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Characterization of *Mesorhizobium ciceri* isolates from chickpea root nodules: A biochemical approach

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Abstract
Root nodules of chickpea plants from 44 locations in Maharashtra and Madhya Pradesh were collected for isolating *Mesorhizobium ciceri*. Isolation involved direct inoculation on Congo Red Yeast Extract Mannitol Agar medium. Isolates underwent screening tests (Glucose Peptone Agar, Hofer's alkaline, Ketolactose agar) for authentication. 22 Positive isolates were further tested biochemically and enzymatically, revealing variability in reactions. While most isolates showed typical reactions for certain tests (e.g., citrate utilization, gelatin liquefaction), they varied significantly in others (e.g., starch hydrolysis, H2S production). Preference for glucose, sucrose, and mannitol as carbon sources was noted, with mixed reactions in the skim milk test. These findings underscore the importance of thorough screening during isolation to authenticate *Mesorhizobium* and highlight the metabolic and indeed genetic diversity among strains.

Keywords: *Mesorhizobium ciceri*, chickpea root nodules, biochemical diversity

Introduction
Biological nitrogen fixation is of paramount importance as it converts atmospheric nitrogen gas (N2) into a biologically usable form, ensuring a sustainable nitrogen supply for living organisms. This process plays a crucial role in maintaining soil fertility, promoting plant growth, and enhancing agricultural productivity. Pulses, such as soybeans, peas, lentils, and chickpeas, are particularly significant for biological nitrogen fixation due to their symbiotic relationship with nitrogen-fixing rhizobia bacteria. This symbiosis occurs in root nodules, enabling these plants to convert atmospheric nitrogen into ammonia, a usable form, through the enzyme nitrogenase. This reduces their reliance on external nitrogen sources and enhances soil fertility. Chickpea (*Cicer arietinum* L.) is an annual plant belonging to the Fabaceae family, subfamily Faboideae. Also known as garbanzo beans, chickpeas are highly nutritious legumes. The nitrogen-fixing bacteria associated with chickpeas is *Mesorhizobium ciceri*. Chickpea rhizobia are classified within the genus *Mesorhizobium*, which falls between *Rhizobium* and *Bradyrhizobium*. The *Mesorhizobium* genus is a well-described rhizobial genus within the Rhizobiales order, comprising a significant number of nodulating rhizobial species (Laranjo *et al*., 2014) [11].

The diversity among various strains of *Rhizobium* is crucial for selecting optimal isolates from diverse geographical locations. This diversity can be assessed in strains isolated from chickpea root nodules through a series of preliminary biochemical tests, laying the groundwork for more in-depth analyses. These screening and biochemical assays are pivotal, not only for discerning variations among strains but also for primary bacterial identification. Native strains of *Rhizobium* are essential for efficient nodulation and other beneficial properties due to their adaptation to local environmental conditions. They exhibit high nodulation efficiency, having evolved in specific soil conditions, ensuring optimal nitrogen fixation. Additionally, their symbiotic specificity with specific legume host plants enhances successful nodulation. These strains are also better adapted to local environmental factors, such as soil pH, temperature, and moisture levels, increasing their competitiveness and survival in the soil. Therefore, the present study was planned with the objective of isolating *M. ciceri* bacteria from chickpea root nodules from different districts of Maharashtra and Madhya Pradesh and their biochemical characterization, to detect the diversity present among the isolates.

Materials and Methods

A) Isolation of *Mesorhizobium ciceri*

The root nodules of chickpea plants were collected from 44 locations in Maharashtra and Madhya pradesh at the flowering stage. The samples were then brought to the laboratory for isolation of *Mesorhizobium ciceri*. Isolation was carried out using the direct inoculation method on Congo Red Yeast Extract Mannitol Agar medium, following the standard procedure described by Vincent (1970) [20]. Chickpea plants at the flowering stage were carefully uprooted and brought to the laboratory. The root nodules were washed under running tap water to remove soil particles, and only pink-colored nodules were selected for isolation. The selected nodules were surface sterilized by dipping in 0.1% $HgCl_2$ for 30 seconds, followed by 3-5 washes with sterile distilled water. These surface-sterilized nodules were then crushed in a drop of sterile water using a sterile glass rod in a test tube. The aliquot was streaked on CRYEMA medium using the quadrant streaking method, and plates were incubated at 28±2 °C for 3-5 days in the dark, following the procedure described by Vincent (1970) [20]. Different single colonies of bacteria were screened for the absorption of Congo red dye from the medium, and characteristic *Mesorhizobium* colonies (white, opaque, raised, gummy, and translucent) were selected for further study.

