African Journal of Microbiology Research Vol 3(1) pp.010-014, January, 2009
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2009 Academic Journals

Full Length Research Paper

Studies on exopolysaccharide and indole acetic acid production by *Rhizobium* strains from *Indigofera*

B. Saritha Kumari, M. Raghu Ram* and K. V. Mallaiah

Department of Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar-522 510, Guntur, A. P., India.

Accepted 9 October, 2008

Rhizobium **strains were isolated from root nodules of five species of** ***Indigofera*** **viz.,** ***Indigofera trita, Indigofera linnaei, Indigofera astragalina, Indigofera parviflora and Indigofera viscosa*** **on Yeast Extract Mannitol Agar (YEMA) medium. The strains were examined for production of acid, exopolysaccharide (EPS) and indole acetic acid (IAA) by utilizing 10 different carbon sources. The** ***Rhizobium*** **strains isolated from** ***I. trita, I. parviflora*** **and** ***I. viscosa*** **showed maximum growth on glucose, while those from** ***I. linnaei*** **and** ***I. astragalina*** **showed maximum growth on fructose and maltose, respectively. All the five strains produced acid, EPS and IAA in Yeast extract mannitol broth. Among the strains studied, maximum EPS production was observed with the strain isolated from** ***I. parviflora*** **and maximum IAA production was observed with the strain isolated from** ***I. viscosa*****.**

Key words: *Rhizobium, Indigofera* species, exopolysaccharide, indole acetic acid, acid production.

INTRODUCTION

The legume-rhizobium interaction is the result of specific recognition of the host legume by *Rhizobium*. Various signal molecules that are produced by both Rhizobia and the legume confer the specificity (Phillips, 1991). Exopolysaccharide (EPA) produced by *Rhizobium* is one such signal for host specificity during the early stage of root hair infection (Olivers et al., 1984; Raghavendrajoshi and Padder, 2002). It also protects the cell from desiccation and predation and helps in nitrogen fixation by preventing high oxygen tension (Jarman et al., 1978). In addition, *Rhizobium* strains 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 EPA and IAA are considered as important traits of plant growth-promoting rhizobacteria.

Indigofera is one of the major nodulated genera in the family Leguminosae. Although *Rhizobium* isolated from *Indigofera* was first described by Joshi in 1920, the type species was characterized only very recently (Wei et al., 2002) and was described as *Rhizobium indigoferae* sp. nov. Reports on the plant growth-promoting characteristics of *Rhizobium* species isolated from *Indigofera* are meager. Hence, the present study was aimed at screening the *Rhizobium* strains from five *Indigofera* species viz., *Indigofera trita, Indigofera linnaei, Indigofera Astragalina, Indigofera parviflora* and *Indigofera viscosa* for the plant growth-promoting characteristics such as utilization of different carbon sources, production of acid, IAA and EPS.

*Corresponding author. E-mail: mraghuram2002@yahoo.co.uk. Mobile: 9441120006.

MATERIALS AND METHODS

Isolation of bacterial isolates

For the present study five species of *Indigofera* viz., *I. trita, I. linnaei, I. astragalina, I. parviflora* and *I. viscosa* growing wild in the University campus, were selected. The seeds were collected and raised in earthen pots and maintained in the experimental botanical garden of our University. The bacteria were isolated from the freshly collected healthy root nodules of all five *Indigofera* species on Yeast Extract Mannitol Agar (YEMA) medium. The cultures were identified by following the tests given in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). After identification as *Rhizobium* strains, pure cultures were maintained on YEMA medium and used for the present study.

Table 1. Effect of different carbon sources on growth of *Rhizobium* strains from *Indigofera* species.

