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Determination of PHB Production in Different Growth Medium by *Cyanobacteria*

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Abstract: In this study, Poly-β-hydroxybutyrate (PHB) accumulation was investigated in different growth medium of 11 strain *Cyanobacteria* and was determined culture medium which yields the more economical for industrial use. These strains were grown in Bristol, BG11 and BG11 modified Allen's Medium aerated under continuous illumination by fluorescent lamps (3000 lux). Cell walls of the cultures were lysed by ultrasonication and PHB converted to crotonic acid by using sulfuric acid. The amount of crotonic acid was measured by UV spectrophotometry. The yield of PHB produced by the strains according to dry cell weight was ranged between 10.80-65.00% in BG11 medium and 11.89-85.45% in Allen's medium. In Bristol medium, PHB accumulation is not determined. The highest yield of PHB production (*Oscillatoria limnosa* 1966/1380: 85.45%) was found in Allen's medium. Allen's medium and *O. limnosa* 1966/1380 is recommended for PHB production.

Key words: *Cyanobacteria*, culture condition, Poly-β-hydroxybutyrate

INTRODUCTION

Poly-β-Hydroxybutyrate (PHB) is a biopolymer found exclusively in prokaryotes, in which it acts as a reserve of carbon and energy. PHB is a polyester of D(-)-3-hydroxybutyric acid and important from an environmental and industrial point of view; recent research has focussed on the possible exploitation of this polymer as a source for biologically degradable plastic products[1].

Several bacteria are able to accumulate PHB in high amounts; as much as 70% of dry weight in *Alcaligenes entrophus*[1]. This polymer has also been identified in some species of *Cyanobacteria*[2]. In many *Cyanobacteria*, a small amount of poly-β-hydroxybutyrate has been found and chemically identified. Electron microscopy revealed that the shapes of PHB granules are very similar to those in PHB accumulating bacteria[3]. The slightly electron dense granules, generally spherical in shape, are surrounded by a single membrane[4].

In *Cyanobacteria*, PHB was first detected in *Chlorogloea fritschii* where in the presence of a reduced carbon source, it constitutes up to 10% of dry weight. The polymer has been identified in other *Cyanobacteria*, either ultrastructurally *Chlorogloea fritschii*, *Aphanocapsa* 6308 and *Anacysits cyanea* or biochemically *Spirulina* sp.[1] .

The present study investigated the abilities to produce PHB of some *Cyanobacteria* in various culture mediums which yields the more economical benefits for industrial use.

MATERIALS AND METHODS

Six strains belonging to *Cyanobacteria* (*Chrococcus* sp. M1, *Chrococcus* sp. K23., *Oscillatoria* sp. F12, *Oscillatoria* sp. B4, *Oscillatoria* sp., M5, *Anabaena* sp. A32) used in this study have been isolated from the different lakes in Turkey. The other strains (*Synechocystis aquatilis* 1965/426, *Oscillatoria limnosa* 1966/1380, *Pseudoanabaena galeatea* 1967/13, *Pseudoanabaena galeatea* 1967/2, *Pseudoanabaena* sp. 1987/15) have been obtained from Scientific Academy of Check Republic, Culture Collection of Botanic Institute. These strains have been incubated for 18 days under room temperature and 3000 lux fluorescent light in BG11, modified Allen's and Bristol medium[5].

Determination of PHB: Determination of the amount of PHB was performed chemically. Bacteria were inoculated with a 1% (v/v), for 14 days in growth medium at room temperature. Suspensions of cultures were centrifuged at 6000 rpm for 30 min. The pellets were dried for 24 h at 100°C. Then total bacterial dry weight was determined. The pellets were suspended in sterile water (5 mL) and homogenized. Bacterial cell walls were destroyed by using ultrasonic treatment (5 min). To 2 mL of the cell suspension was added 2 mL of 2 NHCL and heated to boiling temperature for 2 h in a

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water bath. The tubes were centrifuged 6000 rpm for 20 min and 5 mL of chloroform was added to precipitate. The tubes were left overnight at 28°C on a shaker at 150 rpm. The contents of the test tubes were centrifuged at 6000 rpm for 20 min, extracted with 0.1 mL of chloroform and were dried at 40°C. Five milliliter of concentrated sulfuric acid was added and the mixture was heated at 100°C in a water bath for 20 min. PHB crystals were converted into crotonic acid. The absorbance was measured at 235 nm in a UV spectrophotometer against a sulphuric acid blank. All experiments were repeated two times[6,7].

