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Production of Poly-beta-hydroxybutyrate (PHB) in Different Media by *Streptococcus thermophilus* Ba21S Strain

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Abstract

In this study, accumulated poly-beta-hydroxybutyrate (PHB) was determined in lactic acid bacteria belonging to the genera *Lactobacillus, Lactococcus* and *Streptococcus*. PHB production by these strains was determined by the spectrophotometric method. The yield of PHB produced by *Lactobacillus* species was 0.93-9.00% w/v. The values for *Lactococcus* and *Streptococcus* species were 7.09-16.00 and 5.47-21.15% w/v, respectively. Generally, *Lactococcus* species produced more PHB than the other bacteria species. The highest PHB production and productivity percentage was found in *S. thermophilus* Ba21S (21.15% w/v). The amount of PHB produced by this strain in different carbon and nitrogen sources was analyzed. In this strain, sucrose was the most efficient carbon sources for the production of PHB compared with other carbon (glucose, lactose, sucrose, pyruvate) and nitrogen (protease peptone, L-glycine, L-cysteine, and $(NH_4)_2SO_4$) sources.

Key words: poly-beta-hydroxybutyrate (PHB), different carbon and nitrogen sources, *Lactobacillus, Lactococcus* and *Streptococcus*

INTRODUCTION

Polyhydroxyalkanoates (PHAs) are natural biodegradable polymers which are synthesized and accumulated intracellular during unbalanced growth by a large variety of bacteria. PHAs are similar to synthetic polymers in their physical properties, but unlike the latter they can be fully degraded by microorganisms. One such polymer is poly (beta-hydroxybutyrate) (PHB) which is of considerable technical interest because it is biocompatible and biodegradable [1].

PHB is thermoplastic polyester. In the cell, PHB is an intracellular storage material synthesized during unbalanced growth conditions [2]. A wide variety of microorganisms are known to accumulate PHB as an intracellular energy and carbon storage compound, usually when an essential nutrient (such as nitrogen) is limited in the presence of excess of carbon source. PHB is produced, for instance, in polyphosphate-accumulating organisms under controlled conditions of nutrients such as nitrogen, oxygen and/or minerals. The biosynthesis and degradation of PHB is a cyclical mechanism [3]. PHB synthesis is from acetyl coenzyme A (acetyl-CoA) and is mainly regulated by the availabilities of CoA and acetyl CoA. Furthermore, the attