+	+	+	+	+	+
15	BIK3e	+	+	+	+	+	+	-	+	+	-	-

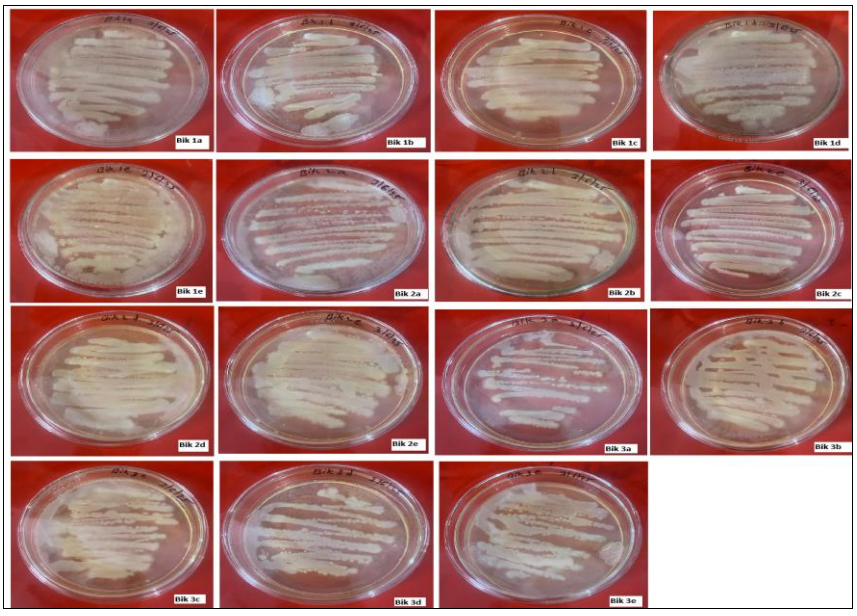

Fig 2: The isolated endophytes from rhizosphere soil collected from desert of Bikaner, Rajasthan

Discussion

The present study demonstrates that the desert soil of Bikaner, Rajasthan, harbors a diverse community of plant growth promoting bacteria (PGPB) capable of expressing multiple beneficial traits essential for plant development under harsh environmental conditions. The predominance of Gram-positive isolates (73.3%) over Gram-negative ones suggests their enhanced ecological fitness in arid soils characterized by high temperature, low organic matter, and osmotic stress. Similar findings were reported by Singh *et al.,* (2020) [10], who observed that Gram-positive *Bacillus* and *Streptomyces* species were dominant in the Thar Desert soil due to their ability to form endospores and withstand desiccation.

The universal presence of catalase activity in all isolates indicates their oxidative stress tolerance and aerobic metabolic adaptability. Catalase-positive PGPB are known to neutralize reactive oxygen species, thereby promoting better plant–microbe symbiosis under stress conditions (Kumar *et al.,* 2021) [6]. The high prevalence of urease (86.7%) and nitrate reduction (73.3%) activities in this study further indicates that these isolates are actively involved in nitrogen transformation processes, supporting sustainable soil fertility in nutrient-poor desert environments. Zhao *et al.,* (2019) [14] also highlighted that urease-producing bacteria play a key role in nitrogen mineralization and enhance nitrogen use efficiency in arid soils.

Phosphate solubilization was one of the most dominant plant growths promoting attributes (73.3%) among the isolates. The ability of these bacteria to release organic acids and convert insoluble phosphate into soluble forms is particularly vital for arid soils where phosphorus bioavailability is often limited. Comparable results were obtained by Tripathi *et al.,* (2022) [12], who reported that *Bacillus megaterium* and *Pseudomonas fluorescens* isolated from semi-arid soils exhibited significant phosphate solubilization efficiency and improved crop biomass under greenhouse conditions.

IAA and siderophore production, recorded in 80% and 60% of isolates respectively, indicate a robust potential for root elongation, iron acquisition, and rhizosphere colonization. IAA-producing rhizobacteria enhance lateral root formation and nutrient uptake, especially under moisture deficit conditions (Spaepen and Vanderleyden, 2011) [11]. Similarly, siderophore production confers a competitive advantage by improving iron availability to plants and suppressing soilborne pathogens. In line with our findings, Qessaoui *et al.,* (2019) [8] reported that siderophore-producing *Bacillus subtilis* and *Pseudomonas putida* from Moroccan desert soils significantly enhanced wheat growth and iron uptake under drought stress.

The identification of three highly active strains—BIK1e, BIK2a, and BIK3d—exhibiting multiple PGP traits underscores their potential as biofertilizer candidates for arid land agriculture. Polyfunctional PGPB with multiple enzymatic and growth-promoting capabilities have been recognized as superior inoculants for improving crop resilience under abiotic stress (Backer *et al.,* 2018) [1]. These results are in agreement with the work of Verma *et al*. (2021) [13], who demonstrated that multifunctional *Bacillus* isolates from the Indian Thar Desert enhanced the germination and growth of pearl millet under saline and drought conditions.

Overall, the findings highlight that the native bacterial isolates from Bikaner desert soil are metabolically versatile and stress-tolerant, showing potential for use as sustainable bioinoculants. Future studies should focus on molecular identification through 16S rRNA gene sequencing, quantification of IAA and siderophore production, and greenhouse trials to confirm their growth-promoting efficacy on local crops.

Conclusion

The present investigation clearly demonstrates that the desert soil of Bikaner, Rajasthan, supports a rich diversity of plant growth promoting bacteria (PGPB) possessing multiple beneficial traits. Out of fifteen isolates obtained, the majority were Gram-positive and exhibited strong catalase, urease, amylase, and phosphate solubilization activities, reflecting their adaptive metabolic versatility under nutrient-deficient and stress-prone desert conditions. High frequencies of IAA (80%) and siderophore (60%) production further indicate their ability to enhance plant root growth and micronutrient uptake, crucial for improving crop performance in arid ecosystems.

Among all isolates, BIK1e, BIK2a, and BIK3d displayed the highest number of growth-promoting and enzymatic traits, designating them as promising candidates for future biofertilizer formulation. Their multifunctional nature suggests that these strains can contribute significantly to nutrient mobilization, stress tolerance, and overall soil fertility improvement in semi-arid and desert agricultural systems.

The findings of this study underscore the ecological and biotechnological potential of indigenous microbial resources from arid regions. Further research involving 16S rRNA sequencing, quantitative estimation of growth-promoting metabolites, and greenhouse or field-level validation is recommended to confirm their efficacy and optimize their formulation for large-scale agricultural applications. Harnessing such native PGPB represents a sustainable strategy to enhance crop productivity while reducing reliance on synthetic fertilizers in challenging environments like the Thar Desert.

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