B) Screening test

The all 35 isolates were screened for following different cultural and biochemical tests for authentication of *Mesorhizobium ciceri*. The tests were carried out to reduce the contamination of common contaminant Agrobacterium which was found in close association in nodules of legume crops.

Growth on Glucose Peptone Agar (GPA) medium

All isolates were streaked on glucose peptone agar medium and incubated at 28 °C for 3-5 days (Vincent 1970) [20]. Rhizobial isolates show no or very poor growth on glucose peptone agar medium and cause very little or no change in pH. The agrobacterium shows the growth on the medium.

Growth on Hoffer's Alkaline Medium

The isolates were inoculated in Hofer's Alkaline Medium (pH 11) and observation for growth was made after 4-5 days (Hofer 1935) [7]. The rhizobial isolates did not grow at pH 11.

Growth on Ketolactose medium

Lactose agar medium was prepared and bacterial isolates were streaked on plates. The plates were incubated at 28 °C for 3-5 days after the growth of culture on the plate these were flooded with benedict solution. The bacteria which produced 3 keto lactose from lactose, exhibited yellow color formation after adding the reagent which was not a characteristic feature of *Rhizobium*.

YEMA – BTB reaction

This test was done for differentiation between fast and slow growers on the Bromothymol blue media: Yeast Mannitol Broth (YMB) incorporated with bromothymol blue was used to distinguish fast-(acid producing) growing strains from slow (non-acid producing or alkali producing) growing rhizobia (Somasegaran *et al*., 1994) [18]. In this medium, the fast growers required 48 hours to produce an acidic reaction by turning the color of the media yellow from green, whereas the slow growers took more than 96 hours to produce alkaline endpoints with or without changing the color of the media from green to blue.

C) Biochemical test

The isolates which shows positive reactions in screening test were selected for the biochemical studies. The various biochemical tests like Methyl Red, KOH test, VP test, starch hydrolysis, Citrate utilization, H_2S production etc. were carried out for biochemical confirmation of *Mesporhizobium ciceri.* The various enzymatic tests also carried out to find the enzyme producing ability of isolates.

D) Testing of carbon sources

Carbon sources viz., glucose, starch, fructose and xylose were incorporated in basal media replaced on molecular basis with mannitol in yeast extract mannitol (YEM) broth. Yeast Mannitol broth was served as control and observations were recorded after 72 hrs of incubation. The YEM broth is supplemented with phenol red which initially remains pink in color and after the production of acid changes to yellow.

E) Skim milk test

The skim milk test were performed by inoculating different isolates of *M. ciceri* in skim milk media. The skim milk media were prepared by dissolving 100 g powdered skim milk in 1000 ml distilled water. The pH indicator methyl red were added to observe the medium for changes, including color, consistency, and gas production, which indicate specific metabolic activities.

These changes can include lactose fermentation (acid production turning the medium pinkish red), alkaline reactions (turning the medium light blue), curd formation, litmus reduction, proteolysis (resulting in a translucent zone), and gas production

Result and Discussion

A) Isolation of *M. Ciceri* chick pea root nodules.

The root nodule isolates were streaked on CRYEMA media and incubated at 28 °C for 3-5 days. They showed pinkish-white colonies with intact margins. Out of 44 isolates, 29 isolates showed fast growth, 6 grew slowly, and 9 showed no growth, likely due to nodule deterioration during transportation or lab contamination. (Table 1.) Isolates that absorbed little or no congo red dye were purified, appearing white or slightly pink. Contaminated colonies were rejected. Isolates were labeled as RC1, RC2, etc. The procedure for isolating *Mesorhizobium ciceri* involved extracting bacteria from chickpea root nodules, following methodologies used in studies by Kucuk *et al*. (2006) [9] and Neamat (2003) [13]. This approach, focusing on root nodules rather than rhizospheric soil, is consistently highlighted in research such as studies by Zafar *et al*. (2017) [23], Nagalingam *et al*. (2020) [12], and Shahzad *et al*. (2012) [15]. Setu *et al*. (2019) [14] also support this method, offering a standardized protocol for *Rhizobium* identification from root nodules.

