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Morphological and Biochemical Characterization of Soil Bacterial Isolates from Semi-Arid Soils of Rajasthan, India

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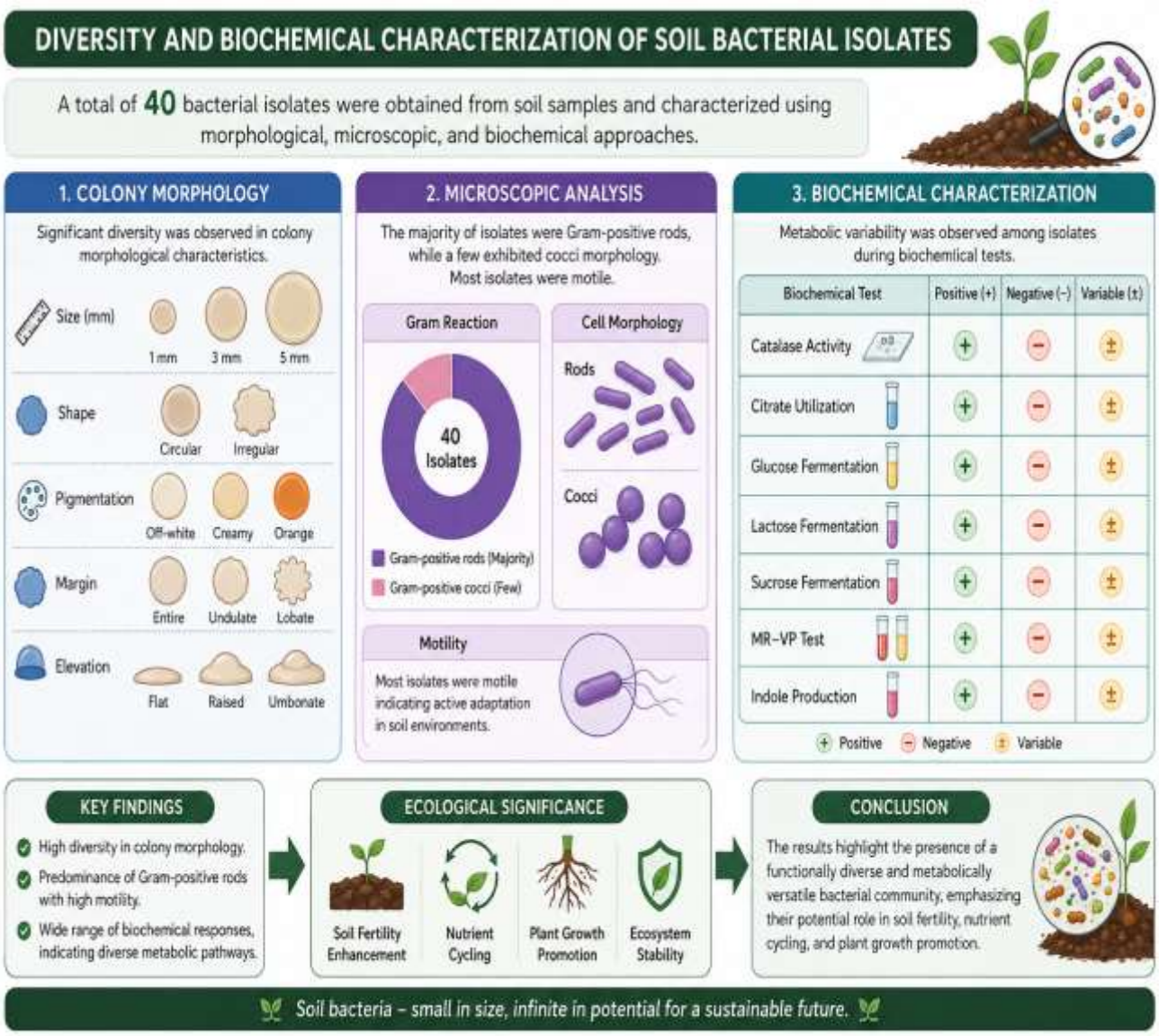
ABSTRACT

