Characterization of *Rhizobium* Isolates from Soybean (*Glycine max* L.) and Assessment of Their Plant Growth-Promoting Attributes in the Malwa Region of Madhya Pradesh

Abstract

Soybean (*Glycine max*) is a leguminous crop that relies on symbiotic nitrogen fixation by rhizobacteria for optimal growth and productivity. This study focuses on the isolation and functional characterization of soybean rhizobacteria from the Ratlam district of Madhya Pradesh, India. Soil samples were collected from different soybean-growing regions, and rhizobium isolates were isolated using selective media. A total of 170 rhizobacteria were isolated from soil samples collected from five villages of Ratlam district, Madhya Pradesh, India. Out of 170 isolates, 15 different bacterial isolates were selected for, colony morphology and characterized on the basis of microscopy and biochemically. The isolates could survive at temperatures between 4 and 45 °C (optimum 30 °C), and pH 5–11 (optimum 8). Additionally we have determine the plant growth promoting trails i.e., phosphate solubilizaion, potassium solubilization, production of indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN). Most of the isolates exhibited plant growth-promoting (PGP) traits. The study concludes that native *Rhizobium* isolates from the Malwa region possess distinct physiological and plant growth-promoting capabilities. These findings highlight their potential as region-specific bioinoculants to improve soil fertility and support sustainable soybean production under local agroclimatic conditions.

Keywords: Soybean, Isolation, Functional Characterization, Rhizobium, Plant Growth Promoting

1. INTRODUCTION

Soybean (*Glycine max* L.), often referred to as the "golden bean," is one of the world's most vital oilseed and forage crops, valued for its high-quality protein and oil content (Lai et al., 2024). Globally, soybeans contribute to more than half of total oilseed production, making them the most important leguminous crop (Pawar et al., 2014). In India, since 2006, soybean has dominated the oilseed sector and played a transformative role in improving the livelihoods of millions of small and marginal farmers, particularly in central regions such as the Malwa Plateau. Its importance lies not only in meeting the country's demand for edible oil but also in enhancing the national economy through foreign exchange earnings from soy meal exports. The success of soybean cultivation in India can be attributed to the collaborative efforts of farmers, researchers, extension workers, and industries engaged in processing and value addition (Singh et al., 2023). Nutritionally, soybean seeds contain approximately 37–42% protein, 6% ash, 29% carbohydrates, and 17–24% oil. They are also rich in dietary fibre, vitamins, and essential minerals. The crop's adaptability to diverse agroecological zones and ease of cultivation further enhance its significance. Soybean plants require substantial nitrogen for optimal growth because the nitrogen accumulated in the shoots is closely linked to seed yield. This nitrogen requirement is primarily met through symbiotic nitrogen fixation and soil mineral nitrogen uptake. Compared to other legumes, soybeans are particularly dependent on rhizobia for nitrogen acquisition (Ntambo et al., 2017). Soybean cultivation across various regions of India has revealed the presence of indigenous rhizobial populations along with some introduced and well-adapted strains. In this study, a total of 22 rhizobial isolates collected from 12 distinct soybean-growing locations, along with 8 reference strains, were analyzed for their biochemical and metabolic characteristics (Sharma et al., 2010). *Rhizobium*, a genus within the Rhizobiaceae family, plays a crucial role in promoting plant growth (Pawar et al., 2014). These bacteria engage in a mutualistic

relationship with legumes through a process known as biological nitrogen fixation (BNF). Assisted by leghaemoglobin, rhizobia convert atmospheric nitrogen into ammonia—a form readily absorbed by plants—within specialized structures called root nodules. In return, the bacteria utilize photosynthetically derived carbon compounds from the host plant. This symbiosis contributes roughly 80% of the biologically fixed nitrogen available in agricultural systems (Wadhwa et al., 2017).

This study focuses on isolating *Rhizobium* strains from soybean nodules obtained from various locations in the Malwa region of Madhya Pradesh. The isolates were examined for their morphological, biochemical, and metabolic characteristics. In addition, their potential as plant growth-promoting rhizobacteria (PGPR) was evaluated to identify effective native strains that could serve as bioinoculants adapted to the local agro-ecological conditions.