	Colony diameter (in mm) after 72 h incubation				
	I. trita	***I. linnaei***	***I.astragalina***	***I. parviflora***	***I. viscosa***
Control	5.0	5.5	4.0	6.0	3.0
Glucose	12.0*	8.5	9.0	11.0	10.3
Galactose	8.5	8.0	5.6	10.3	8.0
Arabinose	6.0	6.2	4.0	5.0	4.5
Raffinose	6.5	6.0	6.1	8.0	8.5
Fructose	11.5	10.0	9.0	10.0	5.0
Xylose	6.0	8.0	5.5	6.5	6.2
Sucrose	9.0	8.0	8.0	9.0	6.5
Maltose	5.0	7.1	10.0	9.5	5.5
Lactose	6.2	6.0	7.0	9.1	4.0
Mannitol	6.0	6.5	5.8	8.0	8.5
Glycerol	5.5	6.0	5.0	6.0	7.0
Starch	5.2	6.0	7.0	9.1	5.1
Cellulose	11.0	5.0	7.1	6.0	4.0
Acetate	4.0	4.5	5.0	5.5	4.0
Citrate	4.0	5.0	4.5	5.0	5.0
Lactate	-	-	-	-	-
Malate	-	-	-	-	-
Oxalate	3.5	-	3.0	5.2	4.0
Pyruvate	5.0	5.0	4.0	5.5	6.0
Succinate	-	4.0	-	-	-

*Each value is an average of three replicates.

Utilization of different carbon sources

All of the *Rhizobium* strains were tested for utilization of different carbon sources by replacing mannitol in YEMA medium with equal amounts of 20 different carbon sources, including seven organic acids. The growth was measured as colony diameter after 72 h of incubation at 30 ± 1°C.

Growth and acid production determinations

To study growth and acid production, *Rhizobium* strains were inoculated into test tubes containing YEM broth. The broth was prepared by supplementing 1% of carbon source and bromothymol blue (1%) as an indicator (Norris, 1965). The inoculated culture tubes were incubated at 30 ± 1°C on a gyro-rotatory shaker (REMI, India) at 200 rpm for 48 h. After incubation, growth was measured at 470 nm using a spectrophotometer (Systronics, India). Acid production was studied by measurement of pH, using a pH meter (Elico, India) and observed visually for the change in colour from green to yellow.

Exopolysaccharide (EPS) production

For the estimation of EPS production, *Rhizobium* strains were inoculated into conical flasks containing 100 ml of YEM broth supplemented with 1% of carbon source. The inoculated flasks were incubated at 30 ± 1°C on a gyrorotatory shaker at 200 rpm for 72 h. After incubation, the culture broth was centrifuged 3500 × g and the supernatant was mixed with two volumes of chilled acetone (Qualigens, India). The crude polysaccharide developed was collected by centrifugation at 3500 × g for 30 min. The EPS was washed with distilled water and acetone alternately, transferred onto a filter paper and weighed after overnight drying at 105°C (Damery and Alexander, 1969).

Indole acetic acid (IAA) production

All five *Rhizobium* strains were further screened for IAA production by inoculating them into 100 ml conical flasks containing YEM broth supplemented with L-tryptophan (0.1%) and 1% of different carbon sources. The flasks were incubated at 30 ± 1°C on gyro-rotatory shaker at 200 rpm for 72 h. After incubation the medium was centrifuged for 5000 × g for 20 min and the cell-free supernatant was used for IAA extraction (Sinha and Basu, 1981). To the 10 ml of supernatant, 2 ml of Salkowsky's reagent was added and incubated for 30 min under darkness. The amount of IAA produced was determined colorimetrically at 540 nm (Gordon and Weber, 1951).

RESULTS AND DISCUSSION

Growth on different carbon sources

All five *Rhizobium* strains were found to utilize a wide range of carbon sources (Table 1). Among the 20 carbohydrates tested, monosaccharides (glucose, galactose,

Table 2. Effect of ten different carbon sources on growth and acid production by *Rhizobium* strains from *Indigofera* species.

Carbon source	Name of the isolate									
	I. trita		***I. linnaei***		***I. astragalina***		***I. parviflora***		***I. viscosa***	
	O.D	**pH**	**O.D**	**pH**	**O.D**	**pH**	**O.D**	**pH**	**O.D**	**pH**
Glucose	0.12*	5.14	0.11	6.44	0.09	6.24	0.10	5.98	0.07	3.26
Galactose	0.15	7.08	0.13	6.74	0.12	5.86	0.07	5.95	0.11	4.04
Arabinose	0.07	5.36	0.10	6.08	0.10	5.56	0.14	6.24	0.13	4.41
Raffinose	0.05	4.87	0.12	6.13	0.05	5.06	0.12	6.18	0.16	5.06
Fructose	0.02	4.64	0.08	5.72	0.03	4.86	0.16	6.86	0.15	4.88
Sucrose	0.09	5.62	0.05	5.48	0.05	4.96	0.07	5.82	0.11	3.58
Maltose	0.06	5.12	0.12	6.44	0.13	5.70	0.19	7.21	0.21	5.16
Lactose	0.11	5.70	0.16	7.02	0.15	5.98	0.14	7.13	0.12	4.98
Mannitol	0.09	5.62	0.11	6.21	0.13	5.86	0.09	6.02	0.12	4.32
Glycerol	0.15	7.02	0.14	6.68	0.16	6.11	0.18	6.78	0.19	5.06
Control	0.09	6.67	0.11	7.05	0.06	6.71	0.09	6.93	0.05	5.17