Table 1: Details of samples collected from different regions of Maharashtra for isolation of rhizobium from chickpea nodules on CR-YEMA.

Sr. No.	Isolate code	District	Tehsil	Location	Variety	Color of Nodules	Type of Growth
1.	RC1	Nagpur	Nagpur	Botany field, COA,	JAKI-9218	Red	Fast
2.	RC2	Yavatmal	Kalamb	Kalamb	JAKI-9218	Pink	Fast
3.	RC3	Amravati	Daryapur	Vadner gangai	JAKI-9218	Red	Slow
4.	RC4	Akola	Akola	Agronomy field, PDKV Akola.	JAKI-9218	Pink	Slow
5.	RC5	Buldhana	Deol gaon raja	Deolgaon raja	VIJAY	Red	Slow
6.	RC6	Nanded	Naygaon	Lohgaon	VIJAY	Pink	Fast
7.	RC7	Baramati	Baramati	ICAR-NCASM, Baramati	PHULE VIKRANT	Red	Fast
8.	RC8	Jabalpur	Jabalpur	Botany Field, JNKVV.	JAKI-9218	Pink	Fast
9.	RC9	Nagpur	Katol	Katol	JAKI-9218	Red	Fast
10.	RC10	Wardha	Wardha	Samudrapur	JAKI-9218	Pink	No growth
11.	RC11	Gadchiroli	Aheri	Aheri	JAKI-9218	Red	Fast
12.	RC12	Chandrapur	Chandrapur	Nandori	VIJAY	Pink	Fast
13.	RC13	Nashik	Niphad	Kothure	VISHAL	Red	Fast
14.	RC14	Jalgaon	Jalgaon	Paladhi	VISHAL	Pink	Fast
15.	RC15	Nashik	Yeola	Yeola	VIJAY	Pink	No growth
16.	RC16	Washim	Washim	Shelu bazar	BDNG-27	Pink	Fast
17.	RC17	Jalana	Ambad	Ambad	JAKI-9218	Pink	Fast
18.	RC18	Katni	Katni	Gugad	JAKI-9218	Red	Fast
19.	RC19	Chindwada	Pandhurna	Pandhurna	JAKI-9218	Pink	Fast
20.	RC20	Kolhapur	Kolhapur	Koparde	JAKI-9218	Pink	Fast
21.	RC21	Sanagli	Miraj	Miraj	JAKI-9218	Pink	Fast
22.	RC22	Satara	Karad	Karad	JAKI-9218	Pink	Fast
23.	RC23	Nandurbar	Shahada	Mhaswad	DIGVIJAY	Pink	Fast
24.	RC24	Solapur	Solapur	Kumbhari	JAKI-9218	Red	Fast
25.	RC25	Jabalpur	Jabalpur	Agronomy field, JNKVV.	DIGVIJAY	Pink	Fast
26.	RC26	Amaravati	Anjangaon	Anjangaon	VIJAY	Pink	Fast
27.	RC27	Sangli	Kasegaon	Kasegaon	JAKI-9218	Pink	No growth
28.	RC28	Dhule	Dhule	Shirpur	DIGVIJAY	Pink	Fast
29.	RC 29	Jalgaon	Jalgaon (kh)	Agronomy field, Dr. UPCA, Jalgaon	VIJAY	Pink	No growth
30.	RC 30	Nagpur	Nagpur	AICRP field	JAKI-9218	Pink	Slow
31.	RC31	Nagpur	Nagpur	Jamtha	JAKI-9218	Red	No growth
32.	RC32	Nagpur	Nagpur	Hingna	JAKI-9218	Pink	Fast
33.	RC33	Nashik	Dindori	Talegaon	JAKI-9218	Red	Fast
34.	RC34	Amravati	Chandur railway	Chandur railway	JAKI-9218	Pink	Slow
35.	RC35	Amravati	Cahndur bazar	Boraj	JAKI-9218	Pink	No growth
36.	RC36	Nanded	Nanded	Ardhapur	DIGVIJAY	Pink	Fast
37.	RC37	Gondia	Sadak arjuni	Sadak arjuni	JAKI-9218	Pink	No growth
38.	RC38	Gondia	Amgaon	Amgaon	VIJAY	Red	Fast
39.	RC39	Bhandara	Tumsar	Tumsar	JAKI-9218	Pink	Fast
40.	RC40	Gadchiroli	Gadchiroli	Chamorshi	JAKI-9218	Pink	No growth
41.	RC41	Akola	Borgaon manju	Borgaon manju	JAKI-9218	Red	Fast
42.	RC42	Wardha	Wardha	Wardha	JAKI-9218	Pink	Fast
43.	RC43	Jalana	Jalana	Ranmurti	VIJAY	Pink	Slow
44.	RC44	Aurangabad	Gangapur	Gangapur	JAKI-9218	Red	No growth

