Isolation and characterization of *Rhizobium* species from cultivated and wild leguminous plants

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Abstract

Plant growth and development requires nitrogen as essential elements, which supplied by symbiotic bacteria rhizobia in association with leguminous plants. Plant growth promoting Rhizobacteria are capable of promoting plant growth by colonizing the plants root and enhances symbiotic and associative nitrogen fixation. This work aimed isolation, characterization and PGPR activity of the bacteria from cultivated and non-cultivated leguminous plants. Seven strains of *Rhizobium* species were isolated from six non-cultivated wild leguminous plants and three *Rhizobium* strains were isolated from cultivated leguminous plants. Plant root´s nodule collected from different regions of Ranchi, Jharkhand. Bacteria were isolated for its characterization and PGPR activities, such as IAA production, Phosphate solublization, HCN production, Ammonia production. During this work six non-cultivated leguminous plants were identified namely *Tephrosia* (*Tephrosia purpurea)*, Tick clover (*Desmodium tortuosum)*, Karanj (*Pongamia pinnata*), Shisham (*Dalbergia sissoo*), Lupin (*Lupinus albus*), Gulmohar (*Delonix regia*). From these plants seven *Rhizobium* species were isolated (RC1, RC2, RC3, RC4, RC5, RC6, RC7) and characterized. Three isolates (RC8, RC9, RC10) were also characterized from cultivated leguminous plants namely Dolichos bean (*Lablab purpureus*), Arhar (*Cajanus arietinum*), Gram (*Cicer arietinum*). These ten isolates showed PGPR activities, which confirmed through IAA, HCN and ammonia production tests. The aim of this study is to isolate Rhizobacteria from wild and cultivated leguminous plants, which have PGPR activity. Inoculum of all these *Rhizobium* species isolated from non-cultivated wild leguminous plants can exploited in plant growth promotion.

Keyword: Indole acetic acid, leguminous, non-cultivated, PGPRs, *Rhizobium* species, wild strain.

1. Introduction

Nitrogen plays an essential role for plant growth and development. Most of the plant do not utilize it in free form. Rhizobia a nitrogen fixing bacteria and has ability to reduce nitrogen. They fix atmospheric nitrogen using the enzyme nitrogenase, which convert this atmospheric nitrogen in simple form so that plant can utilize it [1]. Biological Nitrogen Fixation (BNF) is the cheapest process and environment friendly procedure in which nitrogen fixing bacteria interact with leguminous plants and fix aerobic nitrogen into soil [2]. *Rhizobium* is the well-known group of bacteria that act as the primary symbiotic fixer of nitrogen. Endophytic bacteria are the group of beneficial free-living soil bacterium that colonizes the inside of root cell of plant without showing any external sign of infection on their host [3]. These bacteria infect the roots of leguminous plants; leading to the formation of nodules or lumps where the nitrogen fixation takes place [4]. Rhizobia nod gene determines the host specificity [5].There are different *Rhizobium* species reported mainly from the cultivated leguminous plants. The bacterium's enzyme system supplies the constant source of reduced nitrogen to the host plant and the plant furnishes nutrients and energy for the activities of the bacterium. *Rhizobium* bacteria stimulate the growth of leguminous plants and they are able to fix atmospheric nitrogen into soil by interacting symbiotically with leguminous plants, using the nitrogenase enzyme complex [6]. The legume-*Rhizobium* interaction is the result of specific recognition of the host legume by *Rhizobium*. Various signal molecules produced by both rhizobia and the legume confer the specificity [7]. Exopolysaccharide (EPS) produced by

Rhizobium is one such signal for host specificity during the early stage of root hair infection [8].
Rhizobium strains secrete growth hormones like Indole acetic acid (IAA), which shows positive influence on plant growth and plays an important role in the formation and development of root nodules [9]. *Rhizobium* species also have other various enzymatic activities. Production of EPS and IAA has considered as important traits of plant growth promoting *Rhizobacteria*. *Rhizobium* species also has other various enzymatic activities. These benefits of these species identified biochemically by various researchers [10]. In this study, growth-promoting activities of rhizobium species identified biochemically and screened. The purpose of this work is to isolates and characterizes the *Rhizobium* strains from non-cultivated wild leguminous plants and their exploitation in the agricultural field.

