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Production of PHB by under aerobic dark conditions by two anoxygenic phototrophic purple non sulfur bacteria isolated from tannery effluent

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Abstract: A survey of various tannery effluents for the presence of purple non-sulphur bacteria was undertaken in Warangal district of South India. In all the nine bacterial species, which included *Rhodopseudomonas palustris, R.rutila, R.acdiophila, Rhodopila globiformis, Rhodospirillum rubrum, Rsp.photometricum, Rhodobacter sphaeroides, Rb.capsulatus, Rhodobacter* sp and *Rhodocyclus gelatinosus* were isolated. Among these two strains *Rhodobacter capsulatus* KU002 and *Rhodopseudomonas palustris* KU003 were selected for the production of Polyhydroxybutyrate (PHB). Effects of aerobic dark conditions on the production of PHB were tested. Nitrogen, phosphate and sulphate limitations were investigated for improving the production of the polymer. Nitrogen limitation induced more amounts of the polymer than sulphate or phosphate limitations in *Rhodobacter capsulatus* KU002.Similar trend was observed in *Rhodopseudomonas palustris* KU003. We propose a new method of enhancing the polymer production under aerobic dark conditions with nitrogen limitation. Significance of the above results in the light of existing literature is discussed in this communication.
Keywords: *Rb.capsulatus, Rps.palustris*, Polyhydroxybutyrate, aerobic dark conditions.

Introduction:

Poly hydroxy butyrate (PHB) is an intracellular carbon and energy storage material synthesized by a great variety of bacteria. PHB was originally shown to be a constituent of lipid inclusions in the cells of *Bacillus*[1]. The biosynthesis of PHB occurs through three enzymatic reaction steps involving b-ketothiolase, acetoacetyl-CoA reductase, and PHA-synthetase[2]. Induction studies have revealed that enzymatic activities of both b- ketothiolase

and acetoacetyl-CoA reductase increased markedly in response to PHB-stimulating limitation conditions[3].

PHA (Polyhydroxy alkanoates) production by photosynthetic bacteria is not high. Brandl *et al* .(1991)[3] reported that *Rhodobacter sphaerodies* produced PHB as the major component (97%) and a small amount of PHV(3%) under anaerobic light conditions. PHA production from some waste material has been studied by Yigit *et al.*[4] and Ali Hassan *et al.*[5] from the waste waters of sugar refineries and palm oil waste respectively. PHA production from acetic acid was reported in *Rb.sphaerodies* S and *Rb.sphaerodies* IL 206 by Noparatnaraporn *et al.*[6]. Influence of cultural conditions on the synthesis and accumulation of PHB by *Rps.palustris* SP5212 was investigated by Mahuya *et al*[7]. Combinations of various carbon and nitrogen substrates were used to study poly-β-hydroxybutyrate accumulation and H_2 evolution by *Rhodobacter sphaeroides* strain RV Khatipov *et al.*[8] . In this investigation, an attempt was made to procure PHB from two phototrophic bacteria isolated from tannery effluent and to study the effect of aerobic dark conditions on the production of PHB from these bacteria. This is the first report on the use of nutrient limitation under aerobic dark conditions. We report an enhancement in PHB production from these bacteria in this communication.

Material and Methods:

Phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the Biebl and Pfennig's medium and incubated anaerobically in the light. The cultures obtained by enrichment technique were streaked on to the solid medium repeatedly and colonies were picked up to inoculate into the liquid medium and maintained by subculturing. Bacteria thus isolated were identified by studying the cultural characteristics (colour, size and shape), utilization of carbon and nitrogen sources, vitamin requirements, absorption spectral analysis, bacteriochlorophyll and carotenoids with the help of Bergey's manual of Systematic Bacteriology[9].

Tubes were inoculated with 1ml log phase cultures of two anoxygenic phototrophic bacteria and incubated at 30±2° C under the light intensity of 2000lux in fifteen ml screw cap tubes. Two different media which were found to produce maximum amount of PHB[10] were used in this study. After inoculation, growth and PHB yield was calculated. Bacterial pellet was suspended in 5ml of hypochlorite and incubated for 10 minutes. The suspension was centrifuged at 8000 rpm for 10 minutes. The pellet was washed with diethylether and was then assayed for PHB. PHB extracted by the above method was assayed by Law and Slepcky[11] method. PHB sample was treated with 5 ml of concentrated H_2SO_4 and a placed in a boiling water bath for 20 min. On cooling absorbance was recorded at 236 nm on a UV-Vis spectrophotometer. Standard was run using poly hydroxy butyrate.