B) Screening test

All 35 isolates which showed growth were streaked on glucose peptone agar medium and incubated at 28 °C for 3-5 days. Twenty-two isolates showing no or very poor growth were considered positive for the screening test, while 13 isolates with luxurious growth were discarded. The isolates inoculated in Hofer's Alkaline medium (pH 11), and growth was observed after 3-5 days. 22 isolates showing no growth in the alkaline medium were considered positive, while 13 isolates showing growth were discarded. (Table 2)

All isolates were streaked on ketolactose agar, and growth was observed after 5 days. The plates were flooded with Benedict's solution. Negative isolates for the *Mesorhizobium* screening test showed yellow color formation, while positive isolates showed no reaction. Twenty-two isolates showed a positive reaction, with no yellow color formation. In the BTB test, fast and slow-growing isolates were identified based on acid and alkali production. Out of 35 isolates, 19 produced an alkaline reaction, and 16 produced an acidic reaction in YEM broth supplemented with Bromothymol blue.

In the present study, 22 isolates did not absorb Congo red color when streaked on YEMA-CR media. This characteristic differentiates *Rhizobium* from Agrobacterium and other bacterial contaminants, as noted by Trinick *et al*. (1982) [19]. In the Keto-lactose test, no yellow zone was observed around the colonies after adding Benedict's reagent, which is characteristic of *Rhizobium*. Similar results were observed by Deshwal and Chaubey (2014) [3]. When inoculated in BTB media, color changes from deep green to yellow indicate that the isolates were acid producers and fast growers, which are characteristics of *Rhizobium*. Similar results were observed by De-Vries *et al*. (1980) [4] and Singh *et al*. (2008) [17].

Table 2: Screening tests for authentication of *Mesorhizobium* from collected isolates.

Bacterial Isolate	KLA	Growth On GPA	BTB Broth Reaction	Hoffers Alkaline	Result
RC1	Negative	Very Poor	Alkaline	No Growth	Positive
RC2	Negative	Poor	Alkaline	No Growth	Positive
RC3	Positive	Poor	Alkaline	Growth	Negative
RC4	Negative	Good	Acidic	No Growth	Positive
RC5	Negative	Poor	Acidic	Growth	Negative
RC6	Positive	Poor	Alkaline	Growth	Negative
RC7	Negative	No Growth	Acidic	No Growth	Positive
RC8	Negative	Poor	Alkaline	No Growth	Negative
RC9	Positive	Poor	Alkaline	Growth	Negative
RC11	Negative	Poor	Alkaline	No Growth	Positive
RC12	Negative	Poor	Alkaline	No Growth	Positive
RC13	Negative	Poor	Alkaline	No Growth	Positive
RC14	Negative	Very Poor	Acidic	No Growth	Positive
RC16	Positive	Poor	Alkaline	Growth	Negative
RC17	Negative	Poor	Alkaline	No growth	Negative
RC18	Negative	Very Poor	Alkaline	No Growth	Positive
RC19	Positive	Poor	Alkaline	Growth	Negative
RC20	Negative	Poor	Acidic	No Growth	Positive
RC21	Negative	Poor	Alkaline	No Growth	Positive
RC22	Negative	Poor	Acidic	No Growth	Positive
RC23	Negative	Poor	Alkaline	No Growth	Positive
RC24	Negative	Very Poor	Acidic	Growth	Negative
RC25	Negative	Poor	Alkaline	No Growth	Positive
RC26	Negative	Poor	Acidic	No Growth	Positive
RC28	Negative	Poor	Acidic	No Growth	Positive
RC30	Negative	Very Poor	Acidic	No Growth	Negative
RC32	Negative	Poor	Alkaline	No Growth	Positive
RC33	Negative	Very Poor	Acidic	No Growth	Negative
RC34	Negative	Poor	Acidic	No Growth	Positive
RC36	Negative	No Growth	Acidic	No Growth	Positive
RC38	Negative	Poor	Acidic	Growth	Negative
RC39	Negative	Good	Acidic	No Growth	Positive
RC41	Negative	Poor	Alkaline	Growth	Negative
RC42	Negative	Poor	Alkaline	No Growth	Positive
RC43	Negative	Very Poor	Acidic	No Growth	Positive

