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RESEARCH ARTICLE

CHARACTERIZATION OF *SINORHIZOBIUM AND ENSIFER* SPECIES ISOLATED FROM ROOT NODULES OF *VIGNA TRILOBATA* CULTIVARS

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ABSTRACT

A total of eight rhizobacterial strains were isolated from the root nodules of *Vigna trilobata* plants raised in soils of different districts of Andhra Pradesh, India. Morphologically all the strains were rod shaped gram negative and mucus producing. All these strains were positive for biochemical tests such as ammonia production, catalase test, oxidase test, nitrate reduction test, and urease test. These strains gave negative results for Ketolactose test, growth on glucose peptone agar medium and Hoffer's Alkaline medium. For amylase activity, three strains *Sinorhizobium* sp. MRR 101, *Sinorhizobium* sp. MRR 109 and *E.xinjiangense* MRR 110 showed negative results and rest of the strains showed positive results. For citrate utilization test except *Ensifer* sp. MRR 125 all isolates showed the positive results. Good colony growth was recorded at 0.1% NaCl with pH range of 7-8 and temperatures between 30ºC - 35ºC for all the strains studied. Indole acetic acid and EPS production was common for all the strains with IAA production in range from 8.5-70µg/ml, and EPS production from 379-892 mg/ml. Two out of eight strains i.e. *Sinorhizobium* sp. MRR 101 (250 µg/ml) and *S.kostiense* MRR 104 (510 µg/ml) exhibited phosphate solubilisation ability. Only one strain *E. xinjiangense* MRR 110 showed the chitinase activity (0.30 U/ml) on colloidal chitin agar medium. And three strains viz., *S. kostiense* MRR104, *E.xinjiangense* MRR 110, *S. terange* MRR 121 were HCN producers. None showed siderophore production. Among the eight strains, *Sinorhizobium* sp.MRR 101, *S.kostiense* MRR 104, and *E.xinjiangense* MRR 110 were proved to be better as plant growth promoting rhizobateria.

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INTRODUCTION

Though *Sinorhizobium* and *Ensifer* belongs to a single taxon (Weir, 2010) being the earlier heterotypic synonym, *Ensifer* takes the priority (Young, 2003). There was a taxonomic dispute regarding the systematic position of these two genera. Some suggested merging of all *Sinorhizobium* species in to *Ensifer* (Lindstrom *et al*., 2002) and others supported to keep them as two separate genera (Martens *et al*., 2007). Basing on the recommendations of the 8th European Nitrogen Fixation Conference held on 31 August 2008, these two genera have to be treated as separate, which of course, was later disapproved by Young (2010). For the present study we have treated them as separate genera. Bacteria that colonize the rhizosphere and plant roots, and enhance the plant growth by different mechanisms, are collectively referred to as Plant Growth Promoting Rhizobacteria. They exhibit a variety of characteristics responsible for plant growth. The common traits include production of plant growth regulators (like auxin, gibberellins, and ethylene), siderophores, HCN and antibiotics (Arshad *et al.*, 1992). *Rhizobium* and other related genera, *Rhizobia,* harbouring the nodules of leguminous plants were one among the rhizobacteria which exhibit the plant growth promoting characters. They can stimulate the growth of leguminous plants and by their nitrogen fixing ability (Kiers *et al*., 2003). Several environmental stresses may affect the nitrogen fixation in plants including salinity, water stress, soil pH, temperature and heavy metals (Kucuk and Kivanc, 2008). *Rhizobia* were well adopted for various environments with their symbiotic efficiency (Ali *et al*., 2009). Exopolysaccharide (EPS) produced by *Rhizobia* protects the cell from desiccation and predation and helps in nitrogen fixation by preventing high oxygen tension. In addition, *Rhizobia* secrete growth hormones like indole acetic acid (IAA), which shows positive influence on plant growth and also plays an important role in the formation and development of root nodules (Nutman,1977). Hence, the production of EPS and IAA are considered as important traits of plant growth promoting *Rhizobacteria*. In addition *Rhizobia* also have other enzymatic activities and ability to solubilise the inorganic phosphate in the soil and make it available to the plant.