2. Methods and material

2.1 Collections of samples from studied area

The Ranchi city is located at 23.23^0 N latitude and 85.23^0 E longitude. The temperature of Ranchi during summer varies from a minimum of 20.6^0 centigrade to a maximum of 37.2^0 centigrade. During winter, the temperature varies from a minimum of 10.3^0 centigrade to a maximum of 22.9^0 centigrade.Fresh pink color nodules collected from wild and cultivated leguminous plants in different area of Ranchi and ICAR, RCER Research Centre Plandu, of Jharkhand region (Figure 1A, 1B). Between March to April, wild varieties of leguminous plants, *Tephrosia* (*Tephrosia purpurea)*, Tick clover (*Desmodium tortuosum)*, Karanj (*Pongamia pinnata*), Shisham (*Dalbergia sissoo*), Lupin (*Lupinus albus*), Gulmohar (*Delonix regia*) were taken as Host plant for Rhizobium isolation. Seven isolates namely RC1, RC2, RC3, RC4, RC5, RC6, RC7 were isolated from these plants.(Fig 1A). Three host cultivated leguminous plants, Dolichos bean (*Lablab purpureus*), Arhar (*Cajanus arietinum*), Gram (*Cicer arietinum*) were taken for isolation of rhizobium. These isolates named as RC8, RC9, RC10 (Fig 1B). Color of the nodules varies from brown to pink depending on the state of pigment present in them.

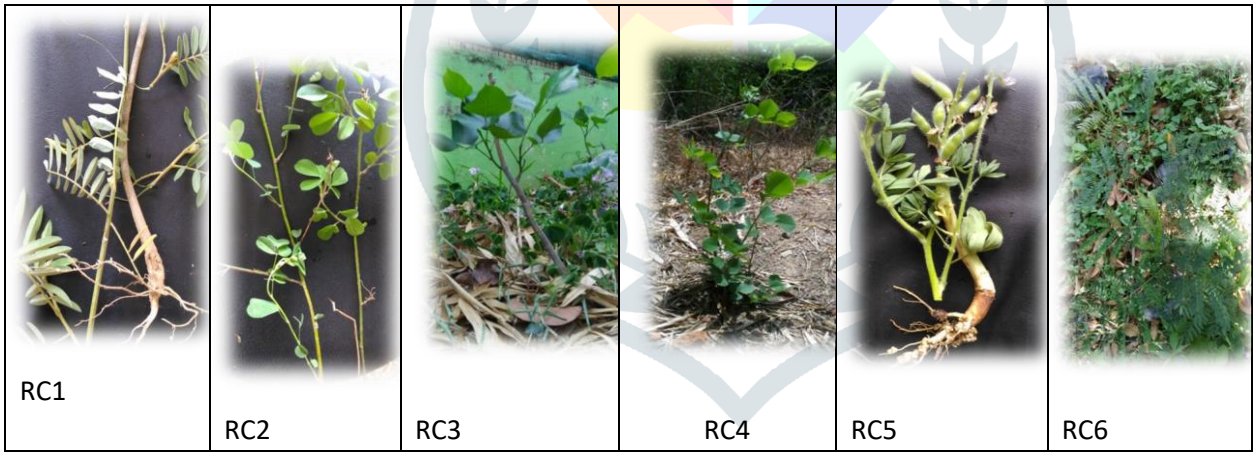

Figure 1 A Host species of non-cultivated leguminous plants RC1 to RC6

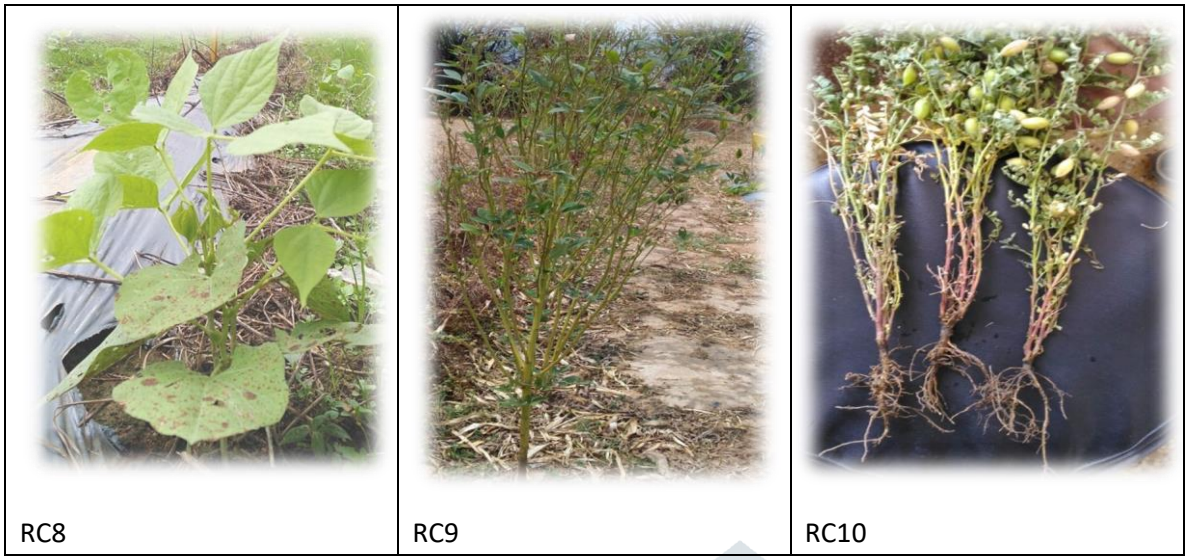

Figure 1 B Host cultivated leguminous plants RC -8, RC- 9, RC -10

2.2 Surface sterilization of nodules

Collected nodules washed under running tap water to remove the adhering soil particles from its surfaces. Nodules were dipped in 0.1% of Mercuric Chloride ($HgCl_2$) solution for 30 seconds and later were washed successively ten times with triple ionized mili Q sterilized distilled water to remove the traces of toxic $HgCl_2$, surface sterilized nodules were transferred in to test tube containing 5ml of sterilized distilled water. These nodules crushed and streaked on Yeast Extract Mannitol Agar (YEMA) Media [11].

2.3 Preparation of culture media

Different microorganisms need different culture media for their growth. Yeast Mannitol Agar Medium with Congo red prepared which generally used for culture of *Rhizobium* species. YEM Agar media prepared and sterilized. The composition used containing Mannitol 10.00 g, $MgSO_4 7H_2O$ 0.20 g, NaCl 0.10 g, K_2HPO_4 0.50 g, $CaCl_2 \cdot 2\ H_2O$ 0.20 g, $FeCl_3 \cdot 6\ H_2O$ 0.01 g, yeast extract 1.00 g, agar 20.00 g and distilled water 1000 ml at pH (6.8-7) [12,13].

2.4 Isolation of Rhizobium by serial dilution

Collected nodules washed in small aliquot of sterile distilled water with the help of brush. Nodules smashed by needles or glass slides and 1-2 drops of distilled water added. 10th fold serial dilution of nodular extract prepared and mixed well to get nodular extract suspension.1ml nodular extract suspension was diluted with 9ml of sterile distilled water making the dilution 10^{-2} and similarly making the dilution upto 10^{-8}. Suspension of 0.1ml of nodular extract suspension from 10^{-3}-10^{-8} dilution inoculated into sterile YEMA plates. The samples spreaded on the YEMA plates and incubated for 4-7 days in an incubator at 37^0c. Bacterial cells from a single colony streaked on the solidified medium with the help of a sterilized inoculation needle. The petri plates incubated at 25^0c for 2-3 days. Usually the separate colonies of the bacteria appeared in plates were used for further studies. Agar slant were prepared and cultures preserved in these tubes for further studies. Cultures subsequently sub-cultured and used regularly. Identification of the isolates were done by morphological and various biochemical methods.

2.5 Morphological Characterization

The colony characteristics (i.e. shape, size, colour, elevation, margin of the bacterial colony and their growth rate) were determined by observing the colonies on YEMA plates of the overnight grown microorganisms at 32°C (Fig 2, Table 2A, 2B).