C) Biochemical Test

Among the 22 different isolates characterized biochemically, they showed varied responses to the various tests performed. *Mesorhizobium* isolates exhibited typical reactions to some tests, such as citrate utilization, gelatin liquefaction, urease test and VP test. However, the majority of isolates in the present study generally showed negative reactions to these tests, with certain exceptions. While majority of the isolates showed positive reactions to starch hydrolysis, H2S production, oxidase, catalase, KOH etc. Biochemical studies revealed that all the isolates performed differently and exhibited diverse reactions towards all the tests conducted. (Table 3.)

Adiguzel *et al*. (2010) [1] determined the phenotypic and genotypic characterization of *R. legumenosorum* sub. sp. *viciae* and reported that strains acidify the medium in bromothymol blue. Ten strains were catalase and oxidase positive. Shahzad *et al*. (2012) [15] also reported similar results in which the *Rhizobium* isolates showed negative reactions to gelatin hydrolysis, citrate utilization, VP test, MR test, and positive reactions to catalase test and urease test.

The results are in agreement with the findings of Wani and Khan (2013) [21], Gachande and Khansole (2011) [5], and Kumari *et al*. (2009) [10], who reported that *Rhizobium* isolates were positive for catalase activities and citrate utilization, and *Rhizobium* sp. were negative for MR-VP and indole reactions. *Rhizobium* strains isolated from root nodules of *Vigna radiata* showed a negative urease test (Deora and Singhal 2010) [2]. Positive results for oxidase, catalase, and urease were obtained by Gauri *et al*. (2012) [6]. Sharma *et al*. (2010) [16] obtained different isolates of *Mesorhizobium* from chickpea nodules. *All Mesorhizobium sp*. were negative for 3-Ketolactose production. These results are consistent with the earlier findings of Wani *et al*. (2009) [22], who reported similar results in *Mesorhizobium sp*. isolated from chickpea.

Table 3: Biochemical test of selected isolates.

Isolate code	MR	VP	CU	H_2S	SH	O	C	U	KOH	G
RC1	+	-	+	-	+	+	+	-	+	+
RC2	+	-	-	+	-	+	+	-	+	+
RC4	+	-	-	+	+	+	+	+	+	-
RC7	+	-	-	+	-	+	+	-	+	-
RC11	+	-	+	-	+	+	+	+	+	-
RC12	+	-	-	+	+	+	+	-	+	+
RC13	+	-	-	+	-	+	+	-	+	-
RC14	-	-	-	+	+	+	+	-	+	-
RC18	+	-	-	+	+	+	+	+	+	-
RC20	+	-	-	-	+	+	+	-	+	-
RC21	-	-	-	+	+	+	+	-	+	-
RC22	-	-	-	+	+	+	+	+	+	-
RC23	-	-	-	+	-	+	+	-	+	-
RC25	+	-	-	-	+	+	+	+	+	-
RC26	+	-	-	+	-	+	+	-	+	-
RC28	+	-	-	+	+	+	+	+	+	+
RC32	+	-	-	+	+	+	+	+	+	-
RC34	+	-	-	+	+	+	+	-	+	-
RC36	-	-	+	-	-	+	+	-	+	-
RC39	+	-	-	+	+	+	+	+	+	-
RC42	+	-	-	+	-	+	+	-	+	-
RC43	-	-	-	+	+	+	+	+	+	-

(**MR**- Methyl red, **VP**- Vogas Proskarus, **CU**- Citrate utilization, **SH**- starch hydrolysis, **O**- oxidase, **C**-catalase, **U**- urease, **G**- Gelatinase)

D) Carbon source utilization

Six different carbon sources were tested among the 22 isolates. The results in Table 4. show that glucose, sucrose, and mannitol produced acid in the test tubes, resulting in a yellow color, indicating that all the bacterial isolates utilized the provided carbon sources. The remaining three sources, xylose, fructose, and arabinose, were also utilized by some isolates, while others failed to utilize these sources. The variability in utilizing the same carbon source among different isolates may be due to the genetic variability present in the isolates.

