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Full Length Research Paper

Production of indole acetic acid by *Rhizobium* isolates from *Vigna trilobata* (L) Verdc.

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Rhizobium **strains were isolated from root nodules of five cultivars of *Vigna trilobata* namely: cultivar-1 from Prakasham District, cultivar-2 from East Godavari District, cultivar-3 from Guntur District, cultivar-4 from West Godavari District and cultivar-5 from Krishna District on yeast extract mannitol broth (YEMB) medium with bromothymol blue indicator. The strains were examined for production of acid and indole acetic acid (IAA) by utilizing different carbon sources. After 48 h of incubation, almost the five isolates produced acid ranging between 4.31 to 7.11. Isolate-4 (from CV-4) showed maximum growth on arabinose, with maximum acidic pH of 4.31. Isolate-1 and 3 (from CV-1 and 3) grow better in all carbon sources, whereas, isolate 2 and 5 (from CV-2 and 5) showed maximum growth on glucose. All the five isolates initiated IAA production immediately after inoculation and maximum was produced at 72 h in YEM broth and showed a decline afterwards. Maximum amount of IAA was produced (92.6 µg/ml) in isolate-3 at 72 h of incubation. These isolates were further tested for the production of IAA in a medium with 0.5, 1.0, 1.5, 2.0 and 2.5 mg/ml of L-Tryptophan. All the five isolates produced maximum growth in a medium containing 1 mg/ml conc. of L-Tryptophan. Among the isolates; isolate-3 produced a maximum of 80.96 µg/ml of IAA. The effect of different carbon sources on IAA production was studied by replacing mannitol in YEM broth with equal quantities of different carbon sources. Isolate-2 produced IAA at a maximum of 82.89 µg/ml followed by isolate-3, 5, 1 and 4.**

Key words: *Rhizobium*, *Vigna trilobata,* indole acetic acid, acid production.

INTRODUCTION

Utilization of wide range of carbon sources was considered as an important trait of plant growth promoting rhizobacteria. *Rhizobium* strains show variation in carbon source utilization. *Rhizobium* was subdivided in to two groups, fast and slow growing strains based on utilization of carbohydrates (Fred et al., 1932). *Rhizobium* strains exhibits strain difference in carbohydrate utilization (George and Ethinger, 1941). Fast growing Rhizobium strains can utilize wide range of pentoses, hexoses, disaccharides, sugar alcohols and organic acids (Stowers and Elkan, 1983, 1984). Whereas lactose and sucrose were utilized by a few slow growers only (Graham, 1963). However, fast growing lupine rhizobial strains were unable to use arabinose or fructose as carbon sources (Miller and Pepper, 1988).

Indole acetic acid is one of the most physiologically active auxin and a common product of L-Tryptophan metabolism by several microorganisms inducing plant growth-promoting bacteria (PGPR) (Ahmad et al., 2005; Datta and Basu, 2000; Gosh and Basu, 2006; Mandal et al., 2007). *Rhizobia* are known to produce significant levels of IAA both in free living conditions and also symbiotically in nodules (Ernstsen et al., 1987). *Vigna* was one of the major nodulating genera in the family leguminoseae, consists of about 150 species, which are annual or perennial legume (Allen and Allen, 1981; Willis,

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Abbreviations: CV; Cultivar, **IS;** Isolate.

1985). Although, nodulation in *Vigna* was reported much earlier in 17th century, very limited work was done on cultural characteristics of *Rhizobium* associated with this legume. *Rhizobium* isolated from *Vigna* was described by by Dakora and Vincent (1984). Hence the present study was aimed at screening the *Rhizobial* strains from five cultivars namely: CV-1,2,3,4 and 5 for the PGPR characters such as utilization of different carbon sources, production of acid and IAA.

MATERIALS AND METHODS

Growth and acid production

All the isolates were examined for acid production in yeast extract mannitol (YEM)-broth containing different carbon sources (1%) with BTB indicator. Acid production was measured by pH depression in 10 ml broth of culture suspension after 48 h of growth at 30 ± 1°C on rotary shaker at 200 rpm. The growth was measured spectrophotometrically at 470 nm.

Determination of Indole Acetic Acid (IAA) production

To study the effect of incubation period on IAA production the YEM broth containing L-tryptophan was inoculated with *Rhizobium* isolates and incubated at 37 ± 1°C on a gyrorotatory shaker at 200 rpm. The amount of IAA produced was estimated for 12 h until the *Rhizobium* strains reach their stationary phase of growth, where they produce maximum IAA (Gordon and Weber, 1951).

To study the ideal concentration of L-tryptophan for production of maximum IAA by the *Rhizobium* isolates, all the isolates were inoculated in to flasks containing 100 ml of YEM broth supplemented with different concentrations of L-tryptophan. The inoculated flasks were incubated at 37 ± 1°C a gyrorotatory shaker at 200 rpm for 72 h.