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The genus *Vigna* with nearly 150 species was one of the major nodulating genera in the family Leguminoseae. *Vigna trilobata*, commonly called as 'Pillipesara', was mainly cultivated as short term Pasteur and green manure crop in India, Pakistan, Sudan and Indonesia. So far, the symbiont in the root nodules was reported as rhizobial strain but not characterized completely. Our study is the first of its kind on characterization of *Sinorhizobium* and *Ensifer* sp. from *Vigna trilobata*. The main objective was to study the morphological, biochemical and PGP characteristics of these microsymbionts.

MATERIALS AND METHODS

Isolation

Rhizobial strains were isolated from the root nodules of *Vigna trilobata* plants raised in earthen pots filled with soils collected from twenty one districts of Andhra Pradesh and maintained properly in the botanical garden of our university. For the isolation pink coloured healthy root nodules were collected by gently uprooting the plants, twenty one days after sowing (DAS), and surface sterilized with 0.1% Mercuric chloride and washed several times with sterile distilled water. Bacterial suspension was prepared by crushing these nodules with sterile glass rod using sterile distilled water. A loopful of suspension was prepared on media plates containing selective medium yeast extract Mannitol agar medium (YEMA) with 0.1% Congo red and incubated at room temperature for 3 days. After incubation, the white translucent, convex, colonies with high mucilage were isolated and pure cultures were maintained after subculturing using the same medium. Pure cultures of all the eight isolates were authenticated as *Rhizobium* by performing the appropriate biochemical tests (Somasegaran and Hoben, 1994), and nodulation ability on homologous hosts by plant infection test (Vincent, 1970). However, by 16S rDNA sequencing (Macrogen, South Korea) the sequences of these eight strains showed 99% similarities with those of *Sinorhizobium* and *Ensifer* species and were identified as three belongs to *Sinorhizobium* sp., two *S. kostiensis,* and one each belongs to *S. terangae, Ensifer xinjiangensis,* and *Ensifer sp.* and the sequences were deposited in the Gene bank. The eight strains identified with accession numbers are - *Sinorhizobium* sp. MRR 101(KC428651), *S. kostiensis* MRR 104(KC428653), *Sinorhizobium* sp.MRR 109(KC503886), *E. xinjiangense* MRR110 (KC415691) (= *E. fredii* as per the latest taxonomic status– www.rhizobia.co.nz/axonomy/rhizobia), *Sinorhizobium sp.* MRR 114 (KC503887), *S. kostiensis* MRR 117(KC415695), *S. terange* MRR 121 (KC503883), and *Ensifer* sp. MRR 125 (KC503885).

Morphological characterization

The colony morphology of the isolates was examined on Yeast Extract Mannitol Agar (YEMA) plates after 72 hrs of incubation at 28ºC. Rhizobial colonies were examined on shape, colour, production of mucus and gram staining (Aneja, 2003).

Bio chemical characterization

The isolates were also tested for different biochemical characteristics namely ammonia production, amylase test, citrate test, catalase test, oxidase test, nitrate reduction test, and urease tests by following the standard procedures (Somasegaran and Hoben 1994, Aneja 2003).

Effect of Salt (NaCl), pH and Temperature Tolerance

The ability of the isolated strains to grow in different concentrations of salt (NaCl) was tested by inoculating them in YEM broth containing (0, 0.05, 0.1, 0.5, 1.0, 1.5, 2.5, 3.0, 3.5 and 4.0% of NaCl) and incubated at room temperature for 72h. pH tolerance of the strains was tested by adjusting the pH of the YEM broth to (3.0 ,4.0, 5.0, 6.0, 7.0 8.0, 9.0, 10.0 and11.0) with either NaOH or HCl. Effect of temperature on growth was studied by incubating the inoculated YEM broth cultures of the strains at different temperatures - 4 ºC, 20 ºC , 25 ºC,30 ºC,35 ºC and 40ºC. The growth was measured by recording the difference in optical density (OD) between the control and the treatment at 610 nm using spectrophotometer (Kucuk *et al*., 2006, Mensah *et al*., 2006 and Ali *et al*., 2009).

Plant growth promoting characteristics

IAA Production

The production of IAA was determined by the method Gordon and Weber (1951). For IAA production, all the eight strains were inoculated separately in to Erlenmeyer flasks (250ml) containing 100 ml of YMB supplemented with L-tryptophan (100mg/ml). The cultures were incubated at 28^0 C on a rotary shaker at 200 rpm for 72hrs. After incubation the culture broth was centrifuged at 10,000 x g for 5 min. and used for IAA extraction (Sinha and Basu, 1981). To the 1 ml of the supernatant, 2 ml of Salkowsky's reagent (0.5 M $FeCl_3$ in 35% perchloric acid) was added and incubated for 30 min. under darkness. The absorbance of the colour developed was measured at 530 nm in a spectrophotometer. The amount of IAA produced was calculated by using the standard graph of authentic IAA (Hi-media).