*Each value is an average of three replicates.

arabinose, fructose, raffinose and xylose) support maximum growth, followed by sugar alcohols (mannitol), disaccharides (lactose, maltose, sucrose) and polysaccharides (starch and cellulose). Much variation in carbon source utilization was observed among the *Rhizobium* strains studied. The strains isolated from *I. trita, I. linnaei* and *I. viscosa* preferred monosaccharides than disaccharides, polysaccharides and sugar alcohols, while those from *I. astragalina* and *I. parviflora* showed better growth on disaccharides than monosaccharides and sugar alcohols. Organic acids like lactate, malate and succinate are not preferred as carbon sources by any of the *Rhizobium* strains studied. However, some of the strains could utilize acetate, citrate, oxalate and pyruvate as carbon sources, but showed very poor growt

Fast-growing rhizobia utilize a wide range of carbon sources compared to slow-growing rhizobia (Graham and Parker, 1964). The *Rhizobium* strains from bean utilize salts of organic acids as carbon sources in addition to wide range of carbon sources (Hungaria et al*.,* 2000). Wei et al. (2002) reported that the *Rhizobium* isolates from *Indigofera amblyantha, Indigofera carlesii* and *Indigofera potaninii* utilize a wide range of carbohydrates like galactose, lactose, sucrose, mannose, xylose, salicin and citrate. Cigdem et al. (2006) reported that *Rhizobium* isolates from root nodules of *Phaseolus vulgaris* were able to grow well in the presence of glucose, galactose, mannitol, sucrose, succinate, rhamnose and mannose. Polysaccharides are generally considered as poor sources of carbon for rhizobial growth. However, in the present study, all five strains showed good growth on starch and cellulose. Similar observations were also reported in *Rhizobium* isolates from *Sesbania sesban* (Helmish et al., 1993). From the present study it is evident that all the strains effectively utilized a wide range of carbohydrates, one of the criteria to be considered as plant growth-promoting rhizobacteria.

Acid production

In Yeast Extract Mannitol (YEM) broth medium with mannitol as carbon source, all the isolates showed acid production (Table 2). The final pH of the medium after 48 h of incubation ranged from 4.32 to 6.21. *Rhizobium* strains grew better in YEM broth when mannitol was replaced with other carbohydrates in equal quantities and produced acid, which was evident by a change in colour of the medium. Among the five *Rhizobium* strains, the strain from *I. viscosa* grow well in all the carbon sources and produced acid with a maximum pH of 3.26 when glucose was used as carbon source. *Rhizobium* strains isoalted from *I. trita* and *I. astragalina* also grew better in all carbon sources and produced acid with a maximum acidic pH of 4.64 and 4.86, respectively, when fructose was used as carbon source. The strains from *I. linnaei* and *I. parviflora* showed better growth in all carbon sources, but could not produce acid in lactose- and maltose-containing medium, respectively. Fred et al. (1932) categorized the *Rhizobia* into fast growers, which can produce acid, and slow growers that cannot produce acid. The strains in the present study can therefore be considered as fast growers as all can produce acid. Generally, the strains with acid-producing ability can be considered as effective stains for nodulation. Therefore, all the strains in the present study may be suitable for better nodulation.

EPS production

The amount of EPS produced by *Rhizobium* strains varied with carbon source in the medium. All five strains

Table 3. Effect of different carbon sources on EPS (mg/g) production by *Rhizobium* strains from *Indigofera* species.