To study the effect of different carbon sources on IAA production, the isolates were inoculated in to YEM broth containing 0.1% of L-tryptophan and 1% of different carbon sources and incubated at 37 ± 1°C a gyrorotatory shaker at 200 rpm for 72 h. For the estimation of IAA the incubated broth cultures were centrifuged at 5000 rpm for 20 min and the cell free supernatant was used for IAA production (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 estimated calorimetrically at 540 nm (Gordon and Weber, 1951).

RESULTS AND DISCUSSION

Growth and acid production

The ability of *Rhizobium* isolates from *Vigna trilobata* to produce acid by utilizing different carbon sources was tested by growing them in YEM broth containing BTB indicator (Table 1).

In yeast extract mannitol broth medium with mannitol as carbon source, all the isolates showed acid production. The final pH of the medium after 48 h incubation was ranged from 4.31 to 7.11. *Rhizobium* isolates grow better in YEM broth when mannitol was replaced with other carbohydrates in equal quantities. Among the five isolates, the isolate-5 grow well in all the carbon sources and produced acid with a maximum pH of 4.56 when starch was used as carbon source. Isolates-1 and-3 also grow better in all carbon sources and produced acid with a maximum acidic pH of 4.78 and 4.80 respectively, when fructose and arabinose were used as carbon source. The isolate-2 and 4 showed better growth in all carbon sources, with maximum acidic pH of 4.87 and 4.31 in mannitol and arabinose supplemented media, respectively. Much variation in acid production was observed in rhizobium isolates from *V. trilobata* cultivars. All the isolates showed relatively poor growth on organic acids and produced acid in the pH range of pH 5 to 5.36.

Indole acetic acid (IAA) production

To study the effect carbon sources on IAA production, the amount of IAA produced was estimated, at every 12 h intervals up to 96 h, in the supernatant of inoculated YEM broth cultures. The *Rhizobium* isolates from *V.trilobata* cultivars initiated IAA production immediately after inoculation and maximum was produced at stationary phase of growth at 72 h (Table 2). The IAA production increased gradually with increase in incubation period up to 72 h and showed a decline afterwards. Thus, 72 h was considered as ideal for maximum IAA production for rhizobium isolated from *V. trilobata.* Among the isolates studied, a maximum of 92.6 µg/ml of IAA was produced in isolate-1 at 72 h of incubation.

The *Rhizobium* isolates from *V. trilobata* cultivars preferred L- tryptophan for growth and IAA production. To identify the ideal concentration of L- tryptophan at which maximum IAA can be produced, the IAA production was recorded at different concentrations of L- tryptophan (Table 3). In all the isolates, maximum IAA production was recorded when 100 mg of L - tryptophan was used in the medium. In the present study, isolate-3 produced a maximum of 80.96 µg/ml of IAA was produced with 100 mg/100 ml. Therefore 100 mg of L- tryptophan was optimum for present IAA production for *V.trilobata* isolates.

The carbon sources present in the medium also influences the IAA production. The effect of different carbon sources on IAA production was studied by replacing mannitol in YEM broth with equal quantities of 12 different carbon sources. The *Rhizobium* isolates from *V.trilobata* cultivars differ in IAA production in different carbon sources as well as in the same carbon source (Table 4). In all the carbon sources studied except arabinose, in majority of isolates, the amount of IAA produced was more than that in the control (without any carbon source). The maximum amount of IAA production was in the range of 30 to 50 µg/ml in all the isolates studied, when different carbon sources was was used, except mannitol, where the maximum reached up to 82.89 µg/ml. The amount of IAA produced was 5 times more than the control in isolate- 1 and 5; while it is 4 times in isolate-2 and 3 and it was only 3 times in isolate-4,

Table 1. Effect of different carbon sources on growth and acid production by *Rhizobium* isolates from five *Vigna trilobata* cultivars.

