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Preliminary Characterization of Rhizobacterial Strains Isolated from Legume [Vigna trilobata (L.) verdc.] Root Nodules

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ABSTRACT

Four Rhizobial strains were isolated from root nodules of Vigna trilobata (L –Verdc) plants raised in soils of different areas in Andhra Pradesh India. These strains were isolated on selective Yeast Extract Mannitol Agar (YEMA) medium. In the present study four Rhizobial strains were further subjected to physiochemical and biochemical characteristics. The Rhizobium strains were rod shaped gram negative and mucilaginous in nature. Except Rhizobium sp. MRR106 all the isolated colonies showed in white colour. Further these four strains were observed for physical conditions like pH, NaCl and temperature for their growth. Optimum conditions of these strains were at 30^0 C temperatures, 0.1% NaCl and a neutral pH 7.0. All the four strains were positive for biochemical tests such as ammonia production test, oxidase test, catalase test, nitrate reductase test and urease test. Except Rhizobium sp. MRR 123 all the strains showed amylase and citrate utilization tests. All these strains showed negative chemical reaction on hofer's alkaline broth test, Ketolactose test and glucose peptone agar tests.

Keywords: Rhizobium, Vigna trilobata, Yeast Extract Mannitol Agar

I. INTRODUCTION

Root nodule bacteria (RNB) or "rhizobia" are a type of plant growth promoting bacteria, typified by their ability to fix nitrogen for their plant host, fixing nearly 65% of the nitrogen currently utilized in sustainable agricultural production of legume crops and pastures. Rhizobia are bacteria capable of fixing nitrogen and forming root nodules in plants belonging to the family Leguminosae (Fabaceae). Currently, this group of bacteria includes 76 species within 13 genera (Weir 2010). A successful symbiosis is the result of a complex series of interactions between the host and the symbiont (Pellock et al. 2000).

Vigna is one of the major nodulating Genera in the family Leguminoseae. Out of 150 species, only 41 were reported to be nodulated (Allen and Allen 1981). Vigna trilobata commonly called as Pillipesara, African gram, Jungle mat bean, Mukni, was mainly cultivated as short term pasture and green manure crop in India, Pakistan, Indonesia and Sudan. In India, Andhra Pradesh is one of the states with highest production of forage crops like Pillipesara and sun hemp .Vigna trilobata is a wild species belonging to the sub genus Ceretotropis in the genus Vigna. So far, the symbiont in the root nodules was reported as rhizobial strains but not characterized completely.

Much work has been done on the Physico chemical, cultural and biochemical characterization of Rhizobial isolates from legume root nodules. Particularly no work has been done on characterization of rhizobacterial isolates from root nodules of Vigna trilobata. Our study is the first of its kind on characterization of the Rhizobial sp. isolated from Vigna trilobata. Therefore our objective was to study the morphological, biochemical and PGP characteristics of Vigna trilobata microsymbionts.

II. MATERIALS AND METHODS

1. 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 the same medium. Pure cultures of all the four isolates were authenticated as Rhizobium by performing the appropriate biochemical tests (Somasegaran and Hoben, 1994), and nodulation ability on homologous hosts by plant infection tests (Vincent, 1970).

2. Morphological characterization

The colony morphology of the isolates was examined on Yeast Extract Mannitol Agar (YEMA) plates after incubation of 72 hrs at 28ºC. Rhizobial colonies were examined on shape, colour, production of mucus and gram staining. (Aneja 2003). Gram's procedure was done as per the method described by (Somasegaran and Hoben 1994).

3. Bio chemical characterization

The isolates were also tested for different biochemical characteristics namely Ammonia production test, Amylase test, citrate test, catalase test, oxidase test, nitrate reduction test, and urease tests (Somasegaran and Hoben 1994, Aneja 2003).

4. Effect of Salt (NaCl), pH and Temperature Tolerance

The ability of the isolated Rhizobial strains to grow in different concentration of salt (NaCl) was tested by inoculating on 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. Differences in pH tolerance were tested in YEM broth by adjusting the pH (3.0 ,4.0, 5.0, 6.0, 7.0 8.0, 9.0, 10.0and11.0) with either NaOH or HCl. Difference in the range of growth temperature were investigated by incubation of bacterial cultures in YEM broth at (4 ºC, 20 ºC , 25 ºC,30 ºC,35 ºC and 40ºC). . Optical density (OD) observation was recorded at 610 nm using spectrophotometer (Kucuk et al., 2006, Mensah et al., 2006 and Ali et al., 2009).

III. RESULTS & DISCUSSION

Four rhizobacterial strains were successfully isolated from the root nodules of Vigna trilobata. These strains were molecularly characterized by 16S rDNA partial gene sequencing i.e. Rhizobium sp. MRR 103 (JX 576499), Rhizobium sp. MRR 106 (KC 428655), Rhizobium sp. MRR 112 (KF 621018), Rhizobium sp. MRR 123 (KC 503884) identified upto the species level, through Macrogen (South Korea) and the sequences were deposited in National Centre for Biotechnology Information (NCBI) Gen Bank. All the four Rhizobacterial strains from root nodules of Vigna trilobata showed a maximum growth on YEMA medium on room temperature at pH. 7.0 after 72 hours of incubation period (Table-1). All the strains were preliminary characterized for colony and cell morphology and Gram staining. Colonies of Rhizobial sp. were found to be round, gram negative and mucilaginous in nature. Among the strains studied except Rhizobium sp. MRR 106, all the strains produced white coloured colonies while this strain

produced light yellow colony. All the Rhizobium strains showed the negative results on Hofer's alkaline medium, growth on Glucose peptone agar medium and production of Ketolactose. Similarly the Rhizobium cannot grow in Hofer's alkaline medium. 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 and citrate test, the Rhizobium sp. MRR 123 showed negative results while all the strains showed positive results. Similar results were also reported by Deka and Azad (2006), Ali et al., (2009) Gachande and Kansole (2011).