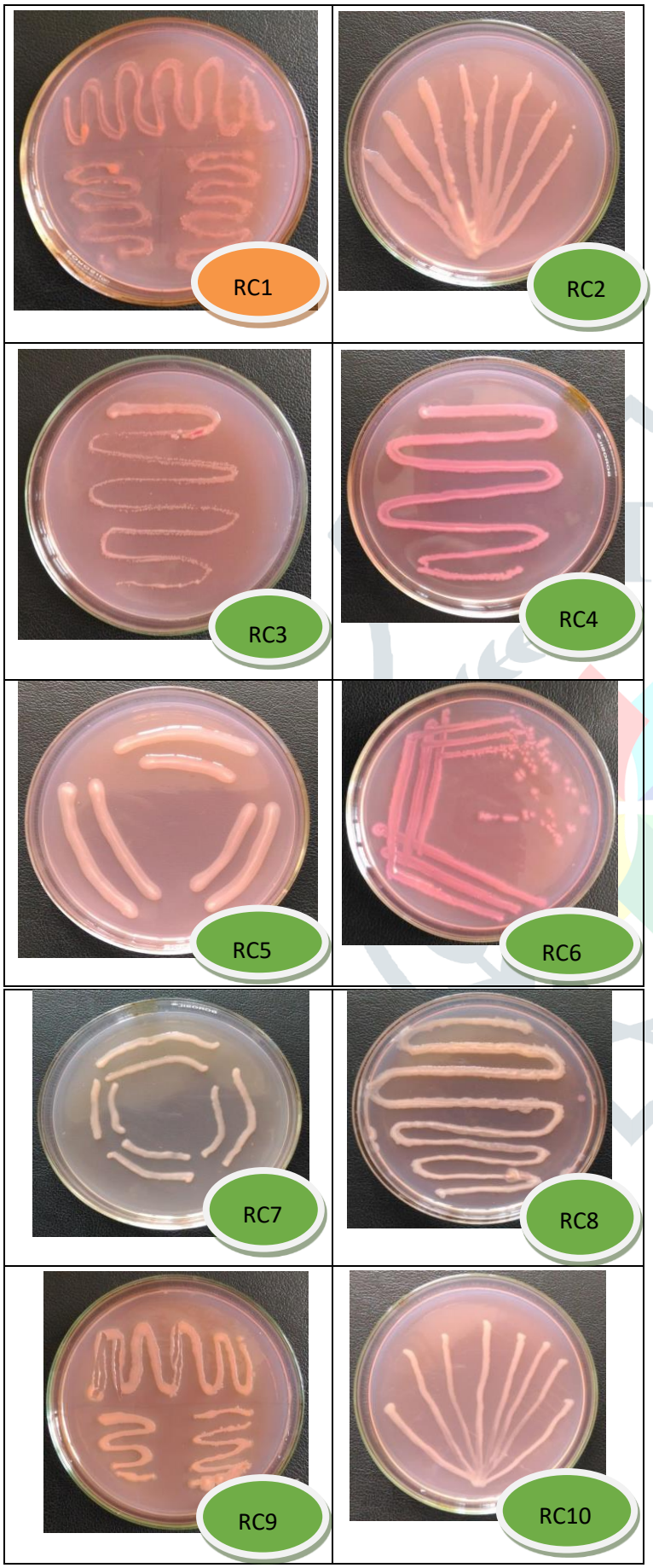

Figure 2. Isolation and purification of strain RC-1 to RC 10 on YEMA medium

2.6 Biochemical Characterization

All Isolates characterized by different biochemical methods. Methyl red test, VP test, Catalase test (cover slip method), starch hydrolysis test, citrate utilization test, and indole test (Figure 3, Table 1A, 1B). Catalase activity test, the presence of the enzyme catalase in the rhizobia isolates examined by suspending one loopful of culture organism in a drop of 3% Hydrogen peroxide on a glass slide [12]. These performed as per standard procedure. Production of bubbles indicates a positive result for catalase test. (Fig 3A, 3B).

2.7 Cultural and Metabolic Characterization

2.7.1 Bromothymol blue media

Yeast Mannitol Agar (YEMA) media incorporated with bromothymol blue was used to distinguish fast (acid producing) growing strains from slow (non-acid producing or alkali producing) growing rhizobia [13]. In this medium, the fast growers require 48 hours to produce an acidic reaction by turning the colour of the media yellow from green, whereas the slow growers take > 96 hours to produce alkaline endpoints with or without changing the colour of the media from green to blue.

2.7.2 Congo red test

The purity of the rhizobia isolates detected by adding Congo red YEMA media. Most rhizobia absorb the dye only weakly whereas contaminants including Agrobacteria, absorbs strongly.

2.7.3 Growth on 2% NaCl

To the basal medium of YEMA, 2% NaCl added to check the growth of isolates. As 2%, NaCl is inhibitory for some rhizobia isolates it may can serve as tools for identification of isolates.

2.8 Screening of isolates of rhizobium for various plant growth promoting

2.8.1 IAA production

IAA production detected by modifying the method described by Brick et al. [14]. 25ml nutrient broth inoculated with freshly grown cultures and kept at 37^{o}c for 36 hr. At 120 rpm in an incubator shaker. Then the cultures centrifuged at 10000 rpm for 15 min at room temperature. Carefully 1 ml supernatant was pipette out and 2 ml Urk van salkowski reagent (2% 0.5m Fe_2cl_3 in 35% per chloric acid) followed by two drops of orthophosphoric acid was added and kept in dark for color formation (Fig 3,Table 3A,3B).

