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PRODUCTION OF INDOLE ACETIC ACID BY THREE ANOXYGENIC PHOTOTROPHIC PURPLE BACTERIA

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ABSTRACT

The present investigation *Allochromatium* sp. GSKRLMBKU-01, *Rhodobacter* sp. GSKRLMBKU-02 and *Rhodobacter* sp. GSKRLMBKU-03 were isolated from different ecological niches and its ability to produce Indole Acetic Acid (IAA) in the presence and absence of tryptophan as inducer was investigated. All the three bacteria produce IAA even in the absence and presence of tryptophan but production was more in tryptophan supplemented medium. The maximum IAA production by *Allochromatium* sp. GSKRLMBKU-01 (165 µg/ml), *Rhodobacter* sp. GSKRLMBKU-02 (140 µg/ml) and *Rhodobacter* sp. GSKRLMBKU-03 (120 µg/ml) was recorded. The highest amount of biomass and IAA production was recorded on 8th day of incubation by *Allochromatium* sp. GSKRLMBKU-01 and *Rhodobacter* sp. GSKRLMBKU-03, while *Rhodobacter* sp. GSKRLMBKU-02 was recorded on 10th day of incubation.

KEY WORDS: Anoxygenic phototrophic purple bacteria, *Allochromatium* sp., *Rhodobacter* sp., Biomass and Indole acetic acid.

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INTRODUCTION

Auxins are the major growth regulators of plants particularly in cell enlargement and cell differentiation. Indole acetic acid (IAA) is the main auxin present in plants which is synthesized through the shikimic acid pathway and form tryptophan in fungi or bacteria also. Although a few purple non sulfur bacteria (PNSB) are reported to produce plant hormones, it is still underexploited. *Rhodospirillum rubrum* is reported to produce 3 Cytokinins, one of them was identified as Zeatinriboside[1]. Kinetin and Zeatin were isolated from culture of *Rhodobacter sphearoides*[2]. Serdyuk *et al*. (1993)[1] reported cytokinin from *Rhodospirillum rubrum*. Yokoyama (1990)[3] reported to produce growth substances by *Rba. sphaeroides*. Production of IAA by different APB was reported by many workers including Sasikala and Ramana (1995a)[4], Rajasekhar *et al.* (1999)[5,6], Srinivas *et al.* (2002)[7] and Ramchander *et al*. (2008 and 2011)[8,9]. Photobiotransformation of IAA by some of the purple non sulfur bacteria was investigated[4]. A new phytohormone Rhodestrin was isolated as a metabolite of anthanilate photobiotransformation by *Rba. sphearoids* O.U 5[10]. Ranjith *et al.* (2007a)[11] reported the production of novel indole terpenoid, Rhodethrin by *Rhodobacter sphaeroides*. Mujahid *et al.* (2010)[12] reported the production of Indole -3-acetic acid (*Rubrivivax benzoatiliticus* JA2), Indole-3-aldehyde (*Rvi. benzoatiliticus* and *Rba. sphaeroides* DSM 158), Indole-3-ethanol (*Rsp. rubrum* ATCC 1170) and Anthranilic acid (*Rba. sphaeroides* DSM 158) by aniline induced tryptophan production. Production of new phenol terpenoid ester Rubrivivaxin, having cytotoxic and cyclooxygenase-I inhibitory effect was produced by *Rubrivivax benzoatilyticus* JA2[13]. Apart from IAA production, anoxygenic phototrophic bacteria (APB) are known for their hydrogen production[14]. Hence, it was considered worthwhile to investigate production of IAA by three anoxygenic phototrophic purple bacteria (APPB).

MATERIALS AND METHODS

Chemicals and Reagents

All the chemicals used in the present investigations were purchased from Sigma Aldrich (Mumbai, India) and Hi Media company (Mumbai, India).

Sampling

Samples for isolation of anoxygenic phototrophic purple bacteria were collected from marine coastal region at Visakhapatnam and different water samples at Chandrapoor District, Maharashtra.

