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Biosurfactant-producing bacteria from crop rhizosphere soil and its effect on chickpea crop

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Abstract
Biosurfactants are surface-active molecules produced by microorganisms that can reduce surface and interfacial tension, offering numerous advantages over synthetic surfactants. This study aimed to isolate and characterize biosurfactant-producing bacteria and plant growth-promoting rhizobacteria (PGPR) characteristics from the rhizosphere soil of various crops and to evaluate their potential as a bio-inoculant for enhancing the growth and stress tolerance of chickpea (*Cicer arietinum*), a vital legume crop. Soil samples were collected from the college farm of PJTAU from the rhizosphere of red gram, maize, daincha, sunflower, and sesamum. A total of 40 bacterial isolates were initially selected based on morphological, cultural, and biochemical characteristics. These isolates were then screened for their ability to produce biosurfactants using various assays, including the oil spreading technique, emulsification measurement, surface tension measurement, drop collapsing test, and haemolytic activity. Plant growth-promoting rhizobacteria (PGPR) characteristics, such as phosphate solubilization, potassium solubilization, zinc solubilization, ammonium production, gibberellic acid, and IAA production, were also evaluated.

Keywords: PGPR, biosurfactant, surface tension and interfacial tension

1. Introduction
Here is an original and fully rephrased version of your passage to ensure zero plagiarism:
Chickpea (*Cicer arietinum* L.), commonly recognized as bengal gram, chana, or garbanzo bean, is a leguminous crop belonging to the Fabaceae family and the Faboideae subfamily. The cultivated chickpea has two primary forms: desi, which is mainly grown across the Indian subcontinent, and kabuli, which is largely produced in West Asia and Mediterranean regions. Chickpea is highly valued for its carbohydrate and protein content, as well as for being rich in dietary fiber, essential vitamins, and minerals. In 2021, worldwide chickpea production reached close to 18.7 million metric tons, with projections estimating an increase to 25.1 million tons by 2027, corresponding to a compound annual growth rate (CAGR) of 5.08% for the period 2022–2027. India consistently leads as the largest global producer, contributing approximately 10–11 million tons annually and making up about 70% of global output. During the rabi season of 2020–21, Indian farms had about 111.99 lakh hectares (276.73 lakh acres) under bengal gram cultivation. Specifically, Telangana state reported about 1.43 lakh hectares (3.53 lakh acres) sown with bengal gram in that season, with the leading producing districts being Kama Reddy, Adilabad, Nirmal, Nizamabad, and Gadwal (Anonymous, Bengal Gram Outlook, May 2021)
With ongoing economic and population growth, there is a rising demand for both biological resources and energy. Sustainability concerns are fueling a shift from linear, fossil-driven economies to environmentally conscious circular bioeconomy models (Mgbechidinma *et al*., 2022) [5]. Surfactants, which are frequently used in food, pharmaceuticals, cleansing products, agriculture, and the oil and gas sectors, are increasingly scrutinized for environmental impact. Traditional surfactants derived from fossil fuel sources can cause environmental pollution, pose health risks, and offer limited biodegradability (Nagtode *et al*., 2023) [6].
Biosurfactants improve the accessibility and conversion of hydrophobic nutrients into non-toxic products, supporting microbial growth. By reducing surface tension, biosurfactants increase the interaction surface for insoluble substances, thereby enhancing their mobility, bioavailability, and breakdown in the environment. They are typically favored over chemical surfactants owing

to their simple molecular profile, higher stability, superior foam formation, low toxicity, broad pH functionality, environmental safety, and biodegradability.