Rhizobia are heterotrophic bacteria that can obtain carbon from a wide variety of organic compounds in the soil. The use of various carbohydrates as a sole carbon source is an effective method for characterizing rhizobial isolates. Kucuk *et al*. (2006) [9] found that *Rhizobium* strains utilize glucose and sucrose more efficiently than mannitol in YEM medium. In this study, mesorhizobia isolates were able to utilize glucose, sucrose, and mannitol. Kaur (2014) [8] reported that most *Mesorhizobium* isolates from chickpea preferentially utilized dextrose (95%) followed by sucrose (67%), with inositol being the least utilized (14%) as a sole carbon source. These findings are consistent with Gauri *et al*. (2012) [6], who also found that chickpea rhizobia could utilize different carbon sources. However, Shahzad *et al*. (2012) [15] reported mixed results for sugars like glucose, lactose, and inositol during the isolation and characterization of R. meliloti.

Table 4: Effect of different sugars on carbon utilization pattern of *Mesorhizobium* isolates.

Isolate Code	Glucose	Sucrose	Xylose	Fructose	Mannitol	Arabinose
RC1	+	+	-	+	+	-
RC2	+	+	-	+	+	-
RC4	+	+	+	+	+	+
RC7	+	+	-	+	+	-
RC11	+	+	+	-	+	+
RC12	+	+	-	+	+	-
RC13	+	+	-	-	+	-
RC14	+	+	+	+	+	-
RC18	+	+	-	+	+	-
RC20	+	+	+	+	+	-
RC21	+	+	-	+	+	+
RC22	+	+	-	+	+	-
RC23	+	+	-	-	+	+
RC25	+	+	+	+	+	-
RC26	+	+	-	+	+	-
RC28	+	+	+	+	+	-
RC32	+	+	+	+	+	-
RC34	+	+	-	+	+	-
RC36	+	+	-	+	+	+
RC39	+	+	+	+	+	-
RC42	+	+	-	+	+	-
RC43	+	+	+	+	+	-

"+" Positive, "-" Negative

E) Skim milk test

The skim milk test is a valuable tool for characterizing *Rhizobium* strains, providing insights into their metabolic capabilities and interactions with plants. Table 5 results indicate that all isolates lack alkaline reaction and gas formation, suggesting a consistent trait. However, 17 isolates exhibit acid production, indicating their ability to use proteins as a nitrogen source. Additionally, 12 isolates can curdle milk in an acidic environment, indicating the presence of caseinases. The reduced curd formation by 4 isolates suggests lower proteolytic activity. These variations in proteolytic activity could be crucial in understanding nutrient cycling and plant-microbe interactions. Different *Rhizobium* strains exhibit varying proteolytic activity levels, which could affect their effectiveness in symbiotic relationships and nutrient cycling. The skim milk test is useful for distinguishing *Rhizobium* strains based on proteolytic activity, highlighting their metabolic diversity and ecological implications.

Table 5: Reactions of different isolates to skim milk test

Isolate Code	Acid	Alkaline	Acid Curd	Reduced curd	Gas formation
RC1	-	-	+	-	-
RC2	+	-	+	-	-
RC4	+	-	-	-	-
RC7	+	-	-	-	-
RC11	+	-	+	-	-
RC12	+	-	+	+	-
RC13	+	-	+	-	-
RC14	+	-	+	-	-
RC18	+	-	+	-	-
RC20	+	-	+	-	-
RC21	-	-	-	+	-
RC22	+	-	+	-	-
RC23	-	-	-	-	-
RC25	+	-	+	-	-
RC26	+	-	+	-	-
RC28	+	-	-	+	-
RC32	-	-	-	-	-
RC34	+	-	+	-	-
RC36	-	-	-	-	-
RC39	+	-	-	+	-
RC42	+	-	-	-	-
RC43	+	-	-	-	-

"+" Positive, "-" Negative

Conclusion

The present study highlights the significance of screening tests such as Congo red, GPA, KLA, Hoffers alkaline test, etc., for isolating *Mesorhizobium* from chickpea root nodules, especially considering the close association of Agrobacterium spp. Various biochemical and enzymatic tests conducted on these isolates reveal the extent of variability among them. Tests involving carbon sources and skim milk further uncover the metabolic diversity, reflecting the genetic variability among the strains.

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