Statistical analysis: The correlation between cell dry weight (g L^{-1}) and PHB production (g L^{-1}) was determined according to Sperman's ρ correlation coefficient test. The ρ value was estimated with the formula and explained using Conover's table[8].

$$\rho = 1 - \frac{6\sum (x_i - y_i)^2}{n(n^2 - 1)}$$

RESULTS AND DISCUSSION

Recently, Poly-β-hydroxybutyrate (PHB) has also been identified in some species of *Cyanobacteria*[2]. PHB is accumulated under conditions of nutrient deprivation or excess reducing power or in the presence of excess acetate. However, the control of PHB accumulation in *Cyanobacteria* is poorly understood.

It was determined that *Synechococcus* sp. MA19 strain produced 33% PHB under light and BG11 medium and that little PHB synthase activity was detected in crude extracts from cell grown under nitrogen-sufficient condition and light but not dark conditions. For the production of PHB by *Chlorogloea fritschii*, it was necessary to supplement the medium with the reduced carbon compound sodium acetate[3]. But, Campbell *et al.*[9] showed that the PHB accumulated without the addition of reduced carbon compounds in *Cyanobacterium Spirulina platensis*.

According to this, *Cyanobacteria* were studied as to whether they accumulated PHB or not in their medium without any other material added to the medium. By this way a more economical and easier PHB supply could be actualized. In this study, PHB yields of *Cyanobacteria* species are compared according to dry weight between BG11 medium (0.017-0.075 g L^{-1}, percentage yields: 10.64-65.00%) and BG11 modified Allen's medium (0.031-0.094 g L^{-1} percentage yields: 11.89-85.45%) and it is found to that Allen's medium is more suitable for PHB production (Table 1 and 2). So far, in the studies made on PHB production, it is observed that BG11 medium has been used[10-12]. After suitable medium are determined PHB production, it is considered that studies have to be developed for more productive results that can contribute to production. As observed in this study, in both biomass and PHB production, Allen's medium is more suitable to be applied. The reason for this is that the amount of sodium nitrate is less in Allen's medium. As known, PHB synthesis in a *Cyanobacterium*, *Synechococcus* sp., strain MA19, is controlled at the enzyme level and is dependent on the C/N balance in the culture medium. The control involves at least two enzymes. The first enzyme is PHB synthase. The second enzyme was phosphotransacetylase which catalyzes the conversion of acetyl coenzyme A to acetyl phosphate. The activity was detected in crude extracts from nitrogen deprived cells[3].

The highest PHB production amount in BG11 medium is found in *Oscillatoria limnosa* 1966/1380 (0.075 g L^{-1}), while the highest PHB percentage yield is found in *Oscillatoria* sp. M5 (65.00%). While the highest PHB amount in BG11 modified Allen's medium is found in *Oscillatoria* sp. M5 (0.094 g L^{-1}), the highest PHB percentage yield is determined in *Oscillatoria limnosa* 1966/1380 (85.45%). Statistical analysis showed that there was no correlation between cell dry weight (g L^{-1}) and

Table 1: The production of PHB of the some *Cyanobacteria* strains in BG11 medium

Strains	Cell dry weight (g L^{-1})	PHB (g L^{-1})	PHB (%)
Chrococcus sp. M1	0.145±0.025	0.027±0.015	18.62
Chrococcus sp. K23	0.280±0.010	0.049±0.006	17.50
Oscillatoria sp. F12	0.049±0.002	0.017±0.001	34.69
Oscillatoria sp. B4	0.139±0.006	0.024±0.002	17.27
Oscillatoria sp. M5	0.080±0.010	0.052±0.015	65.00
*Oscillatoria limnosa*1966/1380	0.705±0.095	0.075±0.045	10.64
Anabaena sp. A32	0.310±0.010	0.053±0.011	17.09
Synechocystis aquatilis 1965/426	0.500±0.100	0.054±0.011	10.80
Pseudoanabaena galeatea 1967/13	0.555±0.005	0.064±0.010	11.53
Pseudoanabaena galeatea 1967/2	0.320±0.030	0.073±0.022	22.81
Pseudoanabaena sp. 1987/15	0.325±0.045	0.063±0.017	19.38

*Values are the means±standard deviations of duplicate measurements

Table 2: The production of PHB of the some *Cyanobacteria* strains in BG11 modified Allen's medium