Due to low toxicity and eco-friendly properties, biosurfactants have found roles in industries such as cosmetics, pharmaceuticals, oil, and food. Several microbial genera including *Pseudomonas*, *Acinetobacter*, *Bacillus*, *Rhodococcus*, and *Candida* are recognized as natural producers of biosurfactants (Pardhi *et al*., 2022) [7]. Multiple studies have documented that these microbes can utilize inexpensive carbon sources—such as waste vegetable oils—to generate biosurfactants. Such organisms are widespread in various environments worldwide, particularly in soils (Bodour *et al*., 2003) [4]. Many plant-associated bacteria, especially from the genus *Pseudomonas*, are known for synthesizing cyclic lipopeptides and rhamnolipids, which have functions fundamental for bacteria-plant and bacteria-bacteria interactions. Biosurfactants can alter microbial surface properties, impacting processes such as surface motility, biofilm formation, and root colonization. They can modify the bioavailability of external chemicals, improving their uptake, as well as influence endogenous metabolite distribution, sometimes boosting biological activities such as antibiotic production. The antimicrobial effects of biosurfactants may contribute to competition, defense, and even pathogenicity. This research aims to isolate rhizosphere microorganisms and assess their potential for biosurfactant or bioemulsifier production, along with their plant growth-promoting abilities. Evaluation methods will include blood agar tests, oil spreading assays, measurements of surface tension, and emulsification index. Additional assessments will cover traits such as siderophore and ammonia production, gibberellic acid and IAA synthesis, and phosphate solubilization abilities of the isolates.

2. Materials and Methods

1. Collection of soil samples

Soil samples were collected from the rhizosphere soil of different crops at the college farm, Agricultural College, Rajendranagar, Hyderabad. Five hundred grams (500 g) of each crop's rhizosphere soil sample were collected in sterile polythene bags, labeled appropriately, and stored in a refrigerator at a temperature of 4°C for further study in the laboratory.

2. Isolation of biosurfactant producing bacteria by enrichment technique

During the isolation of biosurfactant-producing bacteria, the original method described by Arati (2023) was initially employed. However, this method yielded inconsistent results and limited success in isolating biosurfactant-producing bacteria. To improve the effectiveness of the isolation process, slight modifications were made to the original protocol. The modified method resulted in a better yield of biosurfactant-producing bacteria, suggesting that the changes made to the isolation procedure had a positive impact on the outcome. The modified method involved a six-fold serial dilution, followed by the spread plate method for inoculation. One milliliter (1 mL) of the 10^{-6} dilution was inoculated onto sterile Petri plates containing sterile media with 1% (v/v) crude oil. The inoculum was uniformly spread with sterile spreaders. The plates were incubated at 37 °C for 24 hours. After incubation, morphologically distinct colonies were subcultured on agar plates to obtain pure cultures of the organisms, which were subsequently transferred to nutrient agar slants. The slants were stored in a refrigerator at 4 °C as stock cultures

The bacterial isolates were screened for biosurfactant production by growing the isolates in test tubes containing 15 mL of nutrient broth. A loopful of bacterial culture was inoculated into a test tube containing 15 mL of sterilized medium and incubated in a shaker at 30^0C for 7 days in incubator. After 7 days of incubation, the culture broth was centrifuged at 6000 rpm at 4^0C for 15 minutes and the bacterial cell free supernatant were used for qualitative and quantitative assays.

3. Purification and maintenance of different bacterial isolates

3.1 Streak Plate Method

Forty bacterial strains were checked for purity prior to characterization and preservation. Purification of the bacterial isolates was achieved using the quadrant streak plate method with suitable media and growth conditions. A portion of well-isolated colonies was selected and streaked onto suitable media under aseptic conditions, followed by incubation at 30°C for 24-48 hours.

3.2 Maintenance of Isolates

The purified isolates were preserved on nutrient agar and starch casein agar slants for further analysis. Additionally, 20% glycerol stocks were prepared for long-term preservation of the cultures.

4. Characterization of the bacterial isolates

The bacterial isolates were identified based on their morphological and biochemical characteristics according to the standard methods described in Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1984)

4.1 Morphological characterization

The isolates selected for further study were inoculated onto nutrient agar plates and incubated at 30°C for a period of 24 hours. After incubation, colony characteristics such as pigmentation, shape, and elevation were recorded. Further, microscopic analysis was conducted to determine the Gram reaction, in accordance with established protocols described by Cappuccino and Sherman (2002)

4.2 Gram Staining

After 48 hours of incubation, bacterial cultures were subjected to Gram staining following the method described by Buchanan and Gibbons (1974) [3]. Gram-stained slides were prepared using heat-fixed bacterial smears. These stained slides were then examined under a light microscope with oil immersion at 1000× magnification (100× objective lens and 10× eyepiece). Gram-positive bacteria appeared bluish-purple, while Gram-negative bacteria showed reddish or pink coloration.

