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PRODUCTION OF FREE AMINO ACID BY THREE ANOXYGENIC PHOTOTROPHIC PURPLE BACTERIA

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ABSTRACT
Production of Free Amino Acids (FAA) by three anoxygenic phototrophic purple bacteria Allochromatium sp. GSKRLMBKU-01, Rhodobacter sp. GSKRLMBKU-02 and Rhodobacter sp. GSKRLMBKU-03 isolated from different ecological niches was investigated. These three bacteria differed significantly in the amount of FAA produced. The maximum FAA production by Allochromatium sp. GSKRLMBKU-01 (642 µg/ml), Rhodobacter sp. GSKRLMBKU-02 (548 µg/ml) and Rhodobacter sp. GSKRLMBKU-03 (510 µg/ml) was recorded. The biomass and FAA production was maximum on 8th day of incubation by Allochromatium sp. GSKRLMBKU-01 and Rhodobacter sp. GSKRLMBKU-03 and decreased further progress of incubation period, while it was 10th day for Rhodobacter sp. GSKRLMBKU-02. Final pH of the medium was shifted towards alkaline side by all the three phototrophic bacteria.

KEY WORDS
Anoxygenic phototrophic purple bacteria, Allochromatium sp., Rhodobacter sp., Growth conditions and Free amino acids.

INTRODUCTION
For almost 50 years, biotechnological process has been used for industrial production of amino acids. On account of their functionality and special features arising from chirality, this class of compounds are biochemically very important for the chemical industry to be essential [1]. Of the various 20 standard amino acids, 9 amino acid are considered essential amino acids, L-valine, L-leucine, L-isoleucine, L-lysine, L-threonine, L-methionine, L-histidine, L-phenylalanine and tryptophan occupy a key position as they are not able to be synthesized by animals and human beings. Amino acids are important for pharmaceutical and chemical industries. They are used in food industries as flavouring agents and also as food and feed additives [2-4]. They are crucial for the metabolic activities and play important role in the various physiological processes. These constitute the chief building blocks of proteins which are structural and functional components of the living cell. Ninety five percent hormones are amino acids. All neurotransmitters are amino acids in nature. Amino acids play a significant role in the metabolic processes of all living organisms including bacteria and fungi. Most of the antibiotics are made of amino acids. The proteins of the host are hydrolyzed by pathogens like fungi and bacteria to amino acids which may serve as carbon and nitrogen sources. These are among the most important products of microbial biotechnology [5].

Fermentative production of amino acids by different organisms has been investigated by several workers [1, 6, 7]. Anoxygenic phototrophic bacteria are being exploited as single cell protein and produce essential amino acids, vitamins, biological co-factors and fewer amounts of nucleic acids [8] as of their ability to tolerate high BOD and fermentative metabolism. Chemical composition of *Rhodocyclus gelatinosus* biomass produced in poultry slaughter house water was investigated [4]. Salma *et al*. [9] Reported the dietary supplementation of *Rhodobacter capsulatus* to chicken leads to eggs containing low cholesterol. Similarly Ramchander *et al*. [10] reported the hypocholesterolemic effect of the anoxygenic phototrophic bacterium *Rhodopseudomonas palustris* MGU001 in hen laying eggs. Except the reports of Ramchander *et al*. [11] and Srinivas *et al*. [12], further no information is available on FAA production by these bacteria. Therefore, the FAA production by three anoxygenic phototrophic purple bacteria (APPB) was investigated and discussed in this paper.

MATERIALS AND METHODS

Chemicals

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

Isolation of APPB

Samples for isolation of anoxygenic phototrophic bacteria were collected from marine coastal region at Visakhapatnam and Chandrapoor District, Maharashtra. The three anoxygenic phototrophic purple bacterium were isolated by enrichment media [13] by inoculating the collected each sample into the 15 ml Biebl and Pfennig's medium containing screw capped tubes. Strict anaerobic conditions are maintained and incubated under 2000 lux light. The pure cultures were obtained by paired petriplate method, which are flushed with nitrogen gas to maintain the anaerobic condition. Among three phototrophic 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 [14]. The morphologically identified bacterium was further confirmed by precise molecular identification by 16S rRNA sequencing analysis. Sequence thus obtained was submitted in National Centre for Biotechnology Information (GenBank Accession number HF677171.1, HG971782.1 and HF971783.1).

