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Isolation and identification of plant growth promoting bacteria associated with soil collected from Churu

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Abstract
Plant growth-promoting bacteria (PGPB) have gained attention as important contributors to soil fertility and crop productivity as a result of the hunt for environmentally friendly substitutes for chemical fertilizers. In order to isolate and assess PGPB for possible agricultural uses, soil samples were gathered from Churu, Rajasthan, an arid desert region. Gram-negative and catalase-positive isolates were all acquired, indicating that they had adapted to the harsh conditions of the desert. Important PGP characteristics such as the production of indole-3-acetic acid (IAA), phosphate solubilization, siderophore production, nitrate reduction, and urease activity were screened for in the strains.

The findings indicated that IAA production was the most prevalent characteristic, although urease activity and nitrate reduction were also frequently noted. A subset of strains exhibited siderophore production and phosphate solubilization, suggesting variation in their functional capacities. The most effective strains among all the isolates were found to be CH2d and CH2e, which expressed every PGP trait that was tested.

These results show that Churu's desert soils are an important source of multifunctional, stress-tolerant PGPB. The isolates, especially CH2d and CH2e, have a great deal of potential for use as biofertilizers to improve crop resilience, plant growth, and nutrient availability in arid and nutrient-deficient environments. It is advised that more field-based and molecular research be done to confirm their potential for sustainable farming methods.

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

Introduction

Given the rising demand for food worldwide, the deterioration of soil, and the negative environmental effects of excessive use of chemical fertilizers, sustainable agriculture has become imperative. Plant growth-promoting bacteria (PGPB) are environmentally friendly substitutes that boost soil health and crop productivity. Through a number of direct and indirect processes, such as nitrogen fixation, phosphate solubilization, siderophore production, phytohormone synthesis, and biocontrol against phytopathogens, these advantageous microorganisms colonize the rhizosphere and promote plant growth. PGPB are excellent candidates for the development of biofertilizers and sustainable crop production because of these multifunctional qualities (Ramakrishna, 2019; Glick, 2020) [10, 15].

Extreme weather conditions, including high temperatures, little precipitation, nutrient-poor soils, and little organic matter, create major problems for agriculture in arid and semi-arid areas, like those in northwest India. The Thar Desert's Churu district in Rajasthan is a prime example of these circumstances. Despite its harsh environment, desert soils are known to harbor diverse microbial populations that have evolved specialized survival strategies. When used as bioinoculants under agricultural stress conditions, these microbial communities may perform better due to their stress tolerance, which makes them particularly interesting (Walker *et al.*, 2018) [18].

According to earlier research on the microbiota of the desert, even in conditions of oxidative stress and nutrient limitation, stress-tolerant bacteria can produce metabolites that promote plant growth (Chakraborty *et al.*, 2013) [5]. For instance, phosphate-solubilizing strains of desert bacteria increase the availability of phosphorus in alkaline desert soils, while siderophore-producing desert bacteria can help plants with iron deficiency. Moreover, the ability to produce phytohormones such as indole-3-acetic acid (IAA) enhances root development, thereby improving plant access to water and nutrients. Despite the potential of these special isolates to address agricultural challenges in arid regions, systematic studies on PGPB from the Churu desert soils are still rare (Attar *et al.*, 2019; Ashry *et al.*, 2022) [2, 8].

Thus, the goal of the current study was to identify and isolate bacteria that promote plant growth from Churu, Rajasthan's desert soils. A variety of PGP characteristics, such as IAA production, phosphate solubilization, siderophore production, nitrate reduction, and urease activity, were assessed in the isolates. Finding multipurpose strains with potential use as biofertilizers for sustainable crop improvement in nutrient-deficient and stress-prone environments was the aim.

Materials and Methods

Collection and processing of soil samples

Soil was collected from desert area of Churu, 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: Site for soil collection. 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) [6]. 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% H_2O_2 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) [4].

- **H_2S production test**

Lead acetate paper strips from Hi Media are used to demonstrate an organism's capacity to generate H_2S 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, H_2S is being produced (Cappuccino and Sherman, 1992) [4].

- **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) [13].

