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Research Article

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Optimization of Polyhydroxybutyrate production by two phototrophic bacteria *Rhodobacter capsulatus* KU002 and *Rhodopseudomonas palustris* KU003

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ABSTRACT

Purple Non-Sulphur bacteria were isolated from tannery effluents. Among these two strains, Rhodobacter capsulatus KU002 and Rhodopseudomonas palustris KU003 were studied for the production of Polyhydroxybutyrate (PHB). Effect on different carbon and nitrogen sources on the production of PHB were tested and PHB accumulation was more in Glutamate, Malate and Yeast extract (GMY) medium in Rb.capsulatus whereas, Rps. palustris preferred succinate and Yeast extract (SY) medium. The maximum PHB produced was 313 mg/L for Rb. capsulatus and 242 mg/L for Rps. palustris. Rb. capsulatus was comparatively superior than Rps. palustris for the production of bioplastic and can be economically exploited for industrial production.

Keywords: *Rb. capsulatus, Rps. palustris*, Polyhydroxybutyrate, GMY, SY medium.

INTRODUCTION

Polyhydroxy butyrate (PHB) is an energy storage material synthesized by a great variety of bacteria. PHB was first seen in lipid inclusions in the cells of *Bacillus* [1] [12]. Brandl *et al*. (1991) [2] reported that *Rhodobacter sphaerodies* produced PHB as the major component (97%) while PHV (3%) was found under anaerobic light conditions. PHA production has been studied by Yigit *et al.* (1999)[3] [11] and Ali Hassan *et al.*(1996) [4][1] from the waste waters of sugar refineries and palm oil waste respectively. Acetate mediated PHA production was reported in *Rb. sphaerodies* S and *Rb. sphaerodies* IL 206 by Noparatnaraporn *et al.* (2001) [5] [8]. PHB synthesis and accumulation by *Rps. palustris* SP5212 was investigated by Mahuya *et al.*(2005)[6] [7]. Different carbon and nitrogen substrates were used to study poly-β-hydroxybutyrate accumulation and hydrogen evolution by *Rhodobacter sphaeroides* strain RV [7][4]. In this investigation, an attempt was made to procure PHB from two phototrophic bacteria isolated from tannery effluents and to study the effect of different cultural conditions on the production of PHB from these bacteria.

EXPERIMENTAL SECTION

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. Bacteria thus isolated were identified with the help of Bergey's manual of Systematic Bacteriology (1989)[8][3] . Tubes were inoculated with 1ml log phase cultures of two anoxygenic phototrophic bacteria and incubated at 30±2º C at 2000lux in fifteen ml screw cap tubes. Different carbon and nitrogen sources (Himedia, India) were added at the rate at 1gL-1. Influence of pH and incubation period on the production of PHB was also tested. 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 (Remi, India). The pellet was washed with diethylether. It was then assayed for PHB. PHB extracted by the above method was assayed by Law and Slepcky (1960) [9][5] method. PHB sample

was treated with 5 ml of concentrated H_2SO_4 and a placed in a boiling water bath for 20 min. After cooling absorbance was recorded at 236 nm on a UV-Vis spectrophotometer (Elico, India). Standard was run using poly hydroxy butyrate (Himedia, India).

RESULTS AND DISCUSSION

It can be seen from (**figure1**) that yield of PHB was the maximum on the sixth day of incubation. There was no production of polymer on the second day. Fourth and eighth day yield was moderate whereas on tenth day less amounts of PHB was produced. Same pattern of production was also observed in *Rps.palustris*. **Figure 2 and 3** shows that pH of 7.5 was most amenable for the production of the polymer, followed by pH 8.0. There was no production of polymer at pH 4.0, 4.5 and 9.0, but it was increasing trend in PHB production along with an increase in pH till pH 7.5 Moreover lack production of PHB was seen in between pH 4.0 and to 5.0 by *Rps. palustris*. Finally, there is no correlation between pH growth and PHB yield. Production of PHB from *Rb. capsulatus* showed that acetate followed by glucose and succinate produced maximum yield of PHB (**Table 1**). Malate, Fructose and lactate induced less production of the polymer. *Rps. palustris* media produce maximum yield of PHB containing citrate, glucose followed by succinate and acetate. Polymer production was less compared to *Rb. capsulatus*. Lactate followed by fructose and malate induced less amounts of polymer production. Although there is some controversy regarding the influences of carbon sources on PHB production, in this case it can be clearly seen that carbon sources did show some effect on the production of the polymer.