EPS production

The bacterial isolates were inoculated into Erlenmeyer flasks (250 ml) containing 100 ml YEM broth supplemented with 1% Mannitol. The flasks were incubated at RT on orbital shaker at 200 rpm for 72 h. After incubation the broth was centrifuged at 3000 X g and collected supernatant was mixed with 2 volumes of chilled acetone. The crude polysaccharide precipitate was collected by centrifugation at 3000Xg for few minutes. The EPS was washed with distilled water and acetone alternately, transferred into a filter paper and weighed after overnight drying at 105^0C (Damery and Alexander, 1969).

Phosphate Solubilization

Phosphate solubilization ability of isolated strains was detected by spotting separately on Pikovskya's agar plates. Plates were then incubated at 28 ± 1°C for 3 d, and observed for the clearing zone around the colonies (Pikovskaya, 1948). The isolates which formed zone of solubilization on agar medium were further tested in flasks containing 100 ml of Pikovskaya's broth having initial pH 7.0. One ml of the inoculum was inoculated into the broth and the flasks were incubated on rotary shaker (200 rpm) at 28 ± 2ºC for 72 hrs. After incubation, the pH of the broth was measured and the amount of liberated P_2O_5 was estimated. For this, the bacterial cultures were filtered through Whatman No.1 filter paper and centrifuged at 3000 rpm for 15 min. Filtration and centrifugation was repeated until a clear solution was obtained.

Table 1. Morphological, Physiological and Biochemical characteristics of *Sinorhizobium* and *Ensifer* species from root nodules of *Vigna trilobata*

Morphological and Biochemical tests	*Sinorhizobium* sp. MRR 101	*S.kostiensis* MRR 104	*Sinorhizobium* sp.. MRR 109	*E. xinjiangense* MRR 110	*Sinorhizobium* sp. MRR 114	*S.kostiensis* MRR 117	*S.terange* MRR 121	*Ensifer* sp. MRR 125
Grams reaction	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative	Gram negative
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Growth on YEMA	+	+	+	+	+	+	+	+
Growth on Hoffer's Alkaline medium	-	-	-	-	-	-	-	-
Colour of the Colony	White	Light Yellow	White	White	White	White	White	White
Shape of the Colony	Round	Round	Round	Round	Round	Round	Round	Round
Mucilage production	Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Production of Ketolactose test	-	-	-	-	-	-	-	-
Growth on Glucose peptone Agar medium	-	-	-	-	-	-	-	-
Nodulation tests	+	+	+	+	+	+	+	+
Ammonium test	+	+	+	+	+	+	+	+
Amylase test	-	+	-	-	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+
Oxidase test	+	+	+	+	+	+	+	+
Nitrate reductase test	+	+	+	+	+	+	+	+
Urease test	+	+	+	+	+	+	+	+
Citrate test	+	+	+	+	+	+	+	-

Table 2. Effect of Salt (NaCl) concentration on Growth (OD) of the *Sinorhizobium* and *Ensifer* species from root nodules of *Vigna trilobata*

Bacterial Strains	NaCl Concentration										
	0%	0.05%	0.1%	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%	3.5%	4.0%
Sinorhizobium sp. MRR 101	0.017	0.156	0.515	0.412	0.378	0.350	0.286	0.136	0.136	0.055	0.032
S. kostiensis MRR 104	0.024	0.164	0.166	0.130	0.055	0.032	0.012	0.000	0.000	0.000	0.000
Sinorhizobium sp. MRR 109	0.036	0.178	0.342	0.270	0.175	0.132	0.058	0.000	0.000	0.000	0.000
E. xinjiangensis MRR 110	0.056	0.150	0.350	0.232	0.195	0.118	0.094	0.000	0.000	0.000	0.000
Sinorhizobium sp. MRR 114	0.056	0.106	0.371	0.226	0.195	0.151	0.118	0.068	0.000	0.000	0.000
S. kostiensis MRR 117	0.035	0.168	0.573	0.461	0.345	0.335	0.251	0.000	0.000	0.000	0.000
S. terange MRR 121	0.053	0.107	0.583	0.489	0.365	0.365	0.252	0.000	0.000	0.000	0.000
Ensifer sp. MRR 125	0.017	0.118	0.495	0.325	0.231	0.187	0.106	0.092	0.041	0.012	0.000