2. MATERIAL AND METHOD

2.1 Site description

Fifteen soil samples were collected from different sites within the Ratlam district, as summarized in Table 1, based on the availability of soybean (*Glycine max*). Two soybean varieties, JS 335 and JS 93-05, were selected for this study. Sampling sites were chosen based on their proximity and the presence of healthy soybean crops at the flowering stage. Following these criteria, three primary locations were selected for sample collection: Ratlam Local (23.19°N, 75.07°E), Sailana (23°27'24.78"N, 74°52'23.83"E), and Raoti. Each site was further divided into five villages according to their respective tehsils. Detailed information about the sampling locations and crop history of site is provided in Table 1.

Table 1: Site selection and crop history

Sr. no.	Sample no.	Collection site name *	Village name	Collection date	Crop sequence history**	Soybean cultivation	Map marking
1.	RL1	Ratlam local	Mangrol	08/8/24	Wheat +soybean	YES	A
2.	RL2	Ratlam local	Morwani	08/8/24	Wheat +soybean	YES	B
3.	RL3	Ratlam local	Donswas	08/8/24	Wheat +soybean	YES	C
4.	RL4	Ratlam local	Sagod	08/8/24	Wheat +soybean	YES	D
5.	RL5	Ratlam local	Dharad	08/8/24	Wheat +soybean	YES	E
6.	RS1	Sailana,Ratlam	Shivgrah	10/8/24	+soybean	YES	F
7.	RS2	Sailana,Ratlam	Kariya	10/8/24	Wheat +soybean	YES	G
8.	RS3	Sailana,Ratlam	Sarwan	10/8/24	Wheat +soybean	YES	H
9.	RS4	Sailana,Ratlam	Berda	10/8/24	+soybean	YES	I
10.	RS5	Sailana,Ratlam	Garad	10/8/24	+soybean	YES	J
11.	RR1	Raoti, Ratlam	Bid	11/8/24	Wheat +soybean	YES	K
12.	RR2	Raoti, Ratlam	Chainpur	11/8/24	Wheat +soybean	YES	L
13.	RR3	Raoti, Ratlam	Devapada	11/8/24	+soybean	YES	M
14.	RR4	Raoti, Ratlam	Maulawa	11/8/24	+soybean	YES	N
15.	RR5	Raoti, Ratlam	Bhetiya	11/8/24	Wheat +soybean	YES	O

* Collection site was preferred on the basis of distance nearest to the researcher.

**The data on crop sequence history was obtained by survey performed by researcher.

Map 1: location sample collection.

(In the above map, marks from A to O represent all the sample collection sites marked in Table No. 1.)

2.2 Sample collection

For sample collection, carefully uproot the soybean plants and gently wash the roots with water to remove adhering soil particles. Select large, healthy nodules, as these are typically active. Surface-sterilize the nodules by immersing them in 70% ethanol for 30–60 seconds, followed by treatment with 0.1% mercuric chloride for 2–3 minutes. Rinse the nodules thoroughly with sterile distilled water several times to remove any traces of the sterilizing agents. After sterilization, crush the nodules aseptically in a sterile tube containing a small volume of sterile distilled water to prepare a suspension for further isolation of *Rhizobium*.

2.3 Isolation of Rhizobium from extracted root nodules

The isolation of *Rhizobium* was carried out following the method described by Wadhwa et al. (2017). Root nodule extracts from soybean were first prepared, and a serial dilution was performed by adding 1 mL of the sterilized bacteroid solution to 9 mL of sterile distilled water, continuing the dilution series up to 10^{-6}. Aliquots from the 10^{-4} to 10^{-6} dilutions were then plated onto Yeast Extract Mannitol Agar (YEMA) to isolate and identify bacterial colonies. YEMA was prepared by dissolving 10 g mannitol, 0.5 g dipotassium hydrogen phosphate (K_2HPO_4), 0.2 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.1 g sodium chloride (NaCl), 1 g yeast extract, and 15 g agar in 1 liter of distilled water. The plates were incubated at 37 °C for three days, after which colonies were examined for

morphological characteristics and identified. All bacterial isolations and subsequent biochemical assays were conducted under a laminar airflow hood to ensure sterility.