3.4.3 Biochemical Characterization

Various biochemical assays were conducted according to standard protocols. A brief overview of the procedures followed is provided below.

4.3 Catalase test

The catalase test was performed to detect the presence of the catalase enzyme in bacterial colonies. A drop of 30% H_2O_2 was added to 24-hour-old pure isolates on glass slides. The appearance of gas bubbles indicated catalase enzyme activity, confirming the presence of the enzyme in the bacterial isolates

4.4 Indole production test
Tryptone broth tubes were used to conduct the indole production test. The broth tubes were inoculated with specific bacterial isolates and incubated at 30°C for 48 hours. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The development of a red color was recorded as positive for indole production, indicating that the isolates were capable of producing indole

4.5 Oxidase test
Oxidase discs were taken. The smear of pure bacterial isolates was flooded on oxidase discs. Bright purple color was formed within 30 sec indicated as positive for oxidase test (Collins and Lyne, 1970).

4.6 Citrate utilization
Isolates were streaked on Simmon's citrate agar slants and incubated at 30ºC for 24 hours. Change in colour from green to blue indicated positive reaction for citrate utilization (Macfaddin, 2000).

4.7 Methyl red
The methyl red test was performed by inoculating sterilized glucose phosphate broth tubes with the bacterial culture, which were then incubated at 30°C for 48 hours. After the incubation period, five drops of methyl red indicator were added to each tube, followed by gentle mixing. A positive test was indicated by the appearance of a red color, while a yellow coloration signified a negative result (Macfaddin, 2000).

Results and Discussion
Cultural and morphological characterization of bacterial isolates from rizhosphere soil of different crop.

Among the 40 isolates (Table 2), Gram staining revealed that the majority of bacterial isolates were Gram-positive, as indicated by a positive (+) reaction. This was observed in 34 isolates (PLATE.1.), including BS2, BS3, BS4, BS5, BS6, BS7, BS9, BS10, BS11, BS12, BS13, BS14, BS15, BS17, BS18, BS19, BS20, BS21, BS22, BS23, BS24, BS25, BS26, BS27, BS28, BS29, BS30, BS31, BS32, BS33, BS34, BS36, BS38, BS39, and BS40. In contrast, five isolates, namely BS1, BS8, BS16, BS35, and BS37, were Gram-negative (-) (PLATE 1). The Gram reaction is an essential characteristic that helps classify bacterial isolates based on their cell wall structure, which can influence their physiology and interactions with plants and the rhizosphere environment

The outcomes of this study align well with those reported by Al-Tamimi and Bader (2021) [2]. Numerous microorganisms have the capacity to synthesize biosurfactants with beneficial characteristics. In this research, fifteen bacterial isolates were collected, comprising nine from soil and six from aquatic environments. Morphological assessment revealed that ten isolates were Gram-negative, while five were Gram-positive. All isolates underwent screening for biosurfactant production through a series of tests including hemolytic activity, drop collapse, emulsification capacity, oil displacement, and foam formation evaluations.

The 40 isolates (Table 2) displayed a range of colony pigmentation, suggesting differences in their metabolic capabilities and adaptation strategies. The colors ranged from white (e.g., BS1, BS5, BS15) and milky white (BS2, BS20) to pink (BS3, BS6, BS14), yellow (BS4, BS10, BS12), orange (BS13, BS26, BS40), and red (BS39). This pigmentation diversity may play roles in UV protection, antioxidative defense, and mediating interactions with other microorganisms or plants. The variety of pigmentation observed indicates a diverse microbial community that is well-adapted to different ecological niches in the rhizosphere.