2.8.2 Phosphate solubilization

All isolates screened for phosphate solublization [15]. Modified Pikovskya's agar with insoluble Dicalcium phosphate, a loopful of each culture placed on the center of Petri plates and incubated at 37^{0}c for 7 days. Appearance of hollow-zone around the colonies infers positive phosphate solubilizing ability. The solubilization zone was determined by subtracting the diameter of bacterial colony from the diameter of total zone. Phosphate solubilizing index was calculated using formula. [16]. Solubilization index (SI) = (colony diameter + halo-zone diameter)/colony diameter.

2.8.3 HCN production

Bacterial Isolates screened for the production of hydrogen cyanide (HCN) by adapting some method. [17]. Cultures streaked on nutrient agar amended with 4.4gm/l glycine. A whatsmann filter paper no. 1 dipped in 0.5% picric acid solution (in 2% sodium carbonate) placed inside the lid of the plates. Plates were sealed using Para film and incubated at 37^0c for 7 days. If paper turned yellow to brown in color, it showed HCN production by the isolates (Fig 3, Table 3A, 3 B).

2.8.4 Ammonia production

Bacterial isolates tested for the production of ammonia as described earlier [18] grown in peptone water at 37^0 c for 3 days. At the end of incubation period, 0.5ml of Nessler's reagent added to each tube. The development of faint yellow to dark brown color indicated the production of ammonia (Fig 3, Table 3A, 3B).