S. No	Carbon source	Name of the isolate									
		Isolate-1		Isolate-2		Isolate-3		Isolate-4		Isolate-5	
1	Glucose	0.15*	7.08	0.13	6.88	0.15	7.10	0.16	7.06	0.14	7.09
2	Fructose	0.05	**4.78**	0.11	6.25	0.10	6.54	0.11	6.25	0.05	4.81
3	Xylose	0.07	5.02	0.10	6.01	0.07	5.40	0.09	5.65	0.08	5.30
4	Arabinose	0.08	5.01	0.07	5.01	0.05	4.80	0.06	**4.31**	0.10	5.88
5	Rahmnose	0.08	5.01	0.09	5.59	0.08	5.05	0.12	6.25	0.08	5.23
6	Galactose	0.13	6.57	0.11	6.12	0.11	6.25	0.17	7.11	0.10	6.21
7	Sucrose	0.13	6.57	0.11	6.12	0.13	6.89	0.15	7.10	0.09	5.78
8	Maltose	0.05	4.85	0.08	5.14	0.15	7.09	0.14	7.01	0.06	5.12
9	Lactose	0.09	5.17	0.08	5.21	0.09	5.21	0.12	6.22	0.07	5.21
10	Starch	0.06	5.10	0.07	5.43	0.09	5.22	0.11	6.28	0.06	4.56
11	Lactate	-	-	-	-	-	-	-	-	-	-
12	Pyruvate	0.07	5.01	0.06	5.29	0.06	5.12	0.08	5.22	0.07	5.36
13	Citrate	0.06	5.21	0.07	5.26	0.06	5.10	0.08	5.22	0.07	5.36
14	Acetate	0.05	4.80	0.07	5.85	0.07	5.12	0.07	5.15	0.03	4.64
15	Mannitol	0.06	5.24	0.06	4.87	0.08	5.15	0.08	5.21	0.06	4.90
16	Cellulose	0.15	7.07	0.09	5.61	0.09	5.69	0.08	5.22	0.03	4.63
17	Glycerol	0.08	5.10	0.08	5.12	0.08	5.13	0.07	5.32	0.08	5.25

*Each data is an average of three replicates.

Table 2. Effect of incubation period on IAA production (µg/ml) by *Rhizobium* isolates from five cultivars of *V. trilobata.*

S. No	Name of the isolate	Incubation period (h)							
		12	24	36	48	60	72	84	96
1	Isolate-1	17.83*	21.53	37.63	45.2	58.63	82.16	62.4	53.6
2	Isolate-2	21.5	29.3	40.9	53.5	63	86.5	73.9	62.5
3	Isolate-3	20.8	29.6	41.7	53.5	63.89	92.6	76.56	62.6
4	Isolate-4	18.2	23.63	37.4	49.4	58.1	78.56	59.86	40.66
5	Isolate-5	17.85	22.06	32.10	43	52.16	70	5.013	37.7

*Each data is an average of three replicates.

Table 3. Effect of different concentrations of L-Tryptophan on IAA production (µg/ml) by *Rhizobium* isolates from five cultivars of *V. trilobata.*

S. No	Name of the isolate	Different concentrations of L-Tryptophan (mg/ml)				
		50 mg	100 mg	150 mg	200 mg	250 mg
1	Isolate-1	40*	70.7	49.56	38.2	20.12
2	Isolate-2	43.9	78.5	58.86	40.66	22.4
3	Isolate-3	42.66	80.96	68.33	41.44	24.5
4	Isolate-4	40.6	79.56	64.1	40.3	20.3
5	Isolate-5	37.53	66.3	51.46	36.53	19.3

*Each data is an average of three replicates.

Table 4. Effect of different carbon sources on IAA production (µg/ml) by *Rhizobium* isolates from five cultivars of *V. trilobata*.

S. No	Carbon source	Isolate-1	Isolate-2	Isolate-3	Isolate-4	Isolate-5
1	Control	16.8*	17.44	19.4	20.3	16
2	Glucose	58.57	57.68	25.43	46.54	54.93
3	Sucrose	52.4	52.87	46.88	42.56	37.16
4	Galactose	38.56	39.27	41.7	40.53	21.03
5	Lactose	39.87	36.71	41.88	26.18	48.36
6	Starch	38.16	41.24	31.26	41.15	40.23
7	Arabinose	20.53	19.33	21.02	25.24	19.66
8	Raffinose	38.17	39.96	32.07	36.45	47.16
9	Mannitol	79.74	82.89	79.56	77.48	79.9
10	Fructose	57.49	39.96	32.07	36.45	47.16
11	Maltose	33.03	22.43	21.6	30.98	37.13
12	Glycerol	38.48	34.08	29.76	19.50	20.76

*Each data is an average of three replicates.

when mannitol was used as carbon source (Figure 1).

Growth and acid production

Rhizobium isolates were tested for their ability to utilize different carbon sources by replacing mannitol in yeast extract mannitol agar (YEMA) medium with equal amounts of 17 different carbon sources. Much variation in utilization of carbon sources by different isolates was evident in this study.

Fred et al. (1932) reported that *Rhizobia* prefer mannitol and sucrose as best carbon sources for growth. Graham and Parker (1964) opined that strains of fast growing *Rhizobia* generally utilize wide range of carbon sources than slow growing *Rhizobia.* In the present study all the isolates are fast growers and utilize wide range of Carbon sources. Fast growing *Rhizobia* exhibit maximum growth on hexose, pentoses, disaccharides, sugar alcohol and organic acids was reported by Stowers and Elkan (1984). Cigdem et al. (2006) reported that *Rhizobium* isolates from root nodules of *Phaseolus vulgaris* were able to grow well in the presence of glucose, fructose, galactose, mannitol, sucrose, starch, succinate, L. rhamnose and arabinose. The *rhizobium* isolates in the present study also preferred monosaccharides as well as mannitol for proper growth.