Carbon source	Name of the isolate				
	I. trita	***I. linnaei***	***I. astragalina***	***I. parviflora***	***I. viscosa***
Control	28	52	16	32	32
Glucose	428*	920	372	960	280
Galactose	568	408	440	256	352
Arabinose	304	428	860	496	348
Fructose	572	36	396	408	388
Xylose	564	592	488	360	364
Sucrose	348	384	468	356	336
Maltose	540	368	384	244	424
Lactose	656	312	412	360	376
Mannitol	472	476	756	966	688
Glycerol	728	344	248	346	876

*Each value given is an average of three replicates.

Table 4. Effect of different carbon sources on IAA production (µg /ml) by *Rhizobium* strains from *Indigofera* species.

Carbon source	Name of the isolate				
	I. trita	***I. linnaei***	***I. astragalina***	***I. parviflora***	***I. viscosa***
Control	17.7	11.7	6.5	8.2	17.4
Glucose	50.2*	62.2	32.5	54.2	65.7
Galactose	57.1	25.1	10.2	60.5	90.8
Arabinose	19.9	14.8	10.2	12.0	79.9
Raffinose	57.1	37.7	18.8	74.2	34.2
Fructose	63.9	38.8	17.7	89.1	58.8
Xylose	53.1	36.0	31.4	14.8	77.1
Sucrose	33.7	23.4	10.2	37.7	147.1
Maltose	55.9	25.1	41.7	35.4	111.4
Lactose	73.7	27.4	27.4	68.5	95.4
Mannitol	94.8	69.1	82.2	94.8	149.7

*Each value given is average of three replicates.

produced maximum EPS after 72 h of incubation (Table 3). When mannitol was used as carbon source in YEM broth, four out of the five isolates studied showed maximum EPS production, followed by monosaccharides. The strain isolated from *I. parviflora* produced relatively more EPS than other isolates, in all carbon sources, with a maximum of 966 mg/g in mannitol broth. EPS production was inversely related to carbon source utilization (Singh et al., 1967). Singh and Sharma (1991) reported that the *Rhizobium* strains, which produce maximum EPS in sucrose, produced less EPS in glucose and vice versa. Variation in EPS production on different carbon sources was previously reported in *Aechynomene aspera* isolates (Ghosh and Basu, 2001). Similarly, in the present study, variation in EPS production was observed between different strains and also between different carbon sources.

IAA production

The majority of rhizosphere bacteria produces growth-promoting substances like IAA, gibberillins and cytokinins. Rhizobia are known to produce IAA in the culture supernatant and the carbon sources present in the medium influence its production (Gosh and Basu, 2002). All the isolates studied showed variation in IAA production in the same carbon source and different carbon sources (Table 4). The *Rhizobium* strains are known to prefer L-tryptophan for IAA production (Dullaart, 1970). In the present study, it was observed that the maximum production of IAA was observed at 1 µg/ml of L-tryptophan. Among the 10 carbon sources tested, mannitol proved to be the best for growth and IAA production in all five isolates of *Indigofera*. Lactose was found to be the second

best. A maximum of 149.7 µg/ml of IAA was produced when mannitol was used as carbon source in the isolate from *I. viscosa,* which is 760% more than that observed in control without any carbon source. Previously, it has been reported that 107 µg/ml of IAA was produced in *Alysicarpous vaginalis* grown in medium supplemented with L-tryptophan and mannitol as carbon source (Bhattacharya and Pati, 2000). In *Cajanus cajan* an increase of 653% of IAA was reported over the control (Datta and Basu, 2000). Thus, it can be concluded that mannitol stimulated maximal IAA production in all the *Rhizobium* strains, with slight variations in the percentage of increase. However, the amount of IAA produced by the strains in the present study was more when compared to the previous reports. Among the strains studied, the strain from *I. viscosa*, which produced the highest IAA, may be considered as an effective plant growth-promoting bacterial strain.

ACKNOWLEDGEMENTS

Authors are thankful to the Department of Microbiology, Acharya Nagarjuna University, for providing facilities for this work. Saritha Kumari is thankful to Andhra Pradesh State Council of Science and Technology, Hyderabad, A.P., India, for providing Young Scientist Fellowship.

REFERENCES

Bhattacharya RN, Pati BR (2000). Growth behaviour and indole acetic acid (IAA) production by *Rhizobium* isolated from root nodules of *Alysicarpous vaginalis* DC. Acta. Microbiol. Immunol. Hung. 47(1): 41 - 51.