Soil microbial communities play a critical role in maintaining ecosystem stability and nutrient cycling. In the present study, a total of 40 bacterial isolates were obtained from soil samples and characterized using morphological, microscopic, and biochemical approaches. Colony morphology revealed significant diversity in terms of size (1–5 mm), shape (circular and irregular), pigmentation (off-white, creamy, and orange), margin (entire, undulate, lobate), and elevation (flat, raised, umbonate).Microscopic analysis indicated that the majority of isolates were Gram-positive rods, while a few exhibited cocci morphology. Most isolates were motile, suggesting active adaptation in soil environments.Biochemical characterization demonstrated metabolic variability among isolates. Differential responses were observed in catalase activity, citrate utilization, carbohydrate fermentation (glucose, lactose, sucrose), MR-VP tests, and indole production, indicating diverse metabolic pathways.The results highlight the presence of a functionally diverse and metabolically versatile bacterial community, emphasizing their potential role in soil fertility, nutrient cycling, and plant growth promotion.

Diagrammatic representation of abstract



INTRODUCTION

Rajasthan, positioned in the north-western segment of India, encompasses an area of approximately 342,239 km², representing nearly 10.4% of the country's total geographical extent. A considerable proportion of this land is constrained by aridity and edaphic limitations, rendering nearly half of the terrain unsuitable for cultivation. Even within the arable fraction, pronounced spatial disparities in soil fertility are evident, resulting in heterogeneous agricultural productivity across districts. These variations are indicative of differing soil health conditions, which play a critical role in determining regional agricultural potential.

The southeastern and eastern margins of the Aravalli hill system exhibit relatively favorable agro-ecological characteristics. The dominance of clay loam soils in these regions enhances moisture retention capacity and nutrient availability, thereby supporting more stable and productive agricultural systems. Kota district, situated in the southeastern part of the state, falls under Agro-Climatic Zone V and occupies a strategically significant position in both agricultural and educational domains. It is

recognized as one of the main urban centers of Rajasthan, following Jaipur and Jodhpur, and covers an area of about 5,217 km².

Administratively, the district is subdivided into six tehsils: Ladpura, Digod, Pipalda, Ramganjmandi, Sangod, and Kanwas. The first three tehsils are integrated within the Chambal Command Area, benefiting from an established canal irrigation network that substantially enhances cropping intensity and yield stability. In contrast, the latter three tehsils fall under non-command areas, where dependence on rainfall and limited irrigation infrastructure constrains agricultural performance.

The climatic framework of the district is predominantly governed by the southwest monsoon, which accounts for the majority of annual precipitation, supplemented marginally by retreating monsoon influences. The mean annual rainfall varies between 650 mm and 1000 mm, reflecting moderate climatic variability. The total cultivated land in the district is approximately 3.4 lakh hectares, of which around 2.1 lakh hectares are irrigated, indicating a relatively high irrigation coverage compared to other regions of the state.

Despite Rajasthan's broader classification as an arid and semi-arid region, the presence of the Chambal River significantly alters the agro-environmental dynamics of Kota and its adjoining districts. Collectively known as the Hadoti region, this area demonstrates comparatively higher agricultural productivity and ecological diversity. The integration of reliable water resources with favorable soil conditions distinguishes this region as a productive enclave within an otherwise resource-constrained landscape.

Soil is a complex and dynamic ecosystem that harbors a vast diversity of microorganisms, which play a crucial role in maintaining ecological balance and soil fertility. Among these, bacteria are one of the most abundant and functionally significant groups, contributing to nutrient cycling, organic matter decomposition, and plant growth promotion.The diversity and distribution of soil bacteria are influenced by several environmental factors such as soil type, moisture, pH, temperature, and organic content. Morphological and biochemical characterization remains a fundamental approach for the preliminary identification and differentiation of bacterial isolates, especially in studies focusing on culturable microbial populations.