In the present study, the *Rhizobium* isolates from *V. trilobata* showed better growth and acid production when mannitol was replaced with 17 other carbohydrates in equal quantities. In general, the isolates which utilize the carbon sources show good growth and acid production, while those with poor growth show either alkali/near neutral reaction. Ahmad and Smith (1985) studied the utilization of carbon sources and acid/ alkaline production by *Cowpea rhizobia*. This indicates that the acid reaction is a function of utilization of carbon sources. Kasturibai and Raju (1980) also reported that rhizobium isolates collected from soil samples produced acid with medium containing different carbon sources. Norris (1965) reported that the alkali producing *Rhizobium* strains from *Indigofera* species inhabiting the acidic soils are more tolerant than the acid producing strains from alkaline soils. Graham and Parker (1964) categorized the rhizobia into fast growing rhizobia, which can produce acid and slow growing rhizobia that cannot produce acid. In the

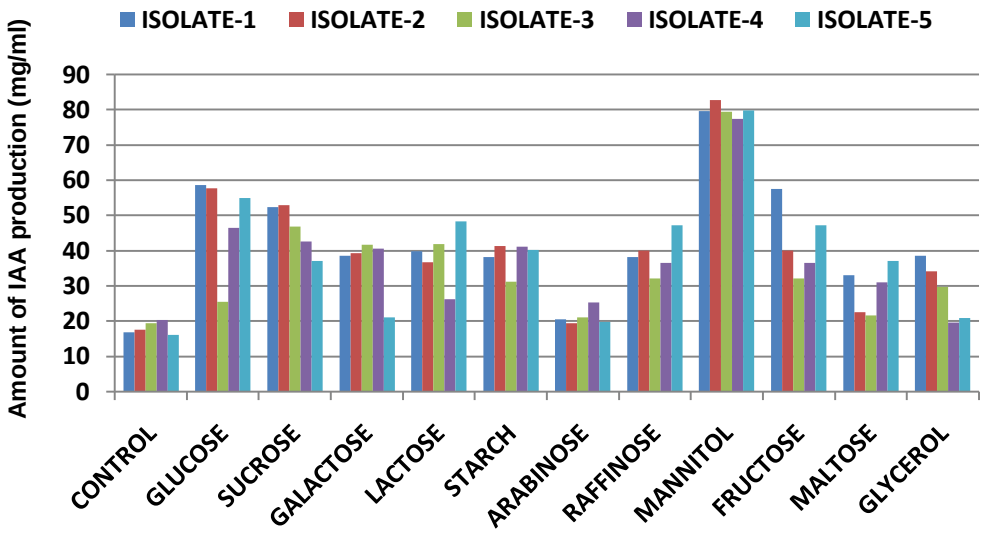

Figure 1. Effect of different Carbon sources on IAA (µg/ml) production by *Rhizobium* isolates from five cultivars of *Vigna trilobata*.

present study the rhizobia isolated from *Vigna trilobata* are fast growers and can produce acid.

IAA production

All the rhizobium isolates from *V. trilobata* produce IAA in L- Tryptophan supplement medium. There is firm evidence that plant growth promoting substances like IAA, Gibberellic acid and cytokinins are produced by number of rhizosphere microorganisms (Brown, 1972) and proper concentration of the hormones essential to induce successful nodulation (Kefford et al., 1960; Nutman, 1997; Planizinski and Rolfe, 1985).

In the present study, rhizobium isolates from *V.trilobata* showed growth and IAA production, after 12 h of inoculation and increased with incubation period up to 72 h and started to decline sharply with increase in incubation period. Similar observations were made by Beltra et al. (1980) in the cultures of *R. legumionsarum* bv. *phaseoli* and *R. legumionsarum* and in *Delbergia lanceolaria* isolate by Gosh and Basu (2002).

Rhizobium stains preferred L- Tryptophan for the growth and IAA production though they were able to produce IAA from other isomers of L –Tryptophane. Preference of L- Tryptophane for IAA production by rhizobium species was reported by earlier workers (Datta and Basu, 1997, 2000; Bhowmic and Basu, 1986). In the present study all the isolates, maximum IAA production was recorded when 100 mg of L - tryptophan was used in the medium.

Roy and Basu (1980, 2004) reported that *Rhizobium* strains from ground nut and *Clitoria ternatea* produced large amounts of IAA when the medium was supplemented with fructose, manganous sulphate, riboflavin and triton X-100 and mannitol. Similarly, in *Dalbergia lanceolaria* isolate, Gosh and Basu (2002) reported the role of mannitol in IAA production.

Conclusion

From this study it is clear that all the *Rhizobium* isolates differ in utilization of carbon source to produce acid and IAA.

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