Isolation and identification of anoxygenic purple phototrophic bacteria

The anoxygenic phototrophic purple bacteria was isolated by enrichment technique[15] by inoculating the collected each sample into the 15 ml of Biebl and Pfennig's medium containing screw capped tubes. Strict anaerobic conditions are maintained and incubated under 2000 lux light. The cultures thus obtained by enrichment technique were streaked on the solid enriched medium repeatedly by paired petriplate method and flushed with nitrogen gas to maintain the anaerobic condition. The colonies were picked up and inoculated into a liquid medium. Among three bacteria thus isolated one was identified as *Allochromatium* sp. and other two as *Rhodobacter* sp. with the help of Bergey's Manual of Systematic Bacteriology[16]. The morphologically identified bacterium was further confirmed by precise molecular method by polymerase chain reaction (PCR) by 16S rRNA sequencing analysis and confirmed as *Allochromatium* sp. strain GSKRLMBKU-01, *Rhodobacter* sp. GSKRLMBKU-02 and *Rhodobacter* sp. GSKRLMBKU-03. Sequence thus obtained was submitted in National Centre for Biotechnology Information (GenBank Accession number HF677171.1, HG971782.1 and HF971783.1).

ESTIMATION OF IAA

Estimation of IAA was determined by inoculating 1ml of each fresh culture of three bacteria into screw capped tubes containing 15ml of Biebl and Pfennigs medium without tryptophan (Medium A) and with 0.1% tryptophan (Medium B) was prepared and incubated for 4, 6, 8, 10, 12 and 15 days at 30 ± 2 ºC under the light intensity of 2000 lux for 15 days. At the end of incubation period cultures were centrifuged at 10,000 rpm for 10 minutes.

The supernatant was collected and the amount of IAA was estimated by the method suggested by Bentley (1962)[17]. To 2 ml of culture filtrate, 8ml of Salkowski reagent (300ml of H_2SO_4 +15ml of 0.5M $FeCl_3$ in 500 ml of distilled water) was added and kept in dark for 30 minutes. The intensity of pink colour thus developed was read at 540 nm. The amount of IAA present in the samples was calculated from a standard graph prepared by using Indole acetic acid. Growth of bacterium was determined by turbidity method by measuring optical density at 660 nm. pH of the culture supernatant was determined by Elico pH meter and the results are expressed in mean of triplicate experiments.

RESULTS AND DISCUSSION

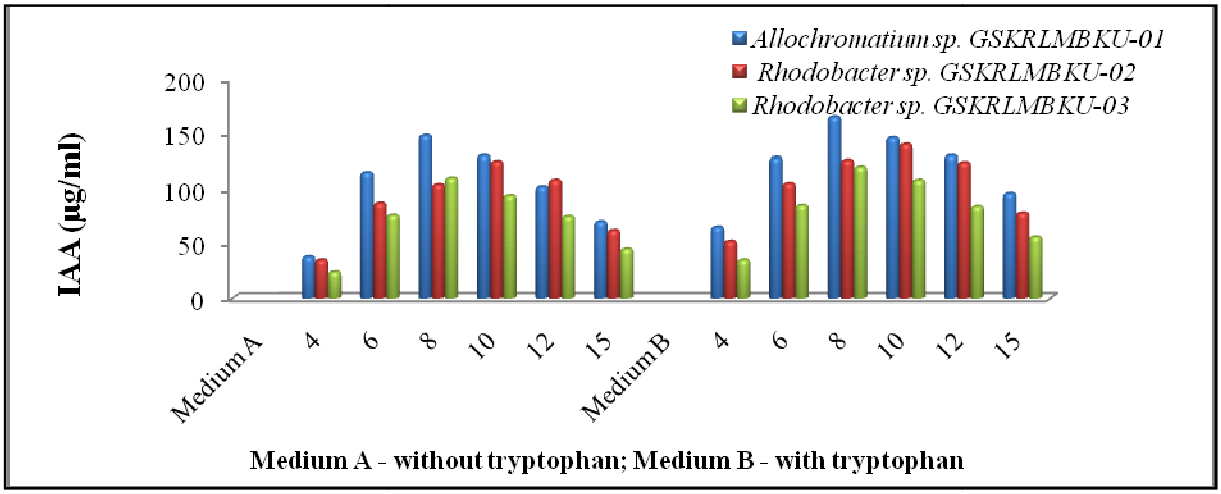
The results presented in Table 1 and Fig.1 reveals that *Allochromatium* sp. GSKRLMBKU-01, *Rhodobacter* sp. GSKRLMBKU-02 and *Rhodobacter* sp. GSKRLMBKU-03 produced IAA even in the absence of tryptophan as precursor (Medium A). The amount of IAA produced by *Allochromatium* sp. GSKRLMBKU-01 was more (148 (µg/ml) in comparison to other two bacteria under investigation. *Rhodobacter* sp. GSKRLMBKU-02 produces maximum IAA (125 (µg/ml) on 10th day of incubation period, while IAA production (110 µg/ml) by *Rhodobacter* sp. GSKRLMBKU-03 was a low. Similarly Ramchander *et al.* (2008)[8] reported the maximum production of IAA in 8 days and 4 days of incubation by *Rhodobacter capsulatus* and *Rhodopseudomonas acidophila* respectively in the absence of tryptophan as precursor. Result of the present investigations was positively correlated with Charya and Reddy (1984)[18]have also reported synthesis of IAA in the absence of tryptophan. The IAA production was significantly more in the medium supplemented with tryptophan (Medium B) by the three APB suggesting its precursors of IAA (Table 1 and Fig. 1) when compared to their production in absence of tryptophan (Medium A). *Allochromatium* sp. GSKRLMBKU-01 could produce more IAA (165 µg/ml) when compared to *Rhodobacter* sp. GSKRLMBKU-02 (140µg/ml) and *Rhodobacter* sp. GSKRLMBKU-03 (120 µg/ml). These results are in agreement with earlier studies of Rajasekhar *et al.* (1999) [5,6], Srinivas *et al.* (2002)[7] and Ramchander *et al.* (2011)[9] who also reported more IAA production in the presence of tryptophan by anoxygenic phototrophic bacteria studied by them. *Allochromatium* sp. GSKRLMBKU-01 and *Rhodobacter* sp. GSKRLMBKU-03 synthesized maximum biomass and IAA by on 8th day of incubation period in both medium, while *Rhodobacter* sp. GSKRLMBKU-02 could achieve maximum growth and IAA by 10th day of incubation period. The final pH of the medium was shifted from neutral to alkaline side. No positive correlation could be observed among the biomass, pH changes and IAA production. The present results are in agreement with those of Ramchander *et al.* (2008)[8] who also recorded maximum IAA production by 8th day of incubation period by *Rba. capsulatus* and *Rps. acidophila.* Similarly Srinivas *et al.* (2002)[7] reported the Indole acetic acid production by anoxygenic phototrophic bacteria at different cultural conditions.

