JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2011. Vol. **5**(2), p. 929-932

Optimisation of Indole Acetic Acid Production by two Anoxygenic Phototrophic Bacteria Isolated from Tannery effluents

Ramchander Merugu[1,2], M.P. Pratap Rudra[2], Atthapu Thirupathaiah[3], S. Girisham[4] and S.M. Reddy[4]

[1]Department of Biochemisty, [4]Department of Microbiology,
Kakatiya University, Warangal - 506 009, India.
[2]Department of Biochemisty, Osmania University, Hyderabad, India.
[3]Department of Pharmaceutical Chemistry, Mahatma Gandhi University, Nalgonda, India.

(Received: 07 August 2010; accepted: 27 October 2010)

Two anoxygenic phototrophic bacteria *Rb.capsulatus* and *Rps.acidophila* were isolated from tannery effluents and their ability to produce Indole acetic acid (IAA) under different nutrient conditions was studied. Illumination conditions favoured the production of IAA than dark conditions. *Rps.acidophila* was a good producer of Indole acetic acid. Maximum amount of IAA on the 8th day of incubation period in *Rps.acidophila*. Neutral pH produced maximum IAA in *Rb.capsulatus* while pH of around 6.0 was favourable for IAA production by *Rps.acidophila.* Glutamic acid was the best nitrogen source for the production of IAA by both the bacteria under investigation. EDTA and SDS induced more release of the phytohormone in both the bacteria under investigation. Significance of above observations in the light of existing literature is discussed.

Key words: Purple non sulphur bacteria, IAA, *Rb.capsulatus*, *Rps. acidophila*.

Diverse soil microorganisms including bacteria, fungi and algae are capable of producing physiologically active quantities of auxins, which may exert pronounced effects on plant growth and establishment. Bastian *et al*.(1998) studied the production of indole-3-acetic acid and gibberellins, A1 and A3 by *Acetobacter diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media**.** Indole acetic Acid production by the indigenous isolates of *Azotobacter* and *Pseudomonas* in the presence and absence of tryptophan was reported by Farah *et al.* (2005). Indole-3-Acetic Acid production from Indole-3-acetonitrile by strains of *Bradyrhizobium* was investigated by Vega *et al*. (2002). IAA produced by bacteria of the genus *Azospirillum* spp. can promote plant growth by stimulating root formation. (Abbas Akbari,2007).

The photobiotransformation of indole to IAA by some of the purple non sulphur bacteria was reported by Sasikala and Ramana,(1995). *Rb.sphaerodies* could produce IAA when grown on amino acids DL glycine or glycine (Rajashekar *et al*.,1999) probably through tryptophan as precursor (Rajasekhar *et al*.,1998). There are no reports on the production of IAA by *Rb.capsulatus* and *Rps.acidophila* except that of our earlier report where IAA production in absence of tryptophan was investigated (Ramchander *et al*.,2008). Hence it was considered worthwhile to investigate the production of IAA under different nutrient conditions and the results are discussed in this communication.

* To whom all correspondence should be addressed.

MATERIAL AND METHODS

The anoxygenic Photosynthetic bacteria were isolated by enrichment techniques (Biebl and Pfennig, 1981) by adding effluent water sample into the Biebl and Pfennig's medium. The culture bottles thus prepared were incubated anaerobically in the light. Identification of bacteria thus isolated was done by studying the culture characteristics, morphological, biochemical characteristics as suggested by Holt *et al.* (1994). Fifteen ml of the Biebl and Pfennig's media with tryptophan was inoculated with 1ml log phase cultures of two anoxygenic phototrophic bacteria and incubated at 30+2º C under the light intensity of 2000lux in screw cap tubes.. At the end of 4,8,12 and 16 days of incubation the content of the vials were harvested by subjecting to centrifugation at 10,000 rpm for 10 minutes. The supernatant was collected and assayed for Indole acetic acid. Growth was determined by measuring optical density at 660 nm using UV-Vis spectrophotometer. Final pH of the culture supernatant was determined with the help of Elico pH meter. All the experiments were run in triplicate. Amount of Indole acetic acid produced by the growing culture was determined by the method of Sousa *et al*.(1962).