Table 1. Morphological, Cultural and Biochemical Characterization of Rhizobacterial strains

Morphological, and Biochemical characterization	**Rhizobium sp. MRR 103**	**Rhizobium sp. MRR 106**	**Rhizobium sp. MRR 112**	**Rhizobium sp. MRR 123**
Grams reaction	Gram Negative	Gram Negative	Gram Negative	Gram Negative
Shape	Rod	Rod	Rod	Rod
Growth on YEMA	+	+	+	+
Growth on Hoffer's Alkaline medium	-	-	-	-
Colour of the Colony	White	Light Yellow	White	White
Shape of the Colony	Round	Round	Round	Round
Mucilage production	High	Medium	High	High
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	+	+	+	-

*Each data is an average of three replicates

1. NaCl, pH and Temperature tolerance

Temperature, NaCl and pH are important parameters for the growth of the organism. Rhizobacterial strains mainly dependent on the growth parameters like incubation period, pH, NaCl and temperature.

i). Effect of NaCl

From the results showed that the optimum NaCl (0.1%) for all the four Rhizobial strains. The rhizobacterial strain Rhizobium sp. MRR 103 tolerate NaCl upto 3.0%. (Table-2.)Rhizobial growth was observed in 0% to 3.0% of NaCl concentration. Similarly, Kucuk et al., (2006) reported that 30 isolates isolated from bean showed tolerance at 3% (w/v) NaCl. Out of thirty isolates, only 20 isolates showed their ability to grow in 4% (w/v) NaCl, and 10 isolates to grow in 5% NaCl. Abo-Aba et al., (2015) have also reported that the Rhizobium leguminosarum bv. trifolii isolates from roots of Trifolium alexandrium were able to tolerate 4% NaCl.

Table: 2. Effect of different Salt (NaCl) concentrations on growth (OD at 610 nm) of the Rhizobial strains from Vigna trilobata

Bacterial strains	NaCl concentration								
	0%	0.05%	0.1%	0.5%	1.0%	1.5%	2.0%	2.5%	3.0%
Rhizobium sp. MRR 103	0.037	0.103	0.468	0.443	0.273	0.187	0.132	0.058	0.024
Rhizobium sp. MRR 106	0.047	0.212	0.237	0.195	0.141	0.094	0.056	0.00	0.00
Rhizobium sp. MRR 112	0.032	0.129	0.333	0.226	0.0178	0.103	0.068	0.024	0.00
Rhizobium sp. MRR 123	0.028	0.119	0.414	0.313	0.205	0.118	0.056	0.017	0.00

ii). Effect of pH

Optimum pH for all the Rhizobium sp. showed the maximum growth at pH 7.0. The growth was observed in acidic to neutral and alkaline pH also. The rhizobial strains also tolerate pH upto 11.0 (Figure-1). Reports of Sethi and adhikari (2014) studied the six different strains isolated from Vigna radiata and seven strains from Arachis hypogea growth pattern of all the strains subjected to various pHs ranging from (5.0- 10.0). All the isolates grew well at pH 7.8.

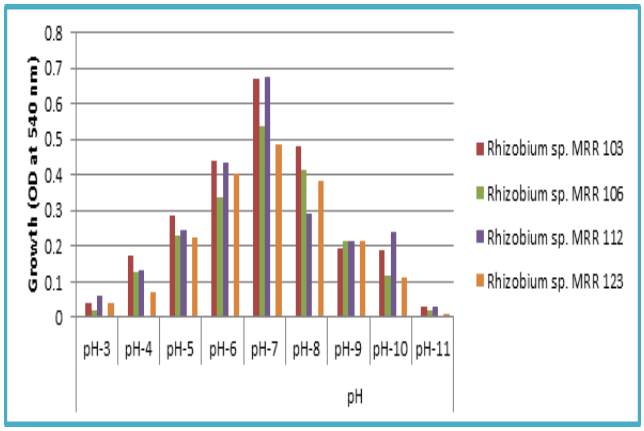

Figure 1. Effect of different pH on growth (OD at 610 nm) of the Rhizobial strains

iii). Effect of temperature

From the results Rhizobium sp. MRR 103 showed the growth on maximum temperature at 45°C (Figure-2). Maximum rhizobial growth was observed in the temperature ranges of 30-35°C. Rhizobium species exhibited much variation in temperature tolerance was evidenced by the earlier reports that Rhizobia can grow at high temperature 50 to 60°C (Abo - Aba et al., 2015) and even at very low temperature of 15°C (Harwani, 2006).

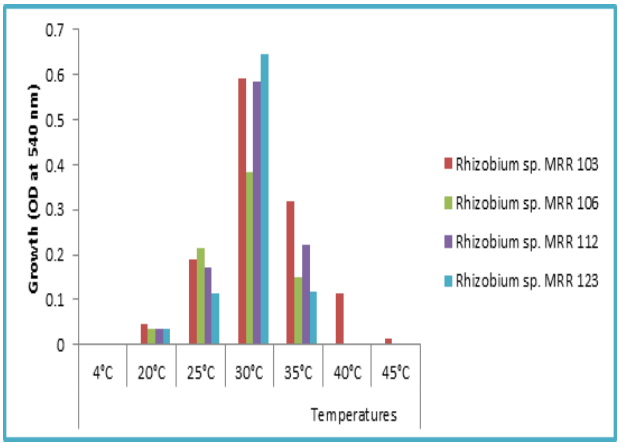

Figure 2. Effect of Temperature on growth (OD at 610 nm) of the Rhizobial strains

IV. ACKNOWLEDGMENTS

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