Table 1
Production of Indole acetic acid (IAA) by three APPB

Organism	Incubation in days	Medium A			Medium B		
		Growth (O.D)	Final pH	IAA (µg/ml)	Growth (O.D)	Final pH	IAA (µg/ml)
Allochromatium sp. GSKRLMBKU-01	4	0.4	8.2	38	0.7	8.3	65
	6	1.3	8.3	115	1.4	8.4	128
	8	1.8	8.5	148	2.0	8.5	165
	10	1.6	8.6	130	1.8	8.6	146
	12	1.2	8.8	102	1.5	8.8	130
	15	0.8	9.0	70	1.2	9.0	96
Rhodobacter sp. GSKRLMBKU-02	4	0.3	7.2	35	0.5	7.3	52
	6	1.1	7.4	87	1.2	7.5	105
	8	1.5	7.5	104	1.6	7.6	126
	10	1.7	7.6	125	1.8	7.8	140
	12	1.4	7.8	108	1.6	8.0	124
	15	1.0	8.0	62	1.2	8.2	78
Rhodobacter sp. GSKRLMBKU-03	4	0.5	7.2	24	0.7	7.2	35
	6	1.2	7.3	76	1.3	7.4	85
	8	1.6	7.5	110	1.7	7.5	120
	10	1.3	7.6	94	1.5	7.6	108
	12	1.0	7.8	75	1.2	7.8	84
	15	0.7	8.0	45	0.8	8.0	56

Medium A = Without tryptophan; Medium B = With tryptophan (0.1%)

Figure 1
Production of Indole acetic acid by three APPB



CONCLUSION

From the present investigation it can be concluded that all the three anoxygenic phototrophic bacteria has the ability to produce IAA even in the absence of tryptophan as precursor but the IAA production was more in medium supplemented with tryptophan. All three phototrophic bacteria were good producers of IAA, among them *Allochromatium* sp. GSKRLMBKU-01 produced significantly more amount of IAA compared to *Rhodobacter* sp. GSKRLMBKU-02 and *Rhodobacter* sp. GSKRLMBKU-03. Further investigation are needed to optimize the conditions for the maximum production of IAA by the three APPB.

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