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[16] 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) [13].

- **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) [4].

- **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

Several bacterial strains that were screened for characteristics that promote plant growth were found in soil samples taken from Churu's desert region. Fifteen isolates in all were obtained as pure colonies. Each was assigned a unique code, as indicated in Table 1. The ability of all the isolates to detoxify hydrogen peroxide and endure oxidative stress was demonstrated by their positive catalase activity reaction and Gram-negative nature.

Other biochemical and plant growth-promoting tests showed variability.

Among the isolates, CH1d, CH1e, CH2d, and CH2e were found to produce siderophores, indicating that they may improve plants' access to iron. With the exception of consistently negative results, urease activity was absent in the CH1 series but positive in isolates CH2a, CH2b, CH2c, CH2d, CH2e, CH3a, CH3b, CH3c, CH3d, and CH3e. The majority of the strains, especially CH1b–e and CH2a, CH2e, produced hydrogen sulfide (H_2S), demonstrating their capacity to mobilize sulfur in the soil.

Differential responses were observed in the Methyl Red (MR) and Voges–Proskauer (VP) tests. While VP was positive in many strains, including CH1a–e and CH2b–e, indicating diversity in fermentative pathways, some strains, such as CH1e, CH2a, CH2c, and CH2e, displayed positive MR activity. With the exception of CH3c and CH3e, nearly all isolates contained indole-3-acetic acid (IAA), a crucial hormone that promotes plant growth, indicating their potent capacity to stimulate root growth.

All isolates exhibited consistent amylase activity, indicating their function in the hydrolysis of starch and the turnover of organic matter. With the exception of CH3b, CH3c, and CH3d, nitrate reductase activity was also detected in most isolates, suggesting their function in nitrogen availability and metabolism. Crucially, isolates CH2d, CH2e, CH3a, CH3b, and CH3c demonstrated a strong phosphate solubilization ability, demonstrating their ability to enhance plant phosphorus uptake.

Strong candidates for bioinoculants that promote plant growth, two strains (CH2d and CH2e) demonstrated positive results for IAA, phosphate solubilization, siderophore production, nitrate reduction, and urease. Overall, the findings show that the bacterial isolates from Churu desert soils have a variety of traits that promote plant growth. CH2e was found to be the most effective strain, showing positive results for nearly every test parameter, including urease, siderophore production, H_2S production, MR, VP, IAA, amylase, nitrate reductase, and phosphate solubilization.

Table 1: Results of all test of the isolated bacteria

Strain Code	Gram's staining	Catalase	Siderophore	Urease	H2S	MR	VP	IAA	Amylase	Nitrate reductase	Phosphate solubilization
CH1a	-	+	-	-	-	-	+	+	+	+	-
CH1b	-	+	-	-	+	-	+	+	+	+	-
CH1c	-	+	-	-	+	-	+	+	+	+	-
CH1d	-	+	+	-	+	-	+	+	+	+	-
CH1e	-	+	+	-	+	+	+	+	+	+	-
CH2a	-	+	-	+	+	+	-	+	+	+	-
CH2b	-	+	-	+	-	-	+	+	+	+	-
CH2c	-	+	-	+	-	+	+	+	+	+	-
CH2d	-	+	+	+	-	-	+	+	+	+	+
CH2e	-	+	+	+	+	+	+	+	+	+	+
CH3a	-	+	-	+	-	-	+	+	+	+	+
CH3b	-	+	-	+	-	-	+	+	+	-	+
CH3c	-	+	-	+	-	-	-	+	+	-	+
CH3d	-	+	-	+	-	-	+	+	+	-	-
CH3e	-	+	-	+		-	+	-	+	+	-

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

Discussion

A wide variety of bacterial isolates exhibiting several plant growth-promoting (PGP) characteristics were found in soil samples taken from Churu's arid desert region. These results are important because it is known that stress-tolerant microbial populations with special adaptations exist in desert ecosystems, despite their harsh climate. Particularly in semi-arid and arid areas where conventional farming is constrained by low soil fertility, low moisture content, and nutrient deficiencies, these microbes can be extremely important to sustainable agriculture (Koberl *et al.*, 2011; AlSharari *et al.*, 2022) [1, 11].