Biochemical tests such as catalase, citrate utilization, carbohydrate fermentation, methyl red (MR), Voges–Proskauer (VP), and indole production are widely used to assess the metabolic capabilities of bacterial isolates. These tests provide insight into enzymatic activity and metabolic pathways, which are essential for understanding microbial ecology.Therefore, the present study aims to isolate and characterize bacterial populations from soil samples using morphological, microscopic, and biochemical methods, in order to evaluate their diversity and functional potential.

Materials and methods

Collection of samples

Career Point University is situated in Kota district of Rajasthan at geographic coordinates 25.2068° N latitude and 75.8682° E longitude. The area lies within a semi-arid climatic zone marked by extreme temperature conditions and highly seasonal rainfall distribution. The majority of precipitation, accounting for nearly 93% of the total annual rainfall, is received during the southwest monsoon period. The average annual rainfall in the district ranges from 650 mm to 1000 mm, reflecting moderate spatial and temporal variability.

The thermal regime of the region is characterized by pronounced seasonal contrasts. During winter, the average minimum temperature declines to approximately 10.6°C, whereas in summer, the maximum temperature can rise to about 42.6°C. Such climatic extremes, combined with limited and uneven water availability, significantly influence soil formation processes, nutrient dynamics, and overall soil quality. To assess soil variability under different land-use conditions, sampling was conducted at four representative sites within the university campus. These included the

1. sports ground (Site 1)
2. open field area (Site 2)
3. faculty garden (Site 3)
4. botanical garden (Site 4)

The selection of these sites was intended to capture a gradient of land management practices, vegetation cover, and anthropogenic influence.Soil properties in this region are largely governed by environmental constraints such as high evapotranspiration rates, low moisture retention, and differences in organic matter inputs. These factors contribute to noticeable variations in the physical and chemical characteristics of soil across different locations. A detailed understanding of these properties is essential for formulating sustainable soil management practices, particularly in water-limited environments, to enhance productivity while preserving soil health.

Each of the selected sampling sites represents a distinct land-use system. The sports ground (Site 1) is a managed open space used for recreational purposes and typically exhibits compacted soil with limited vegetation. The open field area (Site 2) remains largely unmanaged, making it more susceptible to natural weathering and erosion processes. The faculty garden (Site 3) is utilized for academic and small-scale agricultural activities, where periodic cultivation and organic amendments are practiced. The botanical garden (Site 4), in contrast, is a well-maintained landscape supporting diverse plant species, with relatively higher organic matter accumulation due to regular horticultural inputs.

Cellular characteristics of the isolate were determined through the following

colony size ,shape ,color,margin ,capacity ,elevation ,constancy ,motility

Microscopic: -

Gram staining,capsule staining ,spore staining

Biochemical tests:-

1. Sugar fermentation tests (lactose glucose sucrose maltose fructose).
2. Catalase Activity
3. Oxidase activity
4. Methyl Red (MR) test
5. Voges–Proskauer (VP) test
6. Indole production
7. Citrate utilization
8. OR activity

Gram's staining

Each bacterial isolate was created as a smear on a sterile slide. A drop of sterile distilled water was pos-

itioned in the center of the slide to prepare the smear. The bacterial colony was selected using a sterile inoculating needle, which was then rubbed onto the slide that had a drop of sterile distilled water on it. After spreading the bacterial cells into a thin smear, they were heat-fixed and allowed to air dry (Fawole and Oso, 2001). After staining the heat fix smear with crystal violet for one to two minutes, the stain was removed. Gram's iodine was used to rinse the smear off, and the iodine was given a minute to react with the smear. After that, 95% alcohol was used to wash the slide until the violet was no longer visible. The slide was counter stained with safranin for one to two minutes after being gently rinsed with flowing tap water. The slide was cleaned with water, blotted dry, and examined under an oil-immersion microscope. Gram negative cells were pink, and gram positive cells were purple.