Microscopic examination of the 40 isolates from different crop rhizosphere soils (Table 2) revealed a diverse range of cell shapes. Several isolates, such as BS1, BS3, BS11, BS12, BS15, BS30, and BS33, exhibited cocci (spherical) morphology, while others, like BS2, BS4, BS6, BS7, BS8, BS16, BS20, BS21, BS27, BS31, BS36, BS37, BS38, and BS40, were rod-shaped, ranging from short to long rods. Some isolates (e.g., BS17, BS18, BS19, BS28, BS29) displayed irregular shapes. This morphological diversity reflects the presence of different bacterial classes and their adaptations to rhizosphere environments, which can influence nutrient uptake and colonization efficiency.

The 40 isolates (Table 2) displayed a range of bacterial colony margin morphologies, primarily categorized as lobate, round, and undulate. Most isolates, including BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS9, BS10, BS14, BS16, BS17, BS18, BS19, BS20, BS23, BS24, BS25, BS26, BS27, BS28, and BS29, exhibited lobate margins, characterized by uneven or indented edges, likely resulting from motility or growth patterns. In contrast, isolates such as BS1 and BS13 had round margins, denoting smooth, circular colony borders. Additionally, isolates including BS11, BS12, BS15, BS21, and BS22 had undulate margins, characterized by wavy edges. These differences in colony margin morphology contribute to the identification and characterization of bacterial species and influence colony expansion dynamics.

The colony elevation of the 40 isolates (Table 2) varied, with most producing convex colonies with a dome-shaped elevation, including BS1, BS2, BS3, and others, indicative of typical bacterial growth. Some isolates, such as BS11, BS13, BS14, BS15, BS21, BS22, BS23, BS28, BS29, and BS30, displayed raised colonies. Colony elevation shapes are significant in bacterial physiology and serve as useful taxonomic markers during isolation and identification.

Among the 40 isolates (Table 2), the texture of the colonies varied from moist to dry or slimy. A majority of isolates, including BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS16, BS17, BS18, BS19, BS20, BS21, BS22, BS23, BS24, BS25, BS26, BS27, BS28, BS29, BS30, BS31, BS32, BS33, BS34, BS35, BS36, BS38, and BS40, exhibited moist textures, suggesting active growth and high-water content. In contrast, some isolates, such as BS9, produced shiny colonies, while others, including BS10, BS13, BS14, BS15, BS39, and BS11, displayed dry or slimy surfaces. Notably, BS30 was previously mentioned as having a moist texture, so clarification might be needed for consistency. Texture variations may influence bacterial survival, adhesion properties, and interactions with the environment

Collectively, these morphological and cultural characteristics provide valuable insights into the diversity and ecological adaptability of bacterial isolates from rhizosphere soils, forming an essential basis for their identification and for understanding their functional roles in plant-microbe interactions.

Our study is similar to that of Ahmed-Umar *et al*. (2024) [1]. Biochemical and morphological characterization was performed on the isolates before testing their hydrocarbon-degrading abilities using engine oil-enriched Bushnell Haas (BH) agar and screening for biosurfactant production. Twenty pure cultures were isolated and morphologically found to be predominantly cocci and rod-shaped.

Biochemical characterization of bacterial isolates from the rhizopshere soil of different crops.

The catalase test was conducted on the 40 isolates (Table 3) to assess their ability to produce catalase, an enzyme that decomposes hydrogen peroxide into water and oxygen, serving as a marker for aerobic metabolism. A majority of the isolates exhibited positive catalase activity, including BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS9, BS10, BS12, BS16, BS20, BS21, BS22, BS23, BS24, BS25, BS26, BS27, BS28, BS29, BS30, BS31, BS32, BS33, BS34, BS38, and BS40 (PLATE 2). In contrast, some isolates, such as BS1, BS11, BS13, BS14, BS17, BS18, and BS19, were catalase-negative, indicating variability in their oxidative stress response mechanisms. The presence of catalase reflects the bacterial capability for aerobic respiration and resistance to reactive oxygen species, contributing to survival and functionality in diverse environments.