Gram-negative status and catalase positivity were shared by all isolates, suggesting an innate ability to withstand oxidative stress and endure in the harsh desert environment. Because oxidative stress tolerance is essential in both field conditions and rhizosphere interactions, this adaptive feature makes them strong candidates for the development of biofertilizers (Dang, 2024) [7].

With the exception of a few strains (such as CH3c and CH3e), indole-3-acetic acid (IAA) production was the most widely distributed of the PGP traits. This implies that by promoting root elongation, lateral root initiation, and general plant vigor, the majority of the isolates from the Churu desert may have an impact on plant growth. Because improved root systems increase the efficiency of nutrient and water uptake, the widespread presence of IAA-positive strains is especially beneficial for crops grown in nutrient-limited environments (Puri *et al.*, 2020) [14].

Fewer strains, including CH2d, CH2e, CH3a, CH3b, and CH3c, showed phosphate solubilization. Given that phosphate solubilization is a strain-specific characteristic, this variability is not unexpected. Due to high fixation in unavailable forms, phosphorus deficiency is one of the main problems in arid soils. Therefore, the phosphate-solubilizing isolates from Churu have the potential to increase crop phosphorus availability and lessen reliance on chemical fertilizers. Because they demonstrated phosphate solubilization in addition to several other PGP activities, CH2d and CH2e stood out among the others (Meena *et al.*, 2017) [12].

In CH1d, CH1e, CH2d, and CH2e, siderophore production was positive. Siderophores suppress phytopathogens by limiting their access to iron and increase iron uptake by plants in iron-limiting environments. Siderophore-producing isolates can be very helpful for enhancing micronutrient availability and plant health because micronutrient deficiencies are frequently seen in desert soils (Sivashakthi *et al.*, 2014) [17].

Several isolates, including CH2a, CH2b, CH2c, CH2d, CH2e, and CH3a, CH3b, CH3c, CH3d, and CH3e, were found to have urease activity. Their function in nitrogen cycling is further strengthened by the enzymatic activity that converts urea into forms of usable nitrogen. In arid soils where nitrogen losses are common due to volatilization and low organic matter, nitrogen turnover is especially crucial (Fu *et al.*, 2018) [9].

CH2d and CH2e were the most promising isolates when the PGP traits were examined collectively because they demonstrated positive results for all of the important characteristics, including urease activity, phosphate solubilization, siderophore release, nitrate reduction, and IAA production. Their combination of qualities makes them perfect for biofertilizer formulations because they protect plants from nutritional and oxidative stresses, promote root development, and increase nutrient availability (iron, phosphorus, and nitrogen).

The results align with past research on microbes found in deserts and arid regions, where strains possessing multifunctional PGP properties have been shown to considerably enhance plant growth and yield in stressful environments. Such multifunctional isolates are found in Churu desert soils, demonstrating the adaptability of native microbial populations and their potential to support sustainable agriculture.

Overall, the bacterial isolates from Churu desert soils show significant strain-to-strain variability and a broad variety of traits that promote plant growth. Particularly noteworthy as strong bioinoculants for potential application in arid agriculture are the multipurpose strains CH2d and CH2e. Their effectiveness and possible commercial use as environmentally friendly substitutes for chemical fertilizers will be confirmed by additional characterization through field testing, greenhouse bioassays, and molecular identification.

Conclusion

According to the current study, soil samples taken from Churu's arid desert region contain a variety of Gram-negative, catalase-positive bacterial isolates that have several characteristics that promote plant growth. A smaller subset of strains showed phosphate solubilization and siderophore production, while the majority showed urease activity, nitrate reduction, and indole-3-acetic acid (IAA) production. The most promising isolates were strains CH2d and CH2e because they had all of the essential PGP characteristics, such as urease activity, phosphate solubilization, siderophore release, nitrate reduction, and IAA production. According to these results, soils in the Churu desert are a valuable source of multipurpose PGP bacteria that may be used as potential biofertilizers to improve soil fertility and crop growth in nutrient-poor and stress-prone environments.

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