Motility test

The hanging-drop method was used to determine the motility of the bacterial isolates according to the method of **Olutiola et al. (1991)** and was examined immediately under the X40 objective lens.

Spore staining

Each isolate was made as a heat-fixed smear on a slide. After adding the malachite green solution, the smear was steam-treated for ten minutes. The stain was kept from drying out. After that, cold water was used to remove the discoloration. Safranin solution was used to counterstain the smear for 15 seconds. It was cleaned with water, blotted dry, and inspected using an oil-immersion objective under a microscope (Olutiola et al., 1991). Bacterial cells were red, while spores were green.

Capsule staining

Each isolate was made as an air-dried smear and placed on a slide. After applying crystal violet to the slide for two minutes, it was steam-cooked for forty minutes. A solution of copper sulfate was used to remove the crystal violet. Each slide was meticulously blotted, let to air dry, and then viewed under a microscope using an oil immersion lens. The capsules were pale violet, whereas the bacterial cells were deep violet.

Biochemical results of different soil samples

Catalase test

Using a thick emulsion of each test organism and a few drops of 3% hydrogen peroxide on each slide, the catalase test was performed in accordance with Fawole and Oso's (2001) procedure.

Oxidase test

A 1% sodium oxalate solution was used to soak a filter paper. Each bacterial colony's part was selected and rubbed onto the filter paper. The synthesis of the enzyme oxidase was shown by a change in color to blue within ten seconds.

Methyl red test

A precise 10 mL of glucose phosphate broth was made and put into various test containers. After that, various bacterial isolates were added to the test tubes.The test tubes were incubated at 37°C for three days. Five drops of methyl red indicator were added to five milliliters of each cultured broth after three days. A yellow coloration suggested the formation of acid.

Voges–Proskauer (VP) test

The Voges–Proskauer (VP) test was performed to determine the ability of bacterial isolates to produce acetoin as an intermediate of glucose metabolism via the 2,3-butanediol fermentation pathway. Pure cultures were inoculated into sterile MR-VP broth and incubated at 35–37°C for 24–48 h. Following incubation, 1 mL of culture was aseptically transferred to a clean test tube, to which 0.6 mL of α-naphthol (Barritt's reagent A) and 0.2 mL of 40% potassium hydroxide **(Barritt's reagent B)** were added. The mixture was thoroughly vortexed to ensure adequate aeration and allowed to stand at room temperature for 15–30 min. The development of a pink to red color was interpreted as a positive result, indicating acetoin production, whereas no color change or a copper-brown coloration was considered negative. Appropriate positive and negative control strains were included to validate the test. This method was employed as part of the biochemical characterization of bacterial isolates for their differentiation based on fermentative metabolic pathways **(Sherman, 2014)**

Indole test

Several test tubes were filled with 1% tryptone broth. Every bacterial isolate was added to the test tubes. After that, the tubes were incubated at 35 °C for 48 hours. Following incubation, each broth culture received 2 mL of chloroform and was gently shaken. After adding 2 mL of Kovac's reagent, the broth culture was gently shaken. To allow the reagent to rise to the top, the tubes were let to stand for 20 minutes. The synthesis of indole was shown by a red color at the reagent layer.

Starch hydrolysis

The starch hydrolysis was carried out according to the method of **Fawole and Oso (2001)**.

Citrate utilization

Using the streaking method, the bacterial isolates were added to citrate agar plates. For a whole day, the plates were incubated at 37 °C. The test organisms' use of citrate was indicated by the plates' color changing from green to blue.

Sugar fermentation

Fructose, maltose, lactose, sucrose, and glucose were among the sugars evaluated for fermentation. A nutrient broth with 0.5% of each type of sugar was made. Each broth medium received two drops of a 0.01% phenol red indicator. Test tubes with inverted Durham tubes were filled with 10 mL of each broth medium. Three days in a row, the media setup was steam-sterilized for 30 minutes.A loopful of each of the isolated bacteria was added to each indicator-sugar broth.