Cigdem K, Merith K, Engin K (2006) Characterization of *Rhizobium* sp. isolated from bean. Turk. J. Boil. 30: 127-132.

Damery JT, Alexander M (1969). Physiological differences between effective and ineffective strains of *Rhizobium*. Soil. Sci. 103: 209-215.

Datta C, Basu P (2000). Indole acetic acid production by a *Rhizobium* species from root nodules of leguminous shrub, *Cajanus cajan*. Microbiol. Res. 155: 123 127.

Dullaart JO (1970). Quantitative estimation of indole acetic acid and andole carboxylic acid in root nodules and roots of *Lupinus luteus* L. *Act*. Bot. Neer. 16: 222.

Fred EB, Baldwin IL, Mc Coy E (1932). *Root nodule bacteria and Leguminous plants*". Studies in Science, 5. University of Wisconsin, Press, Madison. p. 343.

Gordon SN, Weber RP (1951). Colorimetric estimation of indole acetic acid Plant. Physiol. 26: 192.

Ghosh AC, Basu PS (2001) Extracellular polysaccharide production by *Azorhizobium caulinodans* from stem nodules of leguminous emergent hydrophyte *Aeschynomene aspera*. Indian J. Exp. Biol. 39:155-159.

Gosh AC, Basu PS (2002). Growth behavior and bioproduction of indole acetic acid by a *Rhizobium* sp. from root nodules of leguminous tree *Dalbergia lanceolaria*. Indian J. Exp. Biol. 40: 796 – 801.

Graham, P.H. and Parker, C.A. 1964. Diagnostic features in the characterization of the root nodule bacteria of legumes. Plant Soil. 20: 383- 396.

Helmish, F.A., E1-Mokadem, M.T., Zekry, S.H.A. (1993) Nutritional requirements and invertase activity of *Rhizobium* nodulating *Sesbania seaban* roots. *ZH. Fur-Microbio*. 148: 582- 587.

Hungaria M, Vargas MAT (2000) Environmental factors affecting nitrogen fixation in grain legumes in the tropics with an emphasis on Brazil Field Crops Res. 65: 151- 164.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST (1994). *Bergey's* Manual of Determinative Bacteriology*",* 9th edition published by Williams and Wilkins, A. Wavely company, Baltimore. p. 787.

Jarman TR, Deavin L, Slocombe S, Righelato RC (1978). Investigation of the effect of environmental condition on the rate of EPS synthesis in *Azotobacter vinelandii*. J. Gen. Microbiol. 107: 59- 64.

Norris DO (1965). Acid production by *Rhizobium*, a unifying concept. Plant Soil. 22: 143-166.

Nutman PS (1977). Study frame works for symbiotic nitrogen fixation. In recent developments in nitrogen fixation. Edited by Nutman WJR, Postgate and (Rodriguez Barrueco (Acad. Press, Lond). p. 443.

Olivares J, Bedmar EJ, Martinez ME (1984). Infectivity of *Rhizobium meliloti* as effected by extra cellular polysaccharides. J. Appl. Bacteriol. 56: 389-393.

Phillips DA (1991). Flavonoids: Plant signals to soil microbes. Rec. Adv. Phytochem. 2: 1-33.

Sinha BK, Basu PS (1981). Indole-3-acetic acid and its metabolism in root nodule of *Pongamia pinnata* (L) Pierre. Biochem. Physiol. Pflanzen. 176: 218.

Raghavendrajoshi, Padder US (2002) Effect of lipopolysaccharides and exopolysaccharides on chick pea – *Rhizobium* interaction. Indian J. Microbiol. 42: 327- 328.

Singh A, Sharma PB (1991). Biochemical characteristics and salt tolerance by *Rhizobium* species isolated from legume crops. Indian J. Microbiol. 11: 33- 42.

Singh R, Singh N, Sidhu GS (1967). Biochemical characteristics of *Rhizobium* species isolated from summer legume of Punjab. Indian J. Microbiol. 7: 143- 150.

Wei GH, Chen WX, Wang ET, Tan ZX, Zhu ME (2002). *Rhizobium indigoferae* sp. nov. and *Sinorhizobium kummerowiae* sp. nov., respectively isolated from *Indigofera* spp. and *Kummerowia stipulacea*. Intl. J. Syst. Evol. Microbiol. 52: 2231- 2239.