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PHB (Polyhydroxy butyrate) production under nitrogen limitation by Rhodobacter capsulatus KU002 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 *Rhodobacter capsulatus* KU002 was selected for the production of Polyhydroxybutyrate (PHB). Effect of nitrogen limitation using excess carbon source on the production of PHB was tested. PHB accumulation was more at a nitrogen limitation of 26 mg/L of ammonium chloride and a concentration of acetate at 2.2 g/L. Significance of the above results in the light of existing literature is discussed in this communication.
Keywords: *Rb.capsulatus,* Polyhydroxybutyrate, nitrogen limitation.

Introduction:

Polyhydroxyalkanoates (PHA) are synthesized by numerous bacteria as intracellular carbon and energy storage compounds and accumulated as granules in the cytoplasm of cell under the condition of limiting nutrients such as nitrogen and phosphorus but in the presence of excess carbon source[1]. It is known as a biodegradable polymer having some potential applications in different fields as biomedical and environments. However, the major commercial drawback of the bacterial PHBs is their high production cost, making monomer structures and contents of PHA have them substantially more expensive than synthetic plastics[2] .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. Nutrient limitation is necessary to trigger PHB accumulation, and generally ammonia is used as the critical control factor for uncoupling the growth of cells and PHB production. Because inactivation of inhibition of PHB production by ammonia has industrial potential[1], study was done to optimize its levels in the form of ammonium chloride for enhancing the polymer production.

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 (1989)[4]. Tubes were inoculated with 1ml log phase cultures of two anoxygenic phototrophic bacteria and incubated at 30±2º C under the light intensity of 2000lux in fifteen ml screw cap tubes. Carbon source in the form of acetate was maintained at a concentration of 1.0%. After inoculation, growth and PHB yield was calculated at various concentrations of ammonium chloride. Then excess carbon source was added to the culture medium.

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 (1960) method[5].

Table 1: Effect of Nitrogen limitation on PHB production in *Rb.capsulatus* after eight days incubation.

Organism	Ammonium chloride	Growth	DCW	PHB
	(mg/L)	(O.D)	(g/L)	(mg/L)
Rb. capsulatus	13.0	0.69	1.1	108
	26.0	0.76	1.3	140
	39.0	0.78	1.3	179
	52.0	0.88	1.4	186
	65.0	0.94	1.5	240
	78.0	1.06	1.6	225
	91.0	1.12	1.8	210
	104.0	1.14	1.8	206

Table 2: Effect of excess carbon source with nitrogen limitation on PHB production in *Rb.capsulatus* after eight days incubation.

Organism	Acetate	Growth	DCW	PHB
	(g/L)	(O.D)	(g/L)	(mg/L)
Rb.capsulatus	1.0	1.14	1.7	206
	1.4	1.26	1.8	212
	1.8	1.38	1.9	220
	2.2	1.42	1.9	260
	2.6	1.40	1.9	210
	3.0	1.38	1.9	192

Results and Discussion:

Higher yields of the polymer were observed when nitrogen source in the form of ammonium chloride was limited (table 1) to the organism.

Rb.capsulatus produced 240 mg/L of PHB at 65 mg/L concentration of ammonium chloride. About 4% of enhancement in the accumulation of the polymer was observed. No correlation could be observed between dry cell weight and polymer accumulation. Less than the limitation of nitrogen mentioned above did not result in higher yields of PHB. Excess carbon source in the form of acetate was added to the cultures under limitation to enhance the yield of the PHB (table 2). A concentration of acetate at 2.2 g/L gave 260 mg/L of PHB. Increase in the concentration of the carbon caused an increase in the amount of PHB produced. The linearity was present upto a level, thereafter it decreased. Here also no correlation could be observed between dry cell weight and polymer accumulation. Both these bacteria produced PHB during exponential phase which was similar to *Rhodobacter sphaeroides* ES 16[6], *A.latus* ATCC 29712[7] and different from *Ralstonia eutropha* which accumulated PHB at the stationary phase[8] . Nitrogen limitation in the form of NH_4Cl for PHB production was also reported in *Alcaligenes eutrophus*[9], *Methylobacterium* sp.[10] and *Sinorhizobium fredii*[11]. Excess carbon source with nitrogen limitation could be a more viable approach for maximizing the production of PHB in these group of bacteria.

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