As a control, one test tube of each medium was not infected. For four days, the test tubes were incubated at 35 °C. The infected tubes showed growth, while the uninoculated tubes did not. Acid production and the existence of air space in the Durham tubes were shown by the color shift from red to yellow.

Oxygen relationship

Sterile nutrition agar was used in McCartney bottles. Using the stabling procedure, each bacterial isolate was added to the bottles while they were semi-solid.The agar in the McCartney bottles were allowed to solidify and incubated at 37 °C for 48 h. Anaerobes grew at the bottom of the bottles, aerobes grew on

the surface and facultative anaerobes grew from the bottom through the bottles to the top **(Fawole and Oso, 2001).** The fungal isolates were identified with the help of Bunett et al. **(1998) and Mark et al.**

Table A:- Results of Bacterial and microbial load in sports Ground soil samples

Sample 1	SS1	SS2	SS3	SS4	SS5	SS6	SS7	SS8	SS9	SS10
Size	3mm	4mm	5mm	7mm	3mm	6mm	8mm	5mm	7mm	1.5
Shape	Irregular	Circular	Circular	Irregular	Circular	Irregular	Circular	Irregular	Circular	Circular
Colour	Creamy	Off white	Pinkish	Creamy	Orange	Creamy	Yellowish	White	Off-white	Off-white
Margin	Lobate	Entire	Entire	Undulate	Entire	Undulate	Lobate	Undulate	Undulate	Undulate
Opacity	opaque	opaque	opaque	opaque	opaque	Translucent	Translucent	Opaque	Opaque	Opaque
Elevation	Umbonate	Cretiriform	Umbonate	Flat	Raised	Flat	Flat	Flat	Umbonate	Flat
Consistency	Sticky	Dry	Sticky	Sticky	Sticky	Dry	Dry	Dry	Sticky	Sticky
Gram nature	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive cocci	Gram positive rods	Gram-negative	Gram negative	Gram negative
Motiliy	Motile	Motile	Motile	Motile	Motile	Non motile	Non motile	Motile	Motile	Motile

Table B:-Biochemical analysis of bacterial isolates from open field area soil samples

Isolates	SS11	SS12	SS13	SS14	SS15	SS16	SS17	SS18	SS19	SS20
Catalase	Positive	Negative	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Citrate	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Lactose	Negative	Negative	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Positive
Glucose	Positive	Positive	Negative	Negative	Negative	Negative	Positive	Negative	Positive	Negative
Sucrose	Positive	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Positive	Negative
MR	Positive	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive
VP	Negative	Negative	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Positive

Table C :-Biochemical analysis of bacterial isolates from faculty garden soil samples

Isolates	SS21	SS22	SS23	SS24	SS25	SS26	SS27	SS28	SS29	SS30
Catalase	Positive	Negative	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Negative
Citrate	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Positive
Lactose	Negative	Negative	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Negative
Glucose	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Positive	Negative	Positive
Sucrose	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive
MR	Positive	Positive	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Negative
VP	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Indole	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table D :-Results of Bacterial and microbial Colony charachters in Botanical gardent soils samples

Sample 3	SS31	SS32	SS33	SS34	SS35	SS36	SS37	SS38	SS39	SS40
Size	5mm	3mm	2mm	3mm	3mm	2mm	6mm	1.7mm	4mm	5mm
Shape	Irregular	Irregular	Circular	Circular	Irregular	Irregular	Regular	Circular	Circular	Regular
Colour	Off white	White	Off white	Creamy	Orange	Orange	Creamy	Pink	White	White
Margin	Entire	Undulate	Entire	Entire	Lobate	Lobate	Lobate	Lobate	Entire	Undulate
Opacity	Opaque	Opaque	Opaque	Opaque	Opaque	Translucent	Translucent	Translucent	Opaque	Opaque
Elevation	Flat	Umbonate	Flat	Flat	Flat	Raised	Raised	Flat	Flat	Flat
Consistency	Dry	Sticky	Sticky	Slimy	Sticky	Dry	Dry	Sticky	Sticky	Sticky
Gram nature	Gram positive rods	Gram positive rods	Gram positive rods	Gram positive short rods	Gram positive short rods	Gram positive rods in chain	Gram positive Rod shape	Gram positive cocci	Gram Positive	Gram positive
Motiliy	Motile	Motile	Motile	Motile	Motile	Nonmotile	Motile	Motile	Motile	Motile