2.4 Morphological characterization

The colonies grown on YEMA plates were analyzed to determine their morphological characteristics, including color, opacity, shape, elevation, margin, surface, texture, motility, cellular shape and arrangement, Gram reaction, and the presence of endospores and capsules (Dhiman et al., 2019). For microscopic characterization, Gram staining was performed as a presumptive test for *Rhizobium*. A drop of 12-hour-old culture was evenly smeared on a clean glass slide and gently heat-fixed. Crystal violet was applied and allowed to act for 1 minute, followed by a gentle rinse with tap water. Iodine solution was then added for 1 minute to fix the dye. Decolorization was performed using iodinated alcohol for 5 minutes, followed by counterstaining with safranin for 5 minutes. After a gentle wash with tap water, the slides were air-dried and observed under a light microscope using oil immersion (Paudyal et al., 2021).

2.5 Biochemical and physiological characterization

Biochemical characterization of the isolates, including carbon source utilization, production of extracellular and intracellular enzymes, and antibiotic sensitivity, was conducted following the methods described by Malviya et al. (2012). Catalase activity was assessed by observing the release of oxygen bubbles after adding 3% hydrogen peroxide to the culture, while oxidase activity was confirmed through the oxidation of TMPD (tetramethyl-p-phenylenediamine dihydrochloride) using oxidase reagent discs.

Starch hydrolysis was evaluated by flooding 7-day-old colonies grown on starch agar (containing 2% starch) with Gram's iodine solution and observing the appearance of clear zones around colonies. Citrate utilization was determined by a color change in citrate agar medium, while the ability to metabolize various carbon sources was analyzed using Andrade peptone water supplemented with 0.1% Andrade indicator, where a color shift signified positive utilization.

For evaluation of physiological growth characteristics, isolates were cultured on NAMplates and incubated at varying temperatures (4, 15, 25, 30, 35, 40, and 45 °C) and pH levels ranging from (5.0 to 12.0) to determine their minimum, optimum, and maximum growth conditions. Salt tolerance was examined by growing the isolates on NAM medium amended with different NaCl concentrations (2.0%, 3.0%, 4.0, 5.0%, 6.0%, 7.0%, and 8.0%) and incubating the plates at 30 °C for 3–4 days.

2.6 Qualitative Evaluation of Plant Growth-Promoting traits

2.6.1 Phosphate Solubilizing

The phosphate-solubilizing potential of the bacterial isolates was evaluated using Pikovskaya's agar medium as described by Pikovskaya (1948). An inoculum of 10 µL containing approximately 1×10^{8} CFU mL^{-1} of each isolate was placed on the medium and incubated for three days. The solubilization of phosphate was indicated by the appearance of a clear halo zone surrounding the bacterial colony. This halo formation results from the production of organic acids by the isolates, which dissolve the tri-calcium phosphate present in the medium.

2.6.2 Potassium Solubilization

The potassium-solubilizing capacity of the selected isolates was assessed using a modified Aleksandrov agar medium following the method described by Hu et al. (2006). Approximately 10 µL of bacterial suspension, adjusted to a concentration of about 10^{8} CFU mL^{-1}, was streaked onto the

medium and incubated at 30 °C. Observations were made after 3–5 days of incubation. The ability of the isolates to solubilize muscovite mica, used as an insoluble potassium source, was indicated by the development of a clear halo zone surrounding the bacterial colonies.