RESULTS AND DISCUSSION

Results presented in Table 1 reveals that *Rb.capsulatus* and *Rps.acidophila* produced IAA in the presence of tryptophan as precursor. However they differed significantly in the amount of IAA produced. *Rps.acidophila* was a good producer which produced maximum amount of IAA on the 8th day of incubation period. *Rb.capsulatus* also produced good amounts of IAA but comparatively less. No positive correlation could be observed among the biomass and production of IAA. More production of IAA was observed when tryptophan was added in addition to ammonium chloride as nitrogen source.

Perusal of Table 2 shows that acetate and glucose were more favourable for production of IAA in *Rb.capsulatus* while lactate and succinate were preferred by *Rps.acidophila*.

Glutamic acid was the best nitrogen source for the production of IAA by both the bacteria under investigation. In neutral pH, maximum production of IAA was produced in *Rb.capsulatus* while pH of around 6.0 was favourable for the production by *Rps.acidophila* (Table 3). IAA production was absent above pH 8.0 in *Rb.capsulatus* while in *Rps.acidophila* IAA production was seen upto pH 6.8. Maximum release and production of the phyrohormone took place at a concentration of EDTA 60 µg/ml and SDS at 30 µg/ml in *Rps.acidophila. Rb.capsulatus* required a concentration of EDTA 80 µg/ml and SDS at 20 µg/ml for the same (Table 4 & 5). From the present investigations it is clear that *Rps.acidophila* can be exploited for the production of phytohormones.

Table 1. Production of Indole acetic acid by two anoxygenic phototrophic bacteria with tryptophan

Medium	*Rb.capsulatus*			*Rps.acidophila*			
	Incubation Period (in days)	Growth (in O.D)	Final pH	IAA (µg/ml)	Growth (in O.D)	Final pH	IAA (µg/ml)
	4	0.846	7	20	0.752	6.0	26
BP+Try	8	1.118	7.4	46	0.962	6.4	60
	12	0.924	8	32	0.856	6.8	48
	16	0.721	8.2	18	0.642	7.0	22
	4	0.786	7.2	12	0.678	6	22
BP+Try*	8	1.056	7.6	34	0.897	6.4	48
	12	0.876	8	26	0.841	6.8	42
	16	0.672	8	14	0.654	7.0	16

Try* -Tryptophan as nitrogen source in Bieble and Pfennigs medium

Table 2. Effect of different carbon and nitrogen sources on production of IAA by two anoxygenic phototrophic bacteria

Carbon and nitrogen sources	*Rb.capsulatus*			*Rps.acidophila*		
	Growth (in O.D)	Final pH	IAA (µg/ml)	Growth (in O.D)	Final pH	IAA (µg/ml)
Succinate	0.846	7	48	0.752	6.0	44
Acetate	1.118	7.4	60	0.962	6.4	38
Malate	0.924	8	48	0.856	6.8	10
Lactate	0.721	8.2	20	0.642	7.0	62
Fructose	1.005	7.4	42	0.885	6.6	18
Glucose	0.872	7.6	50	0.702	6.4	42
Ammonium chloride	0.912	7.2	30	0.665	6.2	48
Sodium nitrate	1.002	7.2	28	0.816	6.6	32
Asparagine	0.815	8	36	0.782	6.4	20
Glutamine	0.789	7.4	15	0.764	6.4	18
L-Tyrosine	0.819	7.6	34	—	6.8	18
Glycine	1.12	7.6	22	0.895	6.0	15
Glutamic acid	1.08	7.4	48	0.922	6.0	38

Table 3. Effect of pH on production of IAA on two anoxygenic phototrophic bacteria

pH	*Rb.capsulatus*			*Rps.acidophila*		
	Growth (in O.D)	Final pH	IAA (µg/ml)	Growth (in O.D)	Final pH	IAA ((µg/ml))
4	-	-	-	0.434	4.4	-
4.5	0.421	4.8	-	0.456	4.8	-
5	0.562	5.4	-	0.586	5.2	24
5.5	0.586	6	8	0.685	5.8	32
6	0.765	6.6	14	0.865	6.2	60
6.5	0.986	7.2	26	0.886	6.8	24
7	1.006	7.8	52	-	-	-
7.5	0.896	8	64	-	-	-
8	0.564	8.4	38	-	-	-
8.5	-	-	-	-	-	-
9	-	-	-	-	-	-