Results and Discussion:

IR spectra values: 1290(C-O), 1680(C=O aliphatic), 2670(CH_2),2910-2960(C-H streching)
NMR values: 1.08 (3H,d,-CH_3), 2.35 (2H,d,-CH2-), 5.15(1H,m,-CH-)
Perusal of table 1 shows that the ability of both the bacteria to produce polymer even under aerobic dark conditions, although very less yields were seen. *Rps.palustris* was superior under aerobic conditions than *Rb.capsulatus*. Highest yields were seen in both the bacteria at 6th day of incubation. Perusal of table 1 shows that the ability of both the bacteria to produce polymer even under aerobic dark conditions, although very less yields were seen. *Rps.palustris* was superior under aerobic dark conditions than *Rb.capsulatus*. Highest yields were seen in both the bacteria at 6th day of incubation. Limitations of the sulphate and phosphate could not induce the organisms to produce more amounts of the polymer (table 2, 4). Only nitrogen limitation was effective in enhancing the production of the polymer in both the bacteria under investigation. *Rps.palustris* could produce

128 mg/L of the polymer under nitrogen limitation. On the other hand *Rb.capsulatus* could produce 110 mg/L of the polymer. There have been a number of reports on the PHB production by fed-batch fermentation, only few cases of PHB production by phosphate limitation in a fed-batch mode were reported (Byrom,1987). Production of Poly (3-hydroxybutyrate) by high cell density fed-batch culture of *Alcaligenes eutrophus* with phosphate limitation was reported by Ryu *et al*.[12]. Production of Poly (3-hydroxybutyrate) by *Ralstonia eutrophus* with phosphate limitation was reported by Shang *et al*.[13]. Although production of PHB has been reported under aerobic dark conditions but combinations of both stress conditions was not tried earlier. Eventhough less yields have been reported from the bacteria in this study use of cheaper carbon and nitrogen sources under aerobic dark condition s could make the process economically viable. Hence this approach can be employed for improving the production of the polymer at a larger scale.

Table 1: Production of PHB under aerobic dark conditions by *Rb.capsulatus* and *Rps.palustris*

		Incubation Period (days)			
		2	—	—	—
Rb.capsulatus	Succiante,Malate Yeast Extract Medium(SMY)	4	0.426	0.9	21
		6	0.648	1.1	83
		8	0.725	1.2	87
		10	0.542	1	48
		2	—	—	—
Rps.palustris	Succinate, Yeast Extract Medium (SY)	4	0.482	0.9	29
		6	0.715	1.2	104
		8	0.746	1.3	85
		10	0.624	1.1	62

Table 2: Production of PHB under aerobic dark conditions by two anoxygenic phototrophic bacteria under sulphate limitation

		Incubation Period (days)			
		2	—	—	—
Rb.capsulatus	Succiante ,Malate Yeast Extract Medium(SMY)	4	0.338	0.7	25
		6	0.608	1.0	63
		8	0.685	1.1	77
		10	0.448	0.8	28
		2	—	—	—
	Succinate Yeast Extract Medium (SY)	4	0.412	0.8	36
Rps.palustris		6	0.625	1.1	94
		8	0.718	1.2	83
		10	0.584	0.9	56

Table 3: Production of PHB under aerobic dark conditions by two anoxygenic phototrophic bacteria under nitrogen limitation

		Incubation Period (days)			
		2	—	—	—
Rb.capsulatus	Succiante ,Malate Yeast Extract Medium(SMY)	4	0.336	0.8	32
		6	0.762	1.0	97
		8	0.788	1.1	110
		10	0.558	1	68
		2	—	—	—
	Succinate, Yeast Extract Medium (SY)	4	0.496	0.8	39
Rps.palustris		6	0.768	1.2	128
		8	0.842	1.3	98
		10	0.756	1.1	74

Table 4: Production of PHB under aerobic dark conditions by two anoxygenic phototrophic bacteria under phosphate limitation

		Incubation Period (days)			
		2	—	—	—
Rb.capsulatus	Succiante ,Malate Yeast Extract Medium(SMY)	4	0.384	0.7	10
		6	0.546	0.9	66
		8	0.626	1.0	77
		10	0.464	0.8	38
		2	—	—	—
	Succinate Yeast Extract Medium (SY)	4	0.384	0.9	36
Rps.palustris		6	0.625	1.1	94
		8	0.718	1.2	85
		10	0.434	0.7	54

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