Table 1: Effect of carbon sources on PHB production by two purple Non-Sulphur phototrophic bacteria

Organism	Carbon source	Growth (O.D)	DCW (g/L)	PHB (mg/L)
	Acetate	1.084	1.7	206
	Malate	0.968	1.5	150
Rb.capsulatus	Succinate	1.146	1.8	163
	Lactate	0.942	1.5	78
	Glucose	0.816	1.3	136
	Fructose	0.884	1.5	102
Rps.palustris	Acetate	1.234	1.9	110
	Malate	1.022	1.6	73
	Succinate	0.985	1.6	115
	Lactate	0.962	1.5	23
	Glucose	0.924	1.5	120
	Fructose	0.856	1.4	47

DCW- Dry cell weight

Table 2: Effect of nitrogen sources on PHB production by two purple Non-Sulphur phototrophic bacteria

Organism	Nitrogen source	Growth (O.D)	DCW (g/L)	PHB (mg/L)
	Ammonium sulphate	1.236	1.9	148
	Asparagine	1.184	1.8	144
Rb. capsulatus	Glycine	1.066	1.7	141
	Tyrosine	0.825	1.3	67
	Glutamate	0.935	1.5	129
	Urea	0.796	1.3	14
	Ammonium sulphate	1.108	1.7	98
	Asparagine	0.942	1.5	78
Rps. palustris	Glycine	0.852	1.4	64
	Tyrosine	0.908	1.5	21
	Glutamate	1.112	1.7	51
	Urea	0.865	1.3	30

Table 3: Effect of different media on PHB production by two purple Non-Sulphur phototrophic bacteria

Medium	*Rb.capsulatus*			*Rps.palustris*		
	Growth (O.D)	DCW (g/L)	PHB (mg/L)	Growth (O.D)	DCW (g/L)	PHB (mg/L)
Bieble and Pfennigs	1.125	1.7	184	1.086	1.7	153
Glutamate, malate and Yeast Extract(GMY)	1.092	1.7	313	0.981	1.6	224
Glutamate, Acetate and Yeast extract(GAY)	1.006	1.6	182	1.128	1.7	171
Glutamate, Glucose and Yeast Extract(GGY)	0.988	1.6	197	1.112	1.7	156
Glutamate, Citrate and Yeast Extract(GCY)	0.924	1.5	165	0.886	1.4	132
Succinate and Yeast Extract (SY)	1.108	1.7	225	1.128	1.7	242

Fig 1: Effect of Incubation period on PHB production by two purple non sulphur phototrophic bacteria

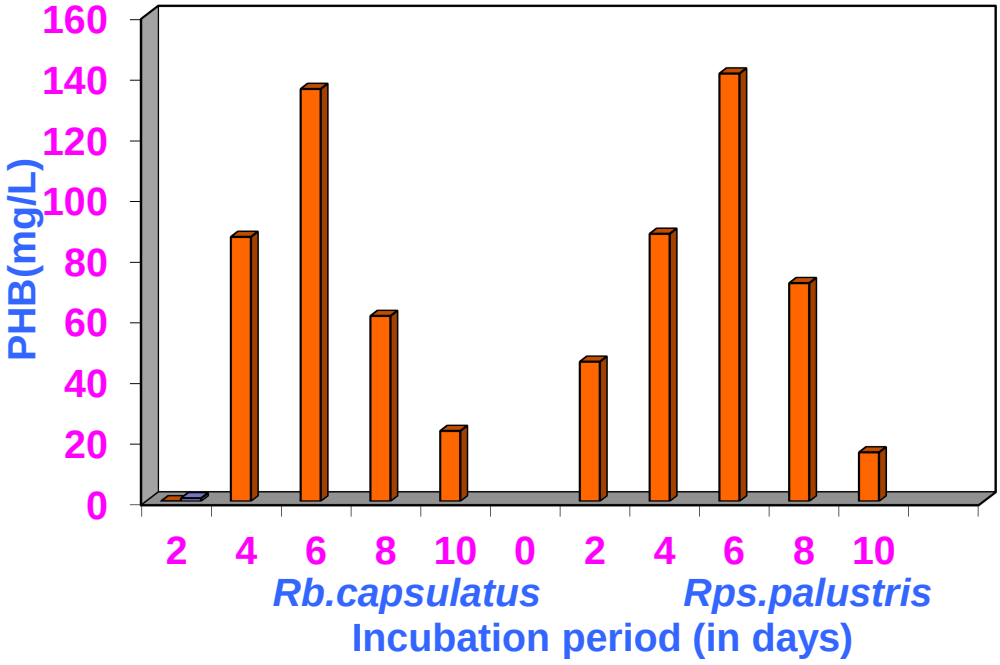
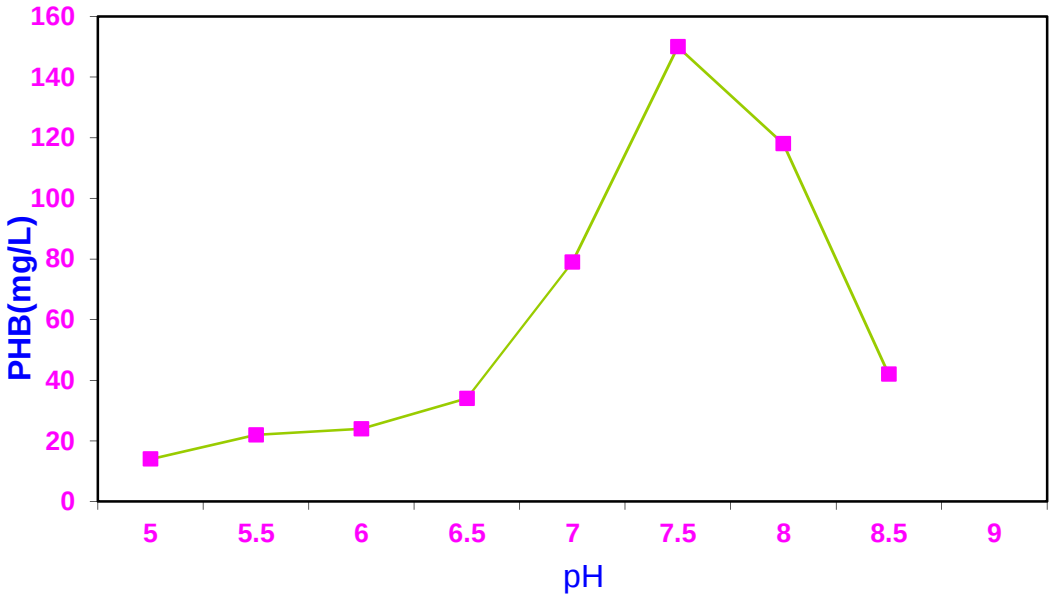