Table 4. Effect of EDTA on production of IAA by two anoxygenic phototrophic bacteria

Concentration of EDTA (µg/ml)	*Rb.capsualtus* IAA (µg/ml)	*Rps.acidophila* IAA (µg/ml)
Control	60	66
20	63	68
40	66	74
60	70	82
80	74	44
100	58	36

Table 5. Effect of SDS on production of IAA by two anoxygenic phototrophic bacteria

Concentration of EDTA (µg/ml)	*Rb.capsualtus* IAA (µg/ml)	*Rps.acidophila* IAA (µg/ml)
10	60	66
20	72	70
30	58	78
40	55	64
50	52	56

ACKNOWLEDGEMENTS

Authors are thankful to the Heads, Departments of Microbiology and Biochemistry, Kakatiya University for providing necessary facilities.

REFERENCES

1. Holt John,G.,Krieg Noel.R., Sneath Peter, Staley.H., James,T. and Willimas Stanley,T., *Bergeys manual of determinative bacteriology*, Fifth edition,Willimas and Wilkins Publishers 1994.
2. Mariá C. Vega-Hernández, Milagros León-Barrios and Ricardo Pérez-Galdona. Indole-3-acetic acid production from indole-3-acetonitrile in *Bradyrhizobium. Soil Biology Biochemistry*. 2002; **34**(5): 665-668
3. Abbas Akbari, Seyyed Mehdi Arab, H.A. Alikhani, I. Allahdadi and M.H. Arzanesh. Isolation and Selection of Indigenous Azospirillum spp. and the IAA of Superior Strains Effects on Wheat Roots. *World. Journ. Agri. Sci* 2007; **3**(4): 523-529,
4. Rajasekhar, N., Sasikala, Ch. and Ramana, Ch.V. Photoproduction of indole 3-acetic acid by *Rhodobacter sphaeroides* from indole and glycine. *Ind. J. Mic. Bio*., 1999; **39**: 39-44.
5. Rajasekhar,N.,Ch.Sasikala,.V.Ramana. Photobiotransformation of indole to its value-added derivatives by *Rhodobacter sphaeroides* OU5, *J.Ind.Microbiol.Biotechnol*., 1998; **20**: 177
6. Sasikala, Ch. and Ch.V.Ramana. Biotechnological potentials of anoxygenic phototrophic bacteria I Production of single –cell protein, vitamins, ubiquinones, hormones and enzymes and use in waste treatment *Advanced Applied Microbiology,* 1995; **41:** 173-226.
7. Ramchander Merugu, M.S.K. Prasad, S. Girisham and S.M.Reddy. Production of Indole acetic acid and free amino acids by two Anoxygenic phototrophic bacteria. *Bioinfolet* 2008; **5**: 82-84
8. Beibl,H. and N.Pfenning., In,The Prokaryotes (eds) M.P.Starr, H.Stolp, H.G.Truper, A.Balows and H.G.Schlegel,Spriger –verlag,Berlin 1981.
9. Bergey's Manual of Systematic bacteriology. "Enrichment and isolation of purple non sulphur photosynthetic bacteria" . Eds:J.T.Staley, M.P.Byrant, N.Pfennig and J.C.Holt 1989.
10. Bentes-Sousa, Jacqueline da Silva Batista, Artur L.C. da Silva and Maria Bentley, J.AS. Analysis of plant hormones. In : *Methods of Biochem Anal.* 1962; **9**: 9-125.
11. Farah Ahmad, Iqbal Ahmad, Mohd Saghir Khan. Indole Acetic Acid Production by the Indigenous Isolates of Azotobacter and Fluorescent Pseudomonas in the Presence and absence of Tryptophan. *Turk J Biol* 2005; **29**: 29-34.
12. Bastian, F., A. Cohen, P. Piccoli, V. Luna, R. Baraldi and R. Bottini. Production of indole-3 acetic acid gibberellins A1 and A3 by *Acetobacter diazitrophicus* and *Herbaspirillum seroopedicae* in chemically defined culture media. *Plant Growth Regul.,* 1998; **24**: 7-11.