2.6.3 Hydrogen Cyanide Production

The production of hydrogen cyanide (HCN) by the bacterial isolates was assessed using the method described by Lorck (1948). Pure bacterial cultures were inoculated into 15 mL of LB broth supplemented with 4.4 g L^{-1} glycine in test tubes. Sterile Whatman No. 1 filter paper soaked in 1% picric acid solution and air-dried was suspended inside each tube. The tubes were sealed with parafilm and incubated at 30 °C for 5–10 days. HCN production was indicated by a color change of the filter paper from yellow to orange-brown or reddish-brown, reflecting cyanide synthesis by the bacterial strains.

2.6.4 Indole-3-Acetic Acid (IAA) Detection

The ability of the bacterial isolates to produce indole-3-acetic acid (IAA) was evaluated using the colorimetric method described by Gordon and Weber (1951). Overnight bacterial cultures were grown in LB broth at 30 °C with shaking at 180 rpm. An inoculum of approximately 10^6 CFU mL^{-1} was prepared and added to LB broth supplemented with L-tryptophan (0.5 and 1.0 g L^{-1}) as a precursor for IAA synthesis. The cultures were incubated at 30 °C for seven days. Following incubation, bacterial cells were removed by centrifugation, and the resulting supernatant was used for quantitative estimation of IAA production by using Salkowski's reagent

2.6.5 Ammonia Production

Fresh bacterial cultures were incubated in peptone water broth at 30 °C for five days. After incubation, the cultures were centrifuged, and the supernatant was collected for ammonia estimation using Nessler's reagent, as described by Goswami et al. (2014). The amount of ammonia produced was quantified by comparing the absorbance with a standard curve prepared using ammonium sulfate, ranging from 0.1 to 1 mM mL^{-1}.

3. RESULTS

3.1 Isolation of Rhizobacteria

A total of 170 isolates were obtained in this study from five distinct locations within the Ratlam district of the Malwa region, Madhya Pradesh, India. Among these, about 15 representative isolates were characterized based on their morphological, biochemical, and plant growth-promoting traits. The highest number of *Rhizobium* isolates was obtained from Ratlam Local, followed by Sailana and Reoti. The results also indicated that sampling sites with a long history of soybean and wheat cultivation showed higher *Rhizobium* populations (Table 1). The isolation of *Rhizobium* on YEMA plates is shown in Figure 1.

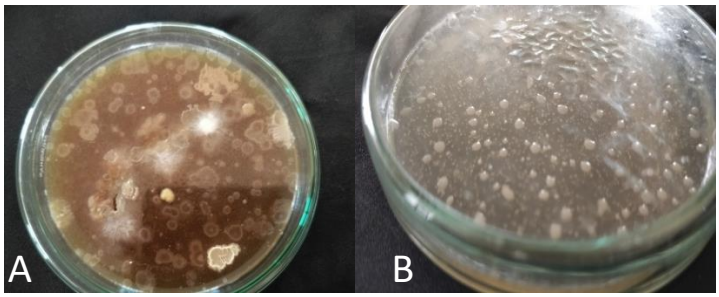

Figure 1 Isolation of Rhizobium on YEMA Media

3.2 Morphological characterization of isolates

A total of 15 isolates were characterized on the basis of morphologically and biochemically. More than one-half of these isolates, observed mostly as milky-white, round, translucent, and often mucoid, with a smooth and entire margin with a diameter of 2-6 mm after 3–5 days of incubation at 30°C. The colony and cell morphology, and growth characteristics of these 15 isolates are presented in Table 2. Most of the isolates were observed gram negative. Five isolates were gram positive. The colony growth characteristics observed on YEMA, along with the respective microscopic features of the three representative genera, are illustrated in Fig. 1.