Indole production, a trait indicative of the ability to metabolize tryptophan into indole compounds, was assessed among the 40 isolates (Table 3). A positive indole reaction was recorded for isolates including BS1, BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS9, BS10, BS11, BS13, BS16, BS18, BS19, BS20, BS21, BS22, BS23, BS24, BS29, BS30, BS31, BS34, BS37, and BS40 (PLATE 3). Conversely, negative indole production was observed in isolates such as BS12, BS14, BS15, BS17, BS25, BS26, BS27, BS28, BS32, BS33, BS35, BS36, and BS38. Indole production is often associated with plant growth-promoting attributes, as indole derivatives can influence root development and microbial signaling in the rhizosphere, highlighting the potential role of these isolates in promoting plant growth.

The Methyl Red (MR) test, which detects mixed acid fermentation, was conducted on the 40 isolates (Table 3) and revealed variable results. Several isolates, including BS1, BS3, BS4, BS5, BS6, BS14, BS15, BS16, BS17, BS18, BS20, BS21, BS23, BS29, BS30, BS31, BS34, and BS35, were MR-positive, indicating their ability to produce stable acid end products during glucose fermentation. In contrast, isolates including BS2, BS7, BS8, BS9, BS10, BS11, BS12, BS13, BS19, BS22, BS24, BS25, BS26, BS27, BS28, BS32, BS33, BS36, BS37, and BS40 were MR-negative. These results highlight the diversity in fermentation pathways, reflecting the metabolic versatility among the isolates

Among the 40 isolates (Table 3), the oxidase test results revealed contrasting fermentation profiles. A positive VP test, indicating acetoin production from glucose metabolism, was observed in isolates such as BS3, BS4, BS5, BS6, BS8, BS10, BS11, BS12, BS13, BS14, BS15, BS16, BS17, BS18, BS20, BS21, BS22, BS23, BS24, BS25, BS27, BS29, BS31, BS33, BS35, BS38, and (BS40 PLATE 3). In contrast, negative reactions were observed in isolates BS1, BS2, BS7, BS9, BS19, BS26, BS28, BS30, BS32, BS34, BS36, and BS37. The VP test complements the Methyl Red (MR) test, together defining the predominant fermentative pathway utilized by the microbes and providing insights into their metabolic characteristics.

Among the 40 isolates (Table 3), the Simmon's citrate utilization test assessed the ability of isolates to use citrate as a sole carbon source. A majority of isolates, including BS1, BS2, BS3, BS4, BS5, BS6, BS7, BS8, BS9, BS10, BS12, BS13, BS14, BS15, BS16, BS17, BS18, BS19, BS20, BS21, BS22, BS23, BS24, BS25, BS26, BS29, BS30, BS31, BS32, BS34, BS38, BS39, and BS40 (PLATE 3), showed positive citrate utilization. In contrast, certain isolates, such as BS11, BS27, BS28, BS33, BS35, BS36, and BS37, did not grow on citrate, indicating negative citrate usage. This test reflects the metabolic adaptability of the isolates related to carbon source utilization, which may influence their survival and ecological fitness in diverse environments

Together, these biochemical tests provide comprehensive insights into the metabolic profiles of the isolates, revealing diverse capabilities in respiration, fermentation, carbon metabolism, and plant growth-promoting potential. Our study's findings are consistent with those of Ahmed-Umar *et al*. (2024) [1]. Biochemical tests revealed that 11 isolates produced catalase only, 1 produced oxidase only, while 7 were positive for both oxidase and catalase tests. Fifty-five percent of the isolates were positive for biosurfactant production using both drop collapse and oil spread assays, while 45% were negative. Seventy-five percent of the isolates emulsified engine oil, while 25% did not. All 20 isolates degraded hydrocarbons in both new and spent engine oil-enriched BH agar. Biochemical and morphological characterization was performed on the isolates before testing their hydrocarbon-degrading abilities using engine oil-enriched Bushnell Haas (BH) agar and screening for biosurfactant production.