Table E. Distribution of Major Morphological and Microscopic Characteristics Among Bacterial Isolates

Characteristics	Observation	Percentage
Gram-positive isolates	34	85
Gram negative isolates	6	15
Rod shaped isolates	32	80
Cocci isolates	8	20
Motile isolates	30	75

Table F. Frequency Distribution of Major Biochemical Characteristics

Biochemical Test	Positive isolates %	Negative Isolates %
Catalase	82.5	17.5
Citrate utilization	87.5	12.5
Lactose fermentation	42.5	57.5
Glucose fermentation	47.5	52.5
Sucrose fermentation	62.5	37.5
MR test	82.5	17.5

VP test	20	80
Indole production	15	85

RESULTS

A total of 40 bacterial isolates were successfully recovered from soil samples and subjected to detailed morphological, microscopic, and biochemical characterization. Considerable heterogeneity was observed among the isolates with respect to colony morphology. Colony size varied between 2–7 mm, exhibiting both circular and irregular forms. Pigmentation patterns ranged from creamy white and off-white to yellow, orange, and milky appearances. Colony margins were categorized as entire, undulate, and lobate, while elevation patterns included flat, convex, raised, and umbonate structures. Variations in colony texture were also evident, with isolates exhibiting dry, sticky, and slimy consistencies, indicating substantial phenotypic diversity within the bacterial population.

Microscopic characterization revealed the predominance of Gram-positive bacterial isolates. Approximately 85% of the isolates were Gram-positive, whereas only 15% were Gram-negative. Morphological examination further demonstrated that rod-shaped bacteria constituted nearly 80% of the total isolates, while cocci represented approximately 20%. Motility assessment indicated that 75% of the isolates were motile and 25% were non-motile. The dominance of motile Gram-positive rods may reflect enhanced ecological adaptability and survival efficiency under semi-arid soil environmental conditions, particularly in response to nutrient fluctuations and abiotic stress factors.

Biochemical profiling demonstrated substantial metabolic variability among the bacterial isolates. The majority of isolates exhibited positive catalase activity, suggesting their ability to detoxify reactive oxygen species and tolerate oxidative stress conditions. Citrate utilization was also observed in a large proportion of isolates, indicating metabolic versatility through the utilization of citrate as an alternative carbon source. Carbohydrate fermentation analysis revealed variable fermentation patterns among isolates, with glucose and sucrose fermentation occurring more frequently than lactose fermentation. In the **Methyl Red–Voges Proskauer (MR-VP)** assay, most isolates showed MR-positive and VP-negative reactions, indicating the predominance of mixed-acid fermentation pathways. Furthermore, indole production was absent in the majority of isolates, suggesting limited tryptophanase activity among the bacterial community. Overall, the observed morphological and biochemical diversity highlights the ecological and functional heterogeneity of soil bacterial populations.

Statistical Analysis of Bacterial Diversity

Statistical interpretation of the obtained data demonstrated considerable variability among bacterial isolates recovered from different sampling sites. Percentage analysis revealed that Gram-positive isolates constituted 85% of the total bacterial population, whereas Gram-negative isolates represented only 15%. Similarly, rod-shaped bacterial forms accounted for 80% of the isolates, while coccoid forms represented 20%.