ESTIMATION OF FAA

Estimation of FAA was determined by inoculating 1ml of each log phase culture of three bacteria into screw capped tubes containing 15ml of Biebl and Pfennig's medium was prepared and incubated at 30 ± 2 °C under the light intensity of 2000 lux for 15 days. The FAA production by three bacteria was estimated at end of 4, 6, 8, 10, 12 and 15 days. At the end of incubation period cultures were centrifuged at 10,000 rpm for 10 minutes. Free amino acids present in the three bacterial cultures were determined by the method suggested by Moore and Stein [15]. To 2 ml of culture filtrate, 2 ml of 2% ninhydrin reagent (pH was adjusted to 6.8) was added and heated in water bath at 60 °C for 2 minutes. Thus the colour intensity developed was read at 570 nm in a spectrophotometer. The amounts of free amino acids were calculated from a standard graph prepared by using tyrosine. The results are expressed in mean of triplicate experiments.

RESULTS AND DISCUSSION

The Critical Persual **Table 1** reveals that *Allochromatium sp*. GSKRLMBKU-01 produced maximum amount of FAA (642 µg/ml) on 8th day of incubation, while *Rhodobacter sp*. GSKRLMBKU-02 produced maximum FAA (548 µg/ml) on 10th day of its incubation period. *Rhodobacter sp.* GSKRLMBKU-03 produced less amount of FAA (510 µg/ml) compared to other two bacteria. Among three bacteria *Allochromatium sp*. GSKRLMBKU-01 was superior in free amino acids production in comparison to *Rhodobacter sp*. GSKRLMBKU-02 and *Rhodobacter sp*. GSKRLMBKU-03. *Allochromatium sp*. GSKRLMBKU-01 and *Rhodobacter sp*. GSKRLMBKU-03 produced maximum biomass on 8 days of incubation, while *Rhodobacter sp.* GSKRLMBKU-02 took 10 days for maximum biomass production. The final pH of the media was shifted towards alkaline side three anoxygenic phototrophic bacteria. These results are in agreement with earlier studies of Srinivas *et al*. [12] who also reported the maximum FAA production at 5 and 10 days incubation by *Rhodobacter sphaeroids, Rhodocyclus gelatinosus*, *Rhodopseudomonas palustris* and *Rcy. tenuis* respectively. Similarly, Ramchander *et al*. [11] reported the maximum free amino acid production by *Rps. acidophila and Rba. capsulatus* was recorded in 8 and 4 days respectively.

Table 1: Production of Free Amino Acids by three anoxygenic phototrophic purple bacteria at different incubation periods

Organisms	Incubation period (in days)	Growth (O.D)	Final pH	Free Amino Acids (µg/ml)
Allochromatium sp. GSKRLMBKU-01	4	0.4	8.2	186
	6	1.4	8.4	320
	8	1.8	8.6	642
	10	1.6	8.8	452
	12	1.2	9.0	315
	15	0.8	9.2	182
Rhodobacter sp. GSKRLMBKU-02	4	0.5	7.2	152
	6	1.1	7.5	285
	8	1.4	7.6	350
	10	1.6	7.8	548
	12	1.1	8.0	386
	15	0.7	8.4	220
Rhodobacter sp. GSKRLMBKU-03	4	0.4	7.2	175
	6	1.0	7.4	275
	8	1.5	7.6	510
	10	1.3	7.8	420
	12	0.8	8.0	289
	15	0.6	8.2	142

The results are expressed in mean of triplicate experiments

CONCLUSION

From the present investigation it is clear that all the three anoxygenic phototrophic purple bacteria are good producers of FAA. Among three phototrophic bacteria, *Allochromatium sp*. GSKRLMBKU-01 produced significantly more amount of FAA compared to Rhodobacter sp. GSKRLMBKU-02 and Rhodobacter sp. GSKRLMBKU-03 at varied incubation periods. Further detailed investigations are needed for the real mechanism of FAA by anoxygenic phototrophic purple bacteria.

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