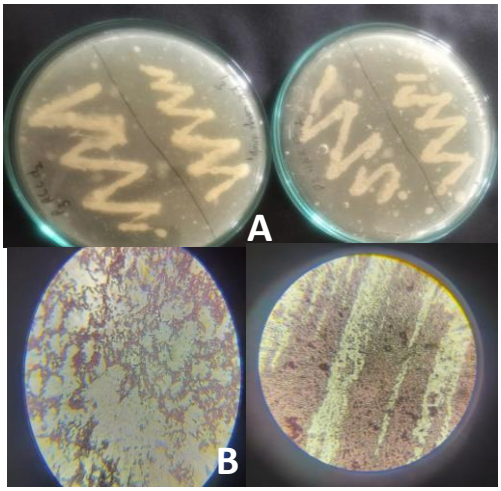

Figure 2 (A)Colony Morphology and (B) Microscopic factures of isolates

Table 2 Colony Morphology and Microscopic characteristics of isolates

Sample no.	Size	Bacterial Shape	Shape of colony	Colour	Margine	Opacity	Bacterium shape	Gram staining
RL1	2.mm	Rod	Circular	Milky white	entire	Translucent	Short Rod	-
RL2	2.6mm	Rod	Circular	Milky white	entire	Translucent	Short Rod	-
RL3	2.0mm	Rod	Circular	Milky	entire	Translucent	Short Rod	-

				white				
RL4	2.mm	Rod	irregular	Off white	Partial	Translucent	Short Rod	-
RL5	2.8mm	Rod	Circular	Transparent	irregular	Translucent	Short Rod	-
RL6	2.4mm	Rod	irregular	Milky white	Full	Translucent	Rod	+
RL7	3.1mm	Rod	irregular	Milky white	Partial	Translucent	Rod	+
RL8	3.0mm	Rod	Circular	Milky white	entire	Translucent	Short Rod	-
RL9	2.9mm	Rod	Circular	Milky white	entire	Translucent	Short Rod	-
RL10	2.8mm	Rod	Circular	Milky white	entire	Translucent	Rod	+
RL11	3.3mm	Rod	Circular	Off whitw	Partial	Translucent	Short Rod	-
RL12	2.4mm	Rod	Circular	Off white	Full	Translucent	Short Rod	-
RL13	2.6mm	Rod	Circular	Milky white	Partial	Translucent	Rod	+
RL14	2.5mm	Rod	Circular	Transparent	entire	Translucent	Short Rod	-
RL15	2.8mm	Rod	irregular	Light yellow	Full	Translucent	Rod	+

3.3 Physiological characters of isolates

The optimum temperature for growth of all the isolates was 30 °C; however, five isolates could not grow at 5 °C, while six of the isolate tolerate up to t 50 °C (Table 3). The optimum pH for growth of all the isolates was 8; none could grow at pH 4 or pH 11 (Table 4). Ten of the isolates tolerated salt concentration up to 6 %, while ten could grow at 7 % salt concentration (Table 5).

Table 3: Temperature tolerance of all the Isolates

Isolates no no.	Temperature (°C)						
	5	15	25	30	35	45	50
RL1	+	-	-	+	+	+	-
RL2	+	+	-	-	+	+	+

RL3	+	+	-	+	-	-	-
RL4	+	+	+	+	-	+	-
RL5	-	+	-	+	+	+	+
RL6	+	+	+	-	+	+	-
RL7	-	+	+	-	-	+	+
RL8	-	+	-	-	+	+	-
RL9	+	+	-	+	+	+	-
RL10	-	+	+	+	+	+	+
RL11	+	+	+	+	-	-	-
RL12	-	+	-	-	+	-	-
RL13	+	+	+	-	+	+	+
RL14	+	+	-	+	-	-	-
RL15	+	+	+	+	+	+	+

* (+) growth was observed, (-) growth was not observed

Table 4: pH tolerance of all the Isolates

Isolates no.	pH						
	4	6	7	8	9	10	11
RL1	-	+	+	+	+	+	-
RL2	-	+	+	+	+	+	-
RL3	-	+	+	+	+	+	-
RL4	-	+	+	+	+	+	-
RL5	-	+	+	+	+	+	-
RL6	-	+	+	+	+	+	-
RL7	-	+	+	+	+	+	-
RL8	-	+	+	+	+	+	-
RL9	-	+	+	+	+	+	-
RL10	-	+	+	+	+	+	-
RL11	-	+	+	+	+	+	-
RL12	-	+	+	+	+	+	-
RL13	-	+	+	+	+	+	-
RL14	-	+	+	+	+	+	-
RL15	-	+	+	+	+	+	-