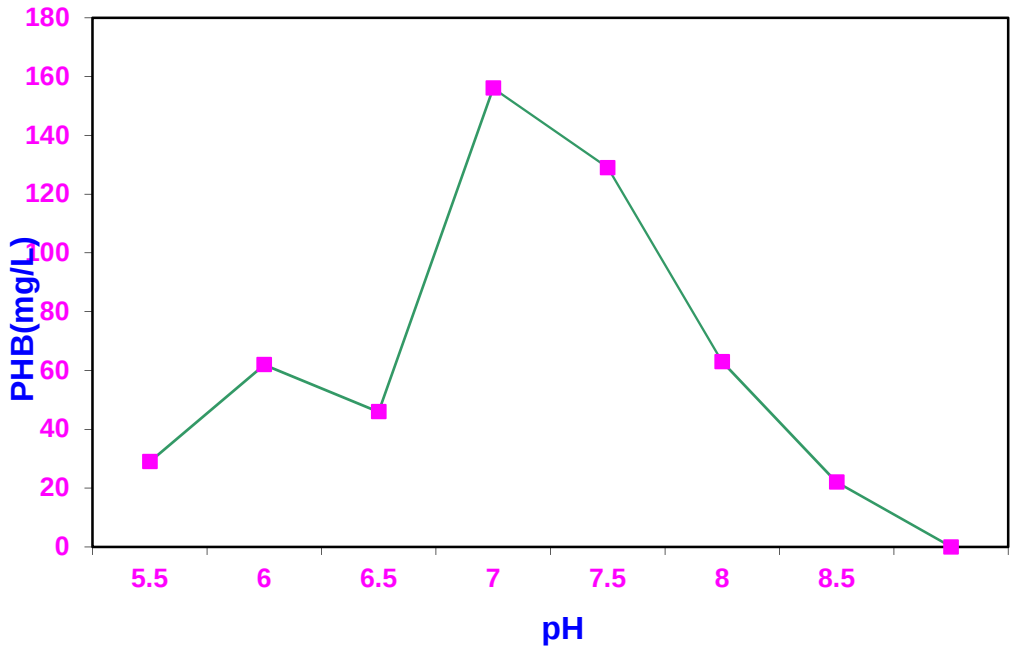
Fig 2 : Effect of pH on PHB production by *Rb.capsulatus*



Effect of nitrogen sources on PHB production showed that glutamate and asparagine induced the maximum production of PHB (**Table 2**). Polymer production was almost same in presence of glycine and ammonium chloride whereas urea induced lowest production of the polymer but presence of tyrosine induce moderate production of the polymer. Moreover, *Rps. palustris* produces maximum polymer in presence of ammonium chloride. Apparently, asparagine and glycine were induced moderate production of the polymer, less amounts of polymer were produced in the presence of tyrosine followed by urea and glutamate. Effect of different media (**Table 3**) showed maximum production of polymer was seen in Glutamate, Malate and Yeast Extract (GMY) medium by *Rb. capsulatus* whereas *Rps. palustris* preferred Succinate and Yeast Extract (SY) medium. PHB yield was comparatively good in GMY medium for both the organisms. It can be seen from the above that Glutamate, Malate and Yeast Extract (GMY) can be selectively used for the production of the polymer. Both these bacteria produced PHB during exponential phase which was similar to *Rhodobacter sphaeroides* ES 16 [10] [8], *A.latus* ATCC 29712 [11][10] and different from