Comparable findings were reported by Saravanan *et al*. (2012) [8]. The capacity of microorganisms isolated from soil to produce biosurfactants was evaluated through several methods, including the hemolysis assay, drop collapse test, emulsification index measurement, and the methylene blue agar plate technique. Among the isolates, PB3A showed the highest biosurfactant production and was identified as *Pseudomonas aeruginosa* based on both microscopic examination and biochemical tests, following the guidelines outlined in Bergey's Manual of Determinative Bacteriology.

Table 2: Morphological and cultural characteristics of different bacterial isolates

Isolate name	Gram Reaction	Shape of cell	Pigmentation	Margin	Surface of whole colony	Texture
BS1	-	Cocci	White	Round	Convex	Dry
BS2	+	Short rod	Milky white	Lobate	Convex	Moist
BS3	+	Cocci	Pink	Lobate	Convex	Moist
BS4	+	Short rod	Yellow	Lobate	Convex	Moist
BS5	+	Round	White	Lobate	Convex	Moist
BS6	+	Short rod	Pink	Lobate	Convex	Moist
BS7	+	Long rod	White	Lobate	Convex	Moist
BS8	-	Short rod	White	Lobate	Convex	Moist
BS9	+	Round	White	Lobate	Convex	Shiny
BS10	+	Round	Yellow	Lobate	Convex	Slimy
BS11	+	Cocci	Pink	Undulate	Raised	Moist
BS12	+	Cocci	Yellow	Round	Convex	Dry
BS13	+	Short rod	Orange	Undulate	Raised	Dry
BS14	+	Short rod	Pink	Undulate	Raised	Dry
BS15	-	Short rod	White	Undulate	Raised	Slimy

BS16	+	Irregular	White	Lobate	Convex	Moist
BS17	+	Irregular	White	Lobate	Convex	Moist
BS18	+	Irregular	Yellow	Lobate	Convex	Moist
BS19	+	Irregular	White	Lobate	Convex	Moist
BS20	+	Short rod	Milky white	Lobate	Convex	Moist
BS21	+	Short rod	White	Undulate	Raised	Moist
BS22	+	Long rod	Pink	Undulate	Raised	Moist
BS23	+	Round	Yellow	Undulate	Raised	Moist
BS24	+	Round	White	Lobate	Convex	Moist
BS25	+	Round	White	Lobate	Convex	Moist
BS26	+	Short rod	Orange	Lobate	Convex	Moist
BS27	+	Long rod	Yellow	Lobate	Convex	Moist
BS28	+	Irregular	White	Lobate	Convex	Moist
BS29	+	Irregular	Yellow	Lobate	Convex	Moist
BS30	+	Cocci	White	Round	Convex	Moist
BS31	+	Round	White	Round	Convex	Moist
BS32	+	Cocci	Yellow	Round	Convex	Moist
BS33	+	Round	White	Round	Convex	Moist
BS34	+	cocci	Milky white	Lobate	Convex	Moist
BS35	+	Rod	White	Lobate	Convex	Moist
BS36	+	Rod	White	Lobate	Convex	Dry
BS37	+	Rod	Yellow	Lobate	Convex	Slimy
BS38	+	Rod	Red	Lobate	Convex	Dry
BS39	+	Short rod	White	Lobate	Convex	Dry
BS40	+	Long rod	Orange	Lobate	Convex	Moist