Table 3. Effect of pH on growth (OD) of *Sinorhizobium* and *Ensifer* species from root nodules of *Vigna trilobata*

Bacterial strains	pH								
	pH-3	pH-4	pH- 5	pH- 6	pH-7	pH- 8	pH- 9	pH- 10	pH- 11
Sinorhizobium sp. MRR 101	0.060	0.174	0.265	0.390	0.623	0.414	0.246	0.168	0.011
S. kostiensis MRR 104	0.010	0.151	0.265	0.430	0.727	0.416	0.219	0.190	0.040
Sinorhizobium sp. MRR 109	0.020	0.124	0.218	0.273	0.527	0.421	0.213	0.129	0.020
E. xinjiangensis MRR 110	0.010	0.110	0.234	0.398	0.517	0.360	0.179	0.151	0.040
Sinorhizobium sp. MRR 114	0.020	0.060	0.196	0.265	0.589	0.333	0.192	0.147	0.060
S. kostiensis MRR 117	0.020	0.129	0.231	0.339	0.539	0.413	0.213	0.119	0.020
S. terange MRR 121	0.020	0.132	0.239	0.342	0.483	0.326	0.254	0.160	0.060
Ensifer sp. MRR 125	0.020	0.059	0.273	0.318	0.593	0.404	0.314	0.132	0.020

Table 4. Effect of Temperature on Growth of *Sinorhizobium* and *Ensifer* species from root nodules of *Vigna trilobata*

Bacterial strains	Temperature						
	4°C	20°C	25°C	30°C	35°C	40°C	45°C
Sinorhizobium sp. MRR 101	0.002	0.052	0.271	0.627	0.412	0.156	0.012
S. kostiensis MRR 104	0.001	0.036	0.149	0.300	0.140	0.100	0.000
Sinorhizobium sp. MRR 109	0.001	0.040	0.150	0.337	0.181	0.136	0.000
E. xinjiangensis MRR 110	0.002	0.045	0.136	0.315	0.154	0.000	0.000
Sinorhizobium sp. MRR 114	0.003	0.041	0.188	0.384	0.036	0.000	0.000
S. kostiensis MRR 117	0.003	0.068	0.226	0.580	0.484	0.116	0.000
S. terange MRR 121	0.005	0.056	0.231	0.558	0.414	0.116	0.012
Ensifer sp. MRR 125	0.003	0.056	0.153	0.553	0.233	0.133	0.019

Table 5. Plant growth promoting characteristics of *Sinorhizobium* and *Ensifer* species from root nodules of *Vigna trilobata*

Bacterial strains	PGP characteristics			
	IAA production (µg/ml)	EPS production (mg/ml)	Phosphate solubilisation (µg/ml)	Chitinase production (U/ml)
Sinorhizobium sp. MRR 101*	24.5	585	250	0
S. kostiensis MRR 104*	45	892	510	0
Sinorhizobium sp. MRR 109	41	642	0	0
E. xinjiangensis MRR 110#	42.5	692	0	0.30
Sinorhizobium sp. MRR 114	70	494	0	0
S. kostiensis MRR 117	38	535	0	0
S. terange MRR 121	8.5	379	0	0
Ensifer sp. MRR 125	46.5	719	0	0

*Phosphate solubilisers, # Chitinase producer

To a 10 ml of aliquot of the clear culture supernatant, 2.5 ml of Barton's reagent was added. After 10 minutes, the resultant yellow colour was read in spectrophotometer meter at 430 nm (Jackson, 1973) and the liberated P_2O_5 was estimated by comparing the values with standard curve prepared with K_2HPO_4.