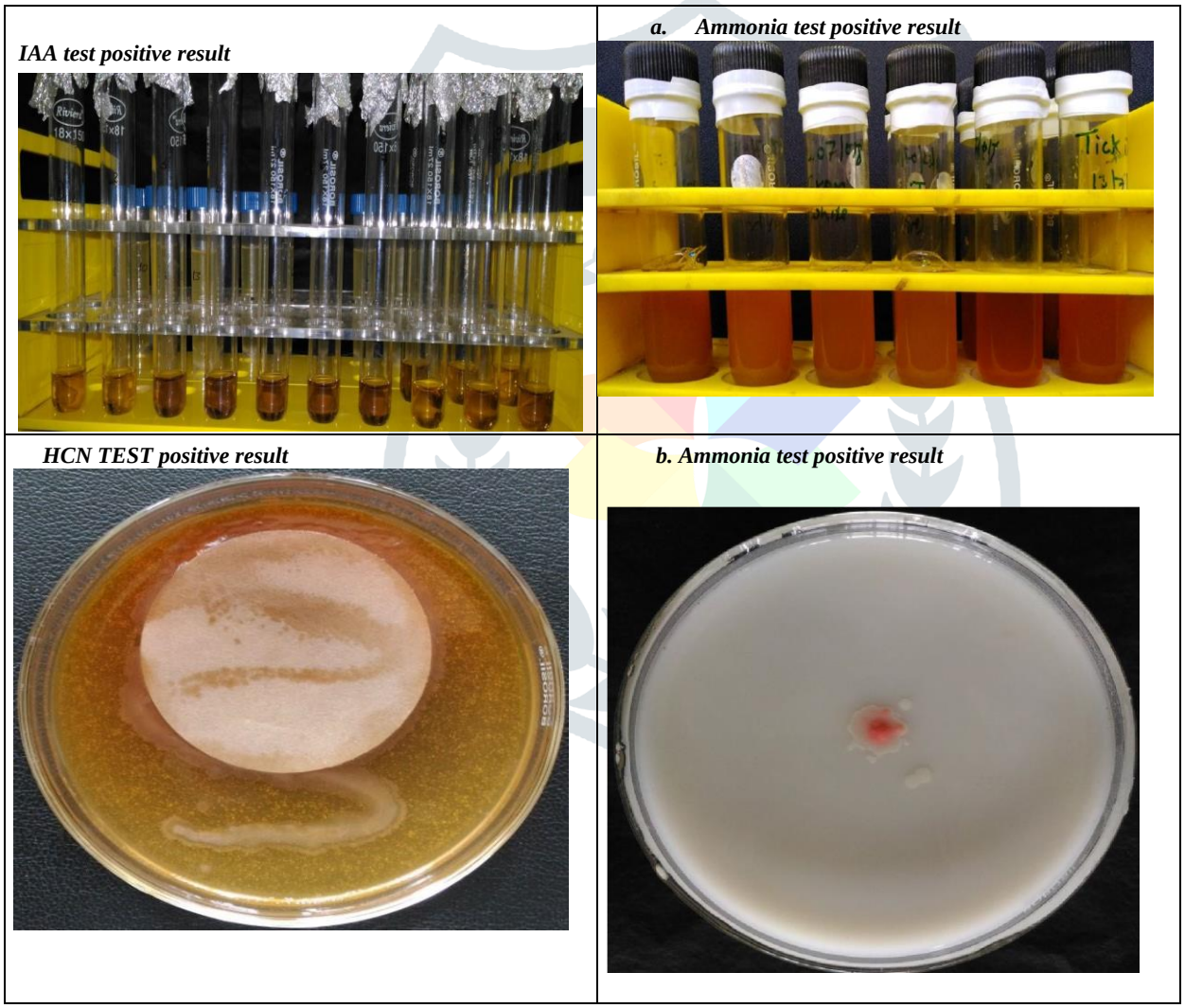

Figure 3 Screening for PGPR activity of isolated *Rhizobium* spp. through ammonia, HCN and IAA production test test.

Table 1A Isolates of *Rhizobium* species (*RC1, RC2, RC3, RC4.RC5, RC6 and RC7)* from wild, noncultivated leguminous plants

Sl No.	**Code of Isolates**	**Host plants**	**Botanical Names**	**Latitude Longitude**	**Morphology of Root Nodules**
1.	RC 1	Tephrosia	*Tephrosia Purpurea*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Elongated and Branch
2.	RC 2	Tick clover (White)	*Desmodium tortuosum*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval
3.	RC 3	Tick clover (Mix)	*Desmodium tortuosum*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval
4.	RC 4	Karanj	*Pongania pinnata*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval
5.	RC 5	Shisham	*Dalbergia sissoo*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval and Elongated
6.	RC 6	Lupin	*Lupinus albus*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval and cluster
7.	RC 7	Gulmohar	*Delonix regia*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval and Elongated

Table 1B Isolates of *Rhizobium* species *(RC8, RC9, RC10)* from cultivated leguminous plants

Sl No.	Code of Isolates	*Rhizobium* species	Botanical Names	Latitude Longitude	Morphology of Root Nodules
1.	RC 8	*R. phaseoli*	*Lablab purpureus*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval
2.	RC 9	*R.leguminosarum*	*Cajanus cajan*	23^0 45' N, 85^0 30' E elevation 620 m amsl	Oval
3.	RC 10	*R. ciceri*	*Cicer arietinum*	23^0 45' N, 85^0 30' E elevation 620 m	Branch

3. Results and Discussion

Seven *Rhizobium* strains (*RC1, RC2, RC3, RC4.RC5, RC6 and RC7)* were isolated from noncultivated wild leguminous plants and three strains *(RC8, RC9, RC10)* were isolated from cultivated leguminous plants (Fig1A, 1B). The colony morphology of isolated *Rhizobium* species monitored on the basis of colony characters by naked eye, all the ten strains of *Rhizobium* species have smooth pink colonies (indicators of *Rhizobium* species) on YEMA medium, with Congo red and white colonies on NA medium (Fig 2,Table-2A, 2B). Ten isolates of Rhizobium species screened for their PGPR activity (Table 3A, 3B). Among all ten isolated strains of Rhizobium species, seven strains were isolated from the non-cultivated wild varieties of leguminous plants that were *Tephrosia purpure*, *Desmodium tortuosu*, *Pongania pinnata*, *Dalbergia sissoo*, *Lupinus albus*, *Delonix regia*. (Fig 1A, 2B, Table 2A).Three strains were isolated from cultivated leguminous plants. Fig 1B2 B, Table 2B). Different biochemical test for screening for PGPR activities used such as IAA, HCN production and Ammonia production tests (Fig 3, Table 3A, 3B). These test result showed positive among all the isolates of *Rhizobium* species while negative result observed in phosphate solubilization (Fig 3, Table 3A, 3B).

Sl No.	Code of Isolates	Host plants	Colony Color on YEMA medium	Colony Color on NA medium
1.	RC 1	Tephrosia (*Tephrosia purpurea*)	Pink (8056)	White (8212)
2.	RC 2	Tick clover (*Desmodium tortuosum*)	Pink (8058)	White (7148)
3.	RC 3	Tick clover (*Desmodium tortuosum*)	Pink (8058)	White (L123)
4.	RC 4	Karanj (*Pongania pinnata*)	Pink (8047)	White (7148)

5.	RC 5	Shisham (*Dalbergia sissoo*)	Pink (8058)	White (L123)
6.	RC 6	Lupin (*Lupinus albus)*	Pink (8099)	White (L136)
7.	RC 7	Gulmohar (Gulmohar)	Pink (8047)	White (7148)

Table 2 A Colony morphology Isolates of *Rhizobium* species (*RC1, RC2, RC3, RC4.RC5, RC6 and RC7)* of wild leguminous plant on YEMA and NA medium*Note: Colour codes are as per colour of Asian paint colour catalogue.