Strains	Cell dry weight (g L^{-1})	PHB (g L^{-1})	PHB %
Chrococcus sp. M1	0.140±0.050	0.089±0.009	63.57
Chrococcus sp. K23	0.185±0.035	0.035±0.018	18.91
Oscillatoria sp. F12	0.145±0.445	0.064±0.011	44.44
Oscillatoria sp. B4	0.130±0.030	0.031±0.007	23.84
Oscillatoria sp. M5	0.132±0.040	0.094±0.029	71.21
*Oscillatoria limnosa*1966/1380	0.055±0.015	0.047±0.005	85.45
Anabaena sp. A32	0.100±0.010	0.040±0.015	40.00
Synechocystis aquatilis 1965/426	0.165±0.045	0.052±0.003	31.52
Pseudoanabaena galeatea 1967/13	0.370±0.000	0.044±0.007	11.89
Pseudoanabaena galeatea 1967/2	0.140±0.010	0.056±0.011	40.00
Pseudoanabaena sp. 1987/15	0.110±0.020	0.072±0.016	65.45

*Values are the means±standard deviations of duplicate measurements

PHB (g L^{-1}) content of the cultures. The ρ value was calculated as 0.127. When this value was compared with the critical value 0.127<0.527. According to this result, there is no correlation between cell dry weight and PHB contents of the *Cyanobacteria*.

To conclude, the difference has been determined between PHB production in both medium. *Oscillatoria* sp. M5 that revealed a maximum production in BG11 medium showed a higher PHB percentage yield in BG11 modified Allen's medium. Similarly, *Oscillatoria limnosa* 1966/1380 that revealed a maximum PHB yield in BG11 modified Allen's medium showed a low yield in BG11 medium. According to this finding, it is possible to put forward that the suitable medium for PHB production is BG11 modified Allen's medium and suitable strain is *Oscillatoria limnosa* 1966/1380. Moreover, it is considered that this strain can be used for PHB production in industry. Recently, *Cyanobacteria* and microalgae are used widely in industry.

REFERENCES

1. Siddiqui, P.J.A., B. Bergmanr, P.O. Björkman and E.J. Carpenter, 1992. Ultrastructural and chemical assesment of PHB in the marine *Cyanobacterium T. thiebautii*. FEMS Microbiol. Lett., 94: 143-148.
2. Vincenzini, M., C. Sili, R. Philippis, A. Ena and R. Materassi, 1990. Occurance of poly-β-hydroxybutyrate-rate in *Spirulina* sp. J. Bacteriol., 172: 2791-2792.
3. Miyake, M., K. Kataoka, M. Shirai and Y. Asada, 1997. Control of poly-β-hydroxybutyrate synthase mediated by acetyl phosphate in *Cyanobacteria*. J. Bacteriol., 179: 5009-5013.
4. Allen, M.M., 1984. *Cyanobacteria* cell inclusion. Ann. Rev. Microbiol., 38: 1-25.
5. Becker, E.W., 1995. Microalgae, Biotechnology and Microbiology. Press Syndicate of the University of Cambridge, USA.
6. Bonartseva, G.A. and V.L. Myshkina, 1985. Fluorescence intensity of strains of nodule bacteria (*Rhizobium meliloti* and *Rhizobium phaseoli*) differing in activity, grown in the presence of the lipophilic vital stain phosphine 3R. Microbiology, 54: 535-541.
7. Aslim, B., F. Calşkan, Y. Beyatli and U. Gündüz, 1998. PHB production by lactic acid bacteria. FEMS Microbiol. Lett., 159: 293-297.
8. Conover, W.J., 1971. Practical Nonparametric Statics. John Wiley and Sons, New York.
9. Campbell, J., J.S.E. Stevens and D.L. Balkwill, 1982. Accumulation of poly-β-hydroxybutyrate in *Spirulina platensis*. J. Bacteriol., 149: 361-363.
10. Asada, Y., M. Miyake, J. Miyake, R. Kurane and Y. Tokiwa, 1999. Photosynthetic accumulation of poly-(hydroxybutyrate) by *Cyanobacteria*-the metabolism and potential for CO_2 recycling. Intl. J. Biol. Macromolecules, 25: 37-42.
11. Du, G., J. Chen, J. Yu and S. Lun, 2001. Continuous production of poly-3-hydroxybutyrate by *Ralstonia eutropha* in a two-stage culture system. J. Biotechnol., 88: 59-65.
12. Miyake, M., K. Takasa, M. Narato, E. Khatipov, J. Schnackenberg, M. Shirai, R. Kurane and Y. Asada, 2000. Poly hydroxybutyrate production from carbon dioxide by *Cyanobacteria*. Applied Biochem. Biotechnol., 84-86: 991-1002.