PRODUCTION OF PHB (POLYHYDROXYBUTYRATE) BY *RHODOPSEUDOMONAS PALUSTRIS* KU003 UNDER NITROGEN LIMITATION

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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 *Rhodopseudomonas palustris* KU003 was selected for the production of Polyhydroxybutyrate (PHB). Effect of nitrogen limitation on the production of PHB was tested. PHB accumulation was more at a nitrogen limitation of 78 mg/L of ammonium chloride. The maximum PHB produced was 180 mg/L of BP medium containing glucose as carbon source. Significance of the above in the light of existing literature is discussed in this communication.

Keywords: *Rps.palustris*, Polyhydroxybutyrate, nitrogen limitation

INTRODUCTION:

Polyhydroxyalkanoates (PHA) are polyesters of hydroxyalkanoates (HA) and consist of β-hydroxyacyl as monomer. PHB is a highly crystalline thermoplastic polymer with a relatively high melting temperature (in the range of 170-180 ºC) and a glass transition temperature in the range of 0-5 ºC. 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* (Winfred and Robards,1973). Brandl *et al* .(1991) reported that *Rhodobacter sphaerodies* produced PHB as the major component (97%) and a small amount of PHV(3%) under anaerobic light conditions. Mahuya *et al.*(2005) investigated the effect of nutrient limitation on accumulation of PHB by *Rps.palustris* SP5212. Combinations of various carbon and nitrogen substrates were used to study poly-β-hydroxybutyrate accumulation and H_2 evolution by *Rhodobacter sphaeroides* RV (Khatipov *et al.,*1998). PHB accumulation of in photosynthetic bacteria depends on optimum C/N ratio (Khatipov et al. 1998). Influence of cultural conditions on the synthesis and accumulation of PHB by *Rps.palustris* SP5212 under nutrient limitation was investigated by Mahuya *et al.*(2005). 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.* (1998). PHB accumulation in photosynthetic bacteria depends on optimum C/N ratio (Khatipov et al. 1998). 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. Hence, in this investigation, an attempt was made to study the effect of nitrogen limitation on the production of PHB.

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) .

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 glucose was maintained at a concentration of 1.0%. After inoculation, growth and PHB was calculated at various concentrations of ammonium chloride.

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. 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:

Highest yields of polymer were observed when nitrogen source in the form of ammonium chloride was used (table 1) by the organism.

Table 1: Effect of Nitrogen limitation on production of PHB by *Rps.palustris* after eight days incubation.

Organism	Ammonium chloride	Growth	DCW	PHB
	(mg/L)	(O.D)	(g/L)	(mg/L)
Rps.palustris	13.0	0.688	1.1	—
	26.0	0.694	1.2	98
	39.0	0.786	1.2	113
	52.0	0.808	1.3	138
	65.0	0.848	1.4	161
	78.0	0.962	1.5	180
	91.0	0.986	1.6	158
	104.0	1.234	2.0	120

Rps. palustris required 78 mg/L for producing 180mg/L of PHB. About 66 % of enhancement in the accumulation of the polymer was observed. No polymer production could be observed at a concentration less than 13 mg/L of ammonium chloride. These phototrophic bacteria produced PHB during exponential phase which was similar to *Rhodobacter sphaeroides* ES 16 (Sangharak and Praserstan,2008), *A.latus* ATCC 29712 (Sayed *et al*.,2009) and different from *Ralstonia eutropha* which accumulated PHB at the stationary phase (Madison and Huisman, 1999). Nitrogen limitation in the form of NH_4Cl for PHB production was also reported in *Alcaligenes eutrophus* (Koutinas *et al*., 2007), *Methylobacterium* sp. (Kim *et al.,* 2006) and *Sinorhizobium fredii* (Liangqi *et al.*, 2006). Mansfield *et al.* (1995) reported that the level of acetyl-CoA remained constant during the growth phase but that of CoASH increased and reached a maximum towards the end of exponential growth leading to inhibit the first enzyme, 3-ketothiolase, in PHB synthesis pathway of *A. eutrophus* during PHB accumulation following nitrogen exhaustion in batch culture. The accumulation of PHB even after exponential growth in this group of bacteria (Ramchander Merugu, 2010) confirms their findings that 3-ketothiolase cannot be completely inhibited by increases in CoASH as production was seen even after exponential phase.

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