Sl No.	Code of Isolates	*Rhizobium* species	Colony Colour on YEMA medium	Colony Colour on NA medium
1.	RC 8	*R. phaseoli*	Pink (8034)	White (L123)
2.	RC9	*R. leguminosarum*	Pink (8034)	White (8212)
3.	RC10	*R. cicero*	Pink (8099)	White (8212)

Table 2 B Colony morphology of *Rhizobium* species *(RC8, RC9 and RC10)* of cultivated leguminous plants on YEMA and NA media *Note: Color codes are as per color of Asian paint color catalogue

Sl no.	Code of Strain	Isolated From	Gram Reaction	Ammonia Production	HCN Production	IAA Production	Phosphate Solubilisation
1.	RC 1	Tephrosia	-	+	+	+	-
2.	RC 2	Tick clover White	-	+	+	+	-
3.	RC 3	Tick clover Mix	-	+	+	+	-
4.	RC 4	Karanj	-	+	+	+	-
5.	RC 5	Shisham	-	+	+	+	-
6.	RC 6	Lupin	-	+	+	+	-
7.	RC 7	Gulmohar	-	+	+	+	-

Table 3A Biochemical tests of *Rhizobium* species (*RC1, RC2, RC3, RC4.RC5, RC6 and RC7)* isolated from wild leguminous plants

***+ = Positive, - = Negative**

Table 3 B Biochemical tests of *Rhizobium* species (*RC8, RC9, and RC10)* isolated from cultivated leguminous plants

***+ = Positive, - = Negative**

Sl no.	Code of Strain	*Rhizobium* species	Gram Reaction	Ammonia Production	HCN Production	IAA Production	Phosphate Solubilisation
1.	RC 8	*R. phaseoli*	-	+	+	+	-
2.	RC 9	*R.leguminosarum*	-	+	+	+	-
3.	RC 10	*R. ciceri*	-	+	-	+	-

One of the main aim of the study of Nitrogen fixing bacteria from the wild leguminous plants is to promote growth of crops by making inoculum of these bacteria to promote the use of Bio fertilizer. Earlier Association of *Rhizobia* with leguminous plants well documented. Exploitation of this beneficial nitrogen-fixing root nodule symbiosis become essential in applied agricultural microbiology. However, little information is available previously regarding the mechanism of association between non-legumes and rhizobia. Ten bacterial strains were isolated from studied area. Growth of bacteria on Yeast Extract Mannitol Agar (YEMA) medium indicates their ability to fix atmospheric Nitrogen to the non- cultivated leguminous plants. The use of YEMA medium for the selective isolation of Nitrogen fixing bacteria had earlier reported by some scientists [19, 20]. All strains inoculated on YEMA as well as nutrient agar media. Pink Colony appeared on YEMA media whereas white in Nutrient agar (Fig 2, Table 2A, 2B). All the isolates from non cultivated (*RC1, RC2, RC3, RC4.RC5, and RC6)* were showed circular, pinhead type small sized colonies on Yeast Extract Mannitol Agar (YEMA). Such strains also showed that positive result for ammonia, HCN, and IAA test. Each isolates found to be negative for phosphate solublization test. Report on *Rhizobium*-mediated defense responses and growth promotion of non-legume (such as rice) provides a novel paradigm of symbiotic plant-microbe interaction [21]. All isolates of noncultivated nodules acted as fast grower and produced acid in bromothymol blue. In Congo red, all the isolates showed pink colour or showed poor absorption of dye Cong red. This fact give further evidence for purity of the *Rhizobium* isolates. For conformation of presence of *Rhizobium strain* all, the isolates showed no growth on the YEMA with 2% NaCl, thus confirming the *Rhizobia*. Therefore, all isolates may confirmed as *Rhizobium* strains.

4. Conclusion

In the present study, ten species of *Rhizobium* from cultivated and non-cultivated leguminous plants were isolated, characterized and identified its PGPR activities. Conventional methods used for its identification. They were cultured in selective media for *Rhizobium* species isolation. All ten isolates identified through different biochemical test. Seven strains were isolated from the noncultivated wild varieties of leguminous plants and three strains were isolated from cultivated leguminous plants. IAA, HCN production and ammonia production test observed positive

in all isolates of *Rhizobium* species. Inoculum of nitrogen fixing *Rhizobia* from these host plants can used as plant growth promoter. This work promote organic farming in the country and is an important factor for the development of the sustainable agricultural system because it is necessary for the agriculture-based country.