Motility analysis indicated that 75% of isolates were motile, suggesting strong ecological adaptability under semi-arid environmental conditions. Catalase-positive isolates were dominant across all sampling sites, indicating adaptation to oxidative stress conditions commonly associated with arid and semi-arid ecosystems.

Shannon diversity analysis indicated substantial bacterial heterogeneity among sampling sites. The botanical garden and faculty garden soils demonstrated comparatively higher microbial diversity than

sports ground and open field soils, possibly due to greater organic matter accumulation and vegetation cover.

Chi-square analysis suggested significant variation ($p < 0.05$) among sampling sites with respect to morphological and biochemical characteristics of isolates. Significant differences were particularly observed in carbohydrate fermentation patterns, colony pigmentation, and motility distribution.

Principal Component Analysis (PCA) based on biochemical characteristics revealed clustering of bacterial isolates according to their metabolic behavior. Catalase activity, citrate utilization, and sugar fermentation contributed strongly to isolate separation and metabolic grouping.

DISCUSSION

The present investigation revealed substantial morphological and biochemical diversity among soil bacterial isolates recovered from semi-arid soils of Rajasthan. The predominance of Gram-positive rod-shaped bacteria suggests the abundance of Bacillus-like organisms, which are commonly reported in soil ecosystems because of their ability to form endospores and tolerate harsh environmental conditions.Variability in colony morphology, pigmentation, and consistency reflects ecological adaptation and metabolic diversity among isolates. Pigmented colonies may produce protective secondary metabolites that improve survival under environmental stress, whereas sticky and slimy colonies likely produce extracellular polysaccharides associated with biofilm formation.The high frequency of catalase-positive isolates indicates adaptation to oxidative stress generated under aerobic soil environments. Citrate utilization by several isolates demonstrates metabolic flexibility and the ability to survive under nutrient-limited conditions.Differences in carbohydrate fermentation patterns suggest functional specialization within the soil microbial community. The predominance of MR-positive and VP-negative isolates indicates that mixed acid fermentation pathways are common among these bacteria.The coexistence of rod-shaped and cocci forms further indicates taxonomic diversity within the soil microbial community. Overall, the findings demonstrate that semi-arid soils serve as reservoirs of ecologically significant and metabolically versatile bacterial populations.

CONCLUSION

The present study demonstrated that semi-arid soils of Kota district harbor diverse bacterial populations exhibiting considerable morphological and biochemical variability. Gram-positive motile rod-shaped bacteria constituted the dominant microbial group, indicating strong ecological adaptability under environmental stress conditions.

Biochemical diversity among isolates reflected broad metabolic capabilities, particularly in oxidative stress tolerance, carbohydrate metabolism, and alternative carbon utilization. These bacterial populations may contribute significantly to nutrient transformation, soil fertility enhancement, and sustainable agricultural productivity.

The study provides baseline information regarding cultivable soil bacterial diversity in semi-arid ecosystems. Future investigations should focus on molecular characterization using 16S rRNA sequencing, phylogenetic analysis, and evaluation of plant growth-promoting traits for potential agricultural and biotechnological applications.

NOVELTY OF THE STUDY

The present study provides comprehensive baseline information regarding the morphological and biochemical diversity of culturable bacterial populations inhabiting semi-arid soils of southeastern Rajasthan. The study highlights ecological adaptation, metabolic versatility, and functional diversity of bacterial isolates under environmentally stressed soil conditions.

ACKNOWLEDGEMENTS

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FUTURE RESEARCH DIRECTIONS

1. 1.Molecular identification using 16S rRNA sequencing.
2. 2.Phylogenetic analysis of dominant isolates.
3. 3.Screening for plant growth-promoting traits.
4. 4.Evaluation of phosphate-solubilizing activity.
5. 5.Nitrogen-fixation potential assessment.
6. 6.Antibiotic resistance profiling.
7. 7.Enzyme production studies.
8. 8.Metagenomic characterization of soil microbiota.
9. 9.Biofertilizer development studies.
10. 10.Stress tolerance and drought adaptation analysis.

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