Chitinase production

The chitinase was assayed by the method described by Vyas and Desh Pande (1989). Chitinase activity was determined by incubating 1 ml of crude enzyme with 1 ml of 1% colloidal chitin in 0.05 M phosphate buffer pH 7.0 at 35 °C for 1 h. After centrifugation, 1 ml of reaction mixture was taken and to this 1 ml of distilled water was added, boiled in a glass ball – covered centrifuge tube for 10 minutes and then centrifused. From the supernatant 0.5 ml of aliquot was taken and to this 0.1 ml of Potassium tetraborate was added and boiled for exactly 3 minutes in a water bath. After cooling, 3 ml of P-Dimethyl Amino Benzaldehyde (P-DMAB) reagent was added, and the absorbance was read at 585 nm against the blank prepared without chitin or enzyme. The amount of N-Acetyl D-Glucose Amine released in the supernatant was determined using N-Acetyl D-Glucosamine as the standard. One unit of the chitinase activity was defined as the amount of the enzyme products 1µ mole of N- Acetate glucosamine in 1 ml of reaction mixture under the standard assay condition (Mathivanan *et al*., 1998). Stranded graph was prepared with curve for authentic N-Acetyl D-Glucosamine to convert the absorbency values to micro moles of N-Acetyl D-Glucosamine liberated from colloidal chitin.

HCN production

Actively growing bacterial cultures were inoculated on YEMA plates amended with Glysine at 4.4 g/l. a Whatman filter paper No.1 (9 cm in diameter) soaked in 2% Picric acid solution was placed in the upper lid of Petri dish. Plates were sealed with parafilm. The plates were incubated for 7 days at room temperature. Change in colour from yellow to light brown, moderate (brown) or strong (reddish brown) indicated hydrogen cyanide production. Control plates did not receive inoculum (Miller and Higgins 1970).

RESULTS AND DISCUSSION

Morphological, Physiological and biochemical characterization

All the strains showed maximum growth on YEMA medium at pH 7.0 after 72 hrs of incubation (Table-1). Colonies were round, translucent with entire margins and mucilaginous in nature. Similar colony characters were reported for *Ensifer* sp. from *Cajanus cajan* by Dubey *et al*. (2010). Our results indicated that except *S. kostiense* MRR 104 all the strains appeared white colour colonies on YEMA medium. All the strains showed the negative results for growth on Hofer's alkaline medium, Glucose peptone agar medium and Ketolactose production. Deka and Azad, (2006) also reported the similar conditions for *Rhizobium* species. All the strains showed positive results for biochemical tests such as ammonia production, catalase test, oxidase test, nitrate reductase test and urease test. For amylase test the three strains *Sinorhizobium* sp. MRR 101, *Sinorhizobium* sp. MRR 109 and *E. xinjiangense* MRR 110 showed negative results while all the strains showed positive results for citrate utilization test except *Ensifer* sp. MRR 125.

Effect of NaCl, pH and Temperature on growth

In the present study, all the eight strains were able to tolerate NaCl concentration upto 2.0% (Table 2). Similarly 2% NaCl tolerance was also exhibited by *Ensifer sp.* from *Cajanus cajan* (Dubey *et al*., 2010), and 5 bacterial strains from root nodules of *Cajanus cajan* (Deb *et al*., 2015). Among the eight, only *Sinorhizobium* sp. MRR 101 showed tolerance up to 4.0% NaCl concentration. However, Kaberi *et al*. (2015) reported that two bacterial strains KRN1 and KRN5 from *Cajanus cajan* showed tolerance at 10% NaCl.

pH

All the eight strains showed maximum growth at pH 7.0 with gradual increase from pH 2.0 and gradual decrease after reaching the peak at pH 7.0 (Table 3). *Sinorhizobia* generally have pH range of 5 -10.5 for optimal growth and growth was adversely affected by low pH (Brenner and Krieg, 2006). Similarly, the strains in the present study showed growth in acidic as well as alkaline conditions. This may be due to their adaptability towards acid tolerance. Sethi and Adhikari (2014) studied the growth pattern of six strains isolated from *Vigna radiata* and seven strains from *Arachis hypogea* and reported that they can grow well at pH 7- 8.

Temperature

An optimum temperature of 30°C was recorded for all the strains in the present study, however, three strains MRR 101, *S. terange* MRR 121 and the *Ensifer* sp. MRR 125 showed the growth even up to 45°C (Table 4). The strains MRR104 and MRR109 showed temperature tolerance up to 40°C. The strains MRR110 and MRR114 showed growth up to 35°C. Optimum temperature of 30 °C was also reported for six strains from *Vigna radiata* and seven strains from *Arachis hypogeae* by Sethi and Adhikari, (2014). *Sinorhizobium* strains generally grow between 25-30°C (Brenner and Krieg, 2006). Majority of the strains in the present study exhibited temperature tolerance of more than 30°C, may be as an adaptation to the environment from which they were isolated.