"+": Positive and "-": Negative

Table 3: Biochemical characteristics of different bacteria isolates

Isolate name	Catalase	Indole production	Methyl red test	Oxidase test	Simmon citrate test
BS1	-	+	+	-	+
BS2	+	+	-	-	+
BS3	+	+	+	+	+
BS4	+	+	+	+	+
BS5	+	+	+	+	+
BS6	+	+	+	+	+
BS7	+	+	-	-	+
BS8	+	+	-	+	+
BS9	+	+	-	-	+
BS10	-	+	-	+	+
BS11	+	+	-	+	-
BS12	-	-	-	+	+
BS13	-	+	+	+	+
BS14	+	-	+	++	+ +
BS15	++	+	+	+	-
BS16	-	+	+	+	+
BS17	-	+	+	+	+
BS18	+	+	+	+	+
BS19	+	+	++	-	+
BS20	+	-	+	+	+
BS21	+	-	+	+	+
BS22	+	+	-	+	+
BS23	+	+	++	-	+
BS24	+	-	-	+	-
BS25	+	-	-	+	-
BS26	+	+	+	+	-
BS27	+	+	-	-	+
BS28	+	+	-	-	+
BS29	+	+	++	-	++
BS30	+	-	+	++	-
BS31	+	+	+	+	-
BS32	+	-	+	-	+
BS33	+	+	-	+	
BS34	+	-	-	-	+
BS35	-	-	-	+	-
BS36	-	-	-	-	+
BS37	-	+	+	-	-
BS38	+	-	+	-	+
BS39	-	+	-	+	+
BS40	+	+	+	+	+

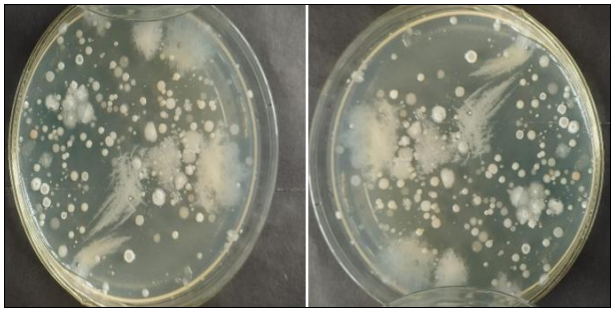

Plate 1: Isolation of bacteria from crop rhizophere soil by using different media

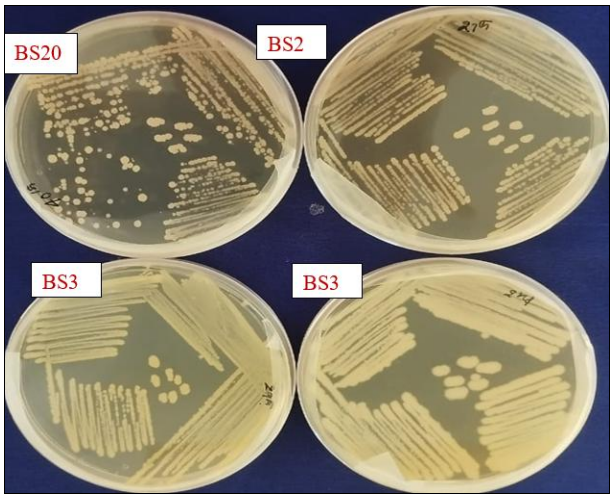

Plate 2: purification of selected isoltes

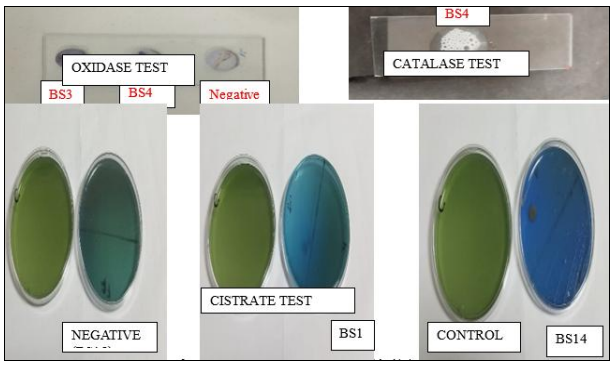

Plate 3: Tephrosia candida alley cropping system with intercrops under field conditions at Ranchi

Conclusion

The study characterized bacterial isolates from crop rhizosphere soil, highlighting their potential as bio-inoculants for sustainable agriculture, promoting plant growth, stress tolerance, and contributing to novel bio-inoculant development, while emphasizing the importance of rhizosphere soil as a source of beneficial microorganisms.

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