Ralstonia eutropha which accumulated PHB at the stationary phase [12][6] (Madison and Huisman, 1999). *Rb.capsulatus* was superior when compared to *Rps.palustris* in the production of the polymer. Further studies using cheaper carbon and nitrogen sources are required. Effect of various nutrient limitations like nitrogen, phosphate and sulphur have been reported by our group earlier [13-20]. PHB production was more in Glutamate, Malate and Yeast extract (GMY) medium in *Rb. capsulatus* whereas *Rps. palustris* preferred succinate and Yeast extract (SY) medium. This could be exploited for the production of the polymer in a large scale. Furthermore, cheaper carbon and nitrogen sources should be investigated for the production of the polymer in a more economical way.

Fig 3 : Effect of pH on PHB production by *Rps.palustris*



REFERENCES

[1] M. Alihassan, Y. Shirai, N. Kusubayashi, M. Karim, K. Nakanishi and K. Hashimoto, *J. Biosci. Bioeng*, **1996**, **82**, 151–156.
[2] H. Brandl, R. Gross, R. Lenz, R. Lloyd and R. C. Fuller, *Arch. Microbiol*, **1991**, **155**, 337–340.
[3] J. Eds, T. Stanley, M. P. Byrant, N. Pfennig and J. C. Holt. Bergey's Manual of Systematic bacteriology (**1989**). "Enrichment and isolation of purple non sulphur photosynthetic bacteria".
[4] E. Khatipov, M. Miyake, J. Miyake, Y. Asada, *FEMS Microbiology Letters*, **1998**, **162 (1)**, 39–45.
[5] J. H. Law and R. A. Slepecky, *J. Bacteriol*, **1961**, **82**, 33-36 .
[6] L. L. Madison and G. W. Huisman, *Microbiology and Molecular Biology Review*, **1999**, 63, 21-53.
[7] M. Mikhopadhyay, A. Patra and A. K. Paul, *World. Journ. Microbiol. Biotechnol*, **2005**, 21, 765-769.
[8] N. Noparatnaraporn, K. Takeno and K. Sasaki. Hydrogen and polyhydroxyalkanoate production from organic acids by photosynthetic bacteria, p. 33–40. In Miyake, J., Matsunaga, T., and Pan Pietro, A. (ed.), Biohydrogen II. Pergamon, Elsevier Science, Amsterdam, London, New York (**2001**).
[9] Kanokphorn Sangkharak Poonsuk Prasertsan *Ejournal of Biotechnology*, 2008, **11**, 3.
[10] El-Sayed, A. Azhar, M. Hemmat. A. M. Abdelhady, A. Hafez and T. A. Khodair *Journal of Applied Sciences Research*, **2009**, **5(5)**, 556-564.
[11] D. O. Yigit, U. Gunduz, L. Turker, M. Yucel and I. Eroglu, *J. Biotechnol*, **1999**, **70**, 125–131.
[12] 1. F. D. Winfred and A. W. Robards, *J Bacteriol*, **1973**, **114**, 1271-1280
[13] Ramchander Merugu, S. Radarapu, D Jayasree, K Sambaru, S Manda. *Journal of life sciences research* 2 (2), 44-48
[14] Ramchander Merugu, MPP Rudra, S. Girisham, S.M Reddy. PHB *Int J Chem Tech Res* 4 (3), 1099-1102
[15] S Radarapu, M.Jayanthi, K. Sambaru, S. Manda, Ramchander Merugu. *Advanced Engineering and Applied Sciences: An International Journal* **2014**; 4(3): 40-43
[16] Ramchander Merugu, S. Girisham, S.M.Reddy (**2012**). *Advanced Biotech, 12*(06), 33-35.
[17] Ramchander Merugu, A.S.Rao, D.Ramesh, S. Girisham, SM. Reddy [**2012**]. *International Journal of ChemTech Research.* Vol. 4 Issue 3, p1103

[18] Ramchander Merugu (**2012**). *International Journal of Chemtech research*.4 (3), 1111-1113.
[19] Ramchander Merugu, S. Girisham, S.M.Reddy (**2010**). *International Journal of Applied Biology and Pharmaceutical Technology.*1(2): 676 to 678.
[20] Ramchander Merugu, S. Girisham, S.M.Reddy (**2010**). *International Journal of Applied Biology and Pharmaceutical Technology.* Volume: I: Issue-3: Nov-Dec -**2010** pp 847-850