Plant growth promoting characteristics

Indole acetic acid and EPS production was common for all the strains (Table 5). Two out of eight strains i.e. *Sinorhizobium* sp. MRR 101 and *S.kostiense* MRR 104 exhibited phosphate solubilisation ability. Only one strain *E. xinjiangense* MRR 110 showed the chitinase activity on colloidal chitin agar medium. And three strains viz., *S. kostiense* MRR104, *E.xinjiangense* MRR 110, *S. terange* MRR 121 were HCN producers (not shown in the table). None showed siderophore production.

IAA production

Majority of the strains in the present study produced IAA in the range of 38- 50 µg/ml. Maximum IAA production was recorded in *Sinorhizobium* sp. MRR 114 (70 µg/ml) and minimum of 8.5 µg/ml was recorded in *S. terange* MRR 121. Similar type of L- tryptophane induced IAA production by *Ensifer* and *Sinorhizobium* sp was also reported by (Kaur *et al*., 2014) and Dubey *et al*., 2010) respectively. However a

maximum of 92.6 µg/ml of IAA production was reported by Rhizobium species isolated from *Vigna trilobata* (Kumar and Ram, 2012). The *Ensifer* sp. of *Vigna trilobata* are proved to be better than the *Ensifer* sp. from soybean which produced only 30.9 µg/ml of IAA (Kaur et al., 2014).

EPS production

The EPS production by the strains in this study was in the range of 379-892 mg/ml. Maximum EPS production of 892 mg/ml was observed in *S. kostiensis* MRR 104 followed by MRR 125 with 719 mg/ml. Tank and Saraf (2003), reported the exopolysaccharide production and tri calcium phosphate solubility by rhizobacteria isolated from *Trigonella foenum-graecum.*

Phosphate solubilization

Among the eight tested only two strains, *Ensifer* sp. MRR 101 (250µg/ml) and *Ensifer* sp. MRR 104 (510 µg/ml) showed the phosphate solubilisation after 72 hours of incubation. *Sinorhizobium meliloti* TR1 was also reported to solubilize TCP in both liquid and solid Pikovskaya's medium with a decline in pH (Tank and Saraf, 2003). These strains can be considered as poor solubilizers when compared to the *Ensifer* sp. LSER 8 with solubilising ability up to 6. 59 mg 100 ml^{-1} on 12th day (Kaur *et al*., 2014). Dubey *et al*. 2010 also reported that maximum phosphate solubilization in *Sinorhizobium* spp. KCC5 from *Cajanus cajan* (L.) at 7^{th} day of incubation.

Chitinase production

Out of eight, only one strain *Ensifer* sp. MRR 110 showed the Chitinase (0.30U/ml) production. Sridevi and Mallaiah (2008) studied the 26 rhizobial strains from root nodules of *Sesbania sesban* for chitinase activity. Among these 12 strains showed chitinase activity after 36 hr of incubation at neutral pH.

HCN production

Three strains i.e. *Ensifer kostisense* MRR 104, *Ensifer xinjiangense* MRR 110 and *Ensifer terange* MRR 121 showed the HCN production. Plant growth promoting rhizobacteria produce HCN to control the growth of different types of pathogens (Bagnasco *et al.*, 1998) in the rhizosphere. Production of HCN by Rhizobia was previously reported by many workers. Beauchamp *et al* (1991) reported that 4 out of 32 rhizobial strains produced HCN. De Brito *et al.* (1995) observed that less than 1% rhizobia isolated from tomato rhizosphere showed positive for HCN production. Deb *et al* 2015 studied the 5 bacterial strains from root nodules of *Cajanus cajan* all isolates were positive for Hydrogen Cyanide (HCN) production.

Conclusion

The root nodules of Vigna trilobata can be colonized by rhizobacteria other than Rhizobium like Sinorhizobium and Ensifer species. This was first report of these strains from the host. Among the eight strains, *Sinorhizobium* sp.MRR 101, *S.kostiense* MRR 104, and *E. xinjiangense* MRR 110 exhibited the maximum PGPR characters and were proved to be better plant growth promoting rhizobateria.

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