* (+) growth was observed, (-) growth was not observed

Table 5: Salt tolerance of all the Isolates

Isolates no	Salt (%)					
	1	2	3	4	5	6
RL1	+	+	+	+	+	+

RL2	+	+	+	+	+	-
RL3	+	+	+	+	+	-
RL4	+	+	+	+	-	+
RL5	+	+	+	+	+	-
RL6	+	+	+	+	+	+
RL7	+	+	+	+	-	+
RL8	+	+	+	+	-	-
RL9	+	+	+	+	+	+
RL10	+	+	+	+	+	-
RL11	+	+	+	+	+	+
RL12	+	+	+	+	-	+
RL13	+	+	+	+	-	-
RL14	+	+	+	+	+	-
RL15	+	+	+	+	+	+

* (+) growth was observed, (-) growth was not observed

3.4 Biochemical test

Biochemical test are presented in Table 6. All the isolates were positive for catalase and oxidase. From 15 isolates, 9 hydrolyzed starch, only 6 were positive for citrate and 10 were positive for Unease test.

Table 6 Biochemical characters of isolates

Sample no.	Catalase test	Oxidase test	Starch hydrolysis test	Citrate utilization test	Urea Hydrolysis
RL1	+	+	+	-	+
RL2	+	+	-	-	+
RL3	+	+	+	-	-
RL4	+	+	+	+	-
RL5	+	+	+	-	+
RL6	+	+	-	+	+
RL7	-	+	-	+	-
RL8	-	+	-	-	+
RL9	+	+	+	-	+
RL10	+	+	+	+	-
RL11	+	+	-	-	-
RL12	-	+	-	-	+
RL13	+	+	-	+	+

RL14	+	+	+	-	-
RL15	+	+	+	+	+

3.5 Utilization of carbon Sources

The isolates showed differences in their ability to utilize various carbon sources, including glucose, fructose, mannitol, arabinose, and sucrose (Table 7). Most of the isolates were capable of utilizing all the tested sugars.

Table 7 Utilization of carbon Sources

Isolates no	Carbon sources					
	D-glucose	D-fructose	Mannitol	L-arabinose	D-mannose	Sucrose
RL1	+	+	+	+	+	+
RL2	+	+	+	+	-	+
RL3	+	+	+	+	+	+
RL4	+	+	+	+	-	+
RL5	+	+	+	+	-	+
RL6	+	+	+	-	+	+
RL7	+	+	-	+	-	-
RL8	+	+	+	+	+	+
RL9	+	+	+	+	+	+
RL10	+	+	+	+	+	+
RL11	+	+	+	-	+	+
RL12	+	+	+	+	+	+
RL13	+	+	-	+	-	+
RL14	+	+	+	+	+	+
RL15	+	+	+	+	+	+

3.6 Plant growth promoting traits

The plant growth promoting traits are presented in Table 8. All the isolates were positive for ammonia, 7 Produce IAA and HCN. 8 solubilized phosphate and 7 solubilized potassium.

Table 8 PGPR activity of all the isolates

Isolate no.	PGPR Traits				
	Ammonia	IAA	HCN	Phosphate Solubilisation	Potassium Solubilization
RL1	+	+	+	+	+
RL2	+	+	-	+	+
RL3	+	-	+	-	-
RL4	+	-	-	-	-
RL5	+	+	+	+	+
RL6	+	+	-	+	+
RL7	+	+	+	+	+
RL8	+	-	-	-	+
RL9	+	-	-	-	_
RL10	+	+	-	+	+
RL11	+	-	-	-	-
RL12	+	+	-	-	-
RL13	+	-	-	+	+
RL14	+	-	+	-	_
RL15	+	+	+	+	+

4. Discussion

In the present study, rhizobium were isolated from soil samples collected from different areas in Ratlam district Malwa region Madhya Pradesh.. A total of 170 different bacterial isolated from several locations of malwa region to observe diversity of rhizobium spp., due to different cultural agronomic practices and geographical distribution. It is hypothesized that there would be greater chances of beneficial bacterial interactions with plant roots from locations which practice minimal

and chemical-free planting systems, namely in Ratlam district Malwa region Madhya Pradesh. In this research, out of 170, 15 different bactera were characterized on the basis of Morphologically, Physiology and Biochemically area. Additionally isolates were tested for their PGP activities.