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REFERENCES

[1] Dixon R O D, Wheeler C T. (1986) Nitrogen fixation in plants. Glasgow, United Kingdom: Blackie and son, Ltd.157. DOI:https://doi.org/10.1016/0092-8674(87)90699-4

[2] Franche, C., Lindstrom, K., Elmerich, C. (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant and Soil 321: 35-59. DOI: 10.1007/s11104-008-9833-8

[3] Hayat, R., Ali, S., Amara, U., Kalid, R., Ahmed, I., (2010). Soil beneficial bacteria and their role in plant growth promotion: a Review. Ann Microbiol 60:579-598.

[4] Zsbrau, H.H.,(1999) Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions and in arid climate, Microbial. Mol. Biol. Rev. 968-989.

[5] Denarie, J., Debelle, F. and Rosenberg, C. (1992). Signaling and host range variation in nodulation. Annual Review Microbiology, 46: 497-531.

[6] Kiers, E.T., Rousseau, R.A., West, S.A., Denison, R.F., (2003) Host sanctions and the legume–rhizobium mutualism. Nature.425: 79-81.

[7] Phillips, D.A., (1991). Flavonoids: Plant signals to soil microbes. Rec. Adv. Phytochem. 2:1-33.

[8] Olivares, J., Bedmar, E.J., Martinez, M.E., (1984). Infectivity of Rhizobium meliloti as affected by extra cellular polysaccharides. J. Appl.Bacteriol. 56: 389-393.

[9] Nutman, P.S., (1977). Study frame works for symbiotic nitrogen fixation. Academic Press London. 443.

[10] Kumari, B.S., Raghu, R.M., Mallaiah, K.V., (2009). Studies on exopolysaccharide and indole acetic acid production by Rhizobium strains from Indigofera. Afr. J. microbiol. Res. 3: 10-14.

[11]Temam, A.H., Alebejo, A.L., (2017) Isolation and Characterization of Rhizobia from Rhizospher and Root Nodule of Cowpea, Elephant and Lab Lab Plants International Journal of Novel Research in Interdisciplinary Studies Vol. 4, Issue 4, pp: (1-7), Available at: www.noveltyjournals.com

[12] Pervin, S., Jannat, B., Sanjee, S., Farzana, T., (2017). Characterization Of Rhizobia From Root Nodule And Rhizosphere Of Lablab Purpureus And Vigna Sinensis In Bangladesh. *Turkish Journal Of Agriculture - Food Science And Technology*, 5(1): 14-17.

[13] Mohammadi, K., Sohrabi,Y., Heidar,i G., Khalesro, S., Majidi, M., (2012). Effective Factors on Biological Nitrogen Fixation. *African Journal Of Agricultural Research*, 7(12):17821788

[14] Brick, J. M., Bostock, R.M,. Silverstone, S.E., (1991) Rapid in situ assay for indole acetic acid production by bacteria immobilized on nitrocellulose membrane. Appl. Environ.
Microbiol, 57 (1991), pp. 535-538

[15] Pikovskaya, R.I., (1948). Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiologiya, 17: 362–370.

[16] Karapagam, K., Nagalakshmi, P.K., (2014). Isolation and characterization of Phosphate solubilizing microbes from Agricultural soil. Int. J. curr. Microbiol app. Sci. 3(30): 601-614

[17] Lorck, H., (1948). Production of hydrocyanic acid by bacteria. Physiol. Plant. 1: 142-146

[18] Cappuccino, S., (1992).Biochemical activities of microorganisms Microbiology, a laboratory manual (1st ed.), The Benjamin/Cummings Publishing Co, California pp. 105-300.

[19] Mujahid, J., Hassan, M., Rahman, M., Rashid, A. N. M., (2013). Isolation and characterization of Rhizobium spp. and determination of their potency for growth factor production. *International Research Journal of Biotechnology*, 4(7):117-123.

[20] Rajpoot, P., Panwar, K.S., (2013). Isolation & Characterization of Rhizobia and their Effect on Vigna radiata Plant. *Octa Journal of Biosciences*, 1(1):69-76

[21] Ravi, P., Mishra, N., Ramesh, K. S., Hemant, K., Vinod K., Maurya, S.
(2006).JournalCurrent microbiology. Springer-Verlag.52 (5) :383-389.