In the present study Out of 15 isolates 10 are Gram –ve and 5 were gram +ve. These 10 isolaes will be the rizobium spp., and 5 will be the bacillus spp. On the basis of mprphological and Biochemcal characterization.. It was found that the strain grown showed the convex elevation in Yeast Extract Mannitol Agar medium. The colonies were 2.5 mm, translucent, whitish pink and glittering (Fig. 2). Roychowdhury et al., (2015) showed the growth of Rhizobium bacteria on Congo red Yeast Extract Mannitol agar medium. Most of these isolates, observed mostly as milky-white, round, convex, and often mucoid, with a smooth and entire margin with a diameter of 2-6 mm after 3–5 days of incubation at 30°C. It was found that the strain grown showed the convex elevation in Yeast Extract Mannitol Agar medium. The colonies were 2.5 mm, translucent, whitish pink and glittering and Rhizobium was Gram negative, motile, rod shaped and were fast growers as they showed convex elevation in Yeast Extract Mannitol medium (Wadhwa et al., 2017). Roychowdhury et al., (2015) showed the growth of Rhizobium bacteria on Congo red Yeast Extract Mannitol agar medium. Rai et al., (2013) and Gauri et al., (2012) also characterized rhizobial isolates on the basis of their colony shape, colour and texture. Temperature is one of the major factors affecting rhizobial growth, survival in the soil and the symbiotic process itself (Niste et al., 2013). High soil temperature in tropical regions is one of the major constraints for BNF in legume crops. In the present study most of the isolates were positive for catalase and oxidase, hydrolyzed starch, unease, and citrate. Paudyal et al., (2021) also reported that most of the rhizobia isolated from legume crop were showing most of the biochemical test which is consistent with preset results. Most of the isolates utilized carbon sources. Deshwal and Chaubey (2014) also reported *Rhizobium leguminosarum* from Root nodule of

Pisum sativum L. utilized most of the sugars. Plant Growth Promoting Rhizobacteria (PGPR) are beneficial soil microorganisms that colonize plant roots, stimulate plant development, and enhance crop productivity (Kasa et al., 2020). Among them, *Rhizobium* plays an important ecological role due to its ability to fix atmospheric nitrogen in symbiotic association with leguminous plants. In this study, *Rhizobium* strains capable of nitrogen fixation were isolated and characterized from soybean root nodules. The main objective of this research was to analyze the bacterial isolates obtained from soybean nodules and evaluate their properties. Most of the isolates exhibited PGPR traits, aligning with the findings of Manasa et al. (2017), who observed similar plant growth–promoting activities in *Rhizobium* species isolated from various rhizospheric soils.

4. CONCLUSION

Present research successfully isolated and evaluated native *Rhizobium* strains associated with soybean (*Glycine max* L.) from the rhizosphere soils of the Malwa region in Madhya Pradesh. From a total of 170 rhizobacterial isolates collected across several villages in the Ratlam district, 15 distinct strains were chosen based on their unique colony characteristics and were further analyzed through microscopic and biochemical methods. Functional assessment revealed that many of these isolates exhibited key plant growth–promoting properties, such as the ability to solubilize phosphate and potassium and to produce indole-3-acetic acid (IAA), ammonia, and hydrogen cyanide (HCN). These traits indicate their potential contribution to improved nutrient cycling and overall plant development through both direct and indirect pathways. The indigenous *Rhizobium* strains identified from the Malwa region show strong physiological resilience and notable plant growth–enhancing capabilities. These characteristics highlight their promise as locally adapted bioinoculants that could enhance soil fertility, decrease reliance on synthetic fertilizers, and support sustainable soybean cultivation under regional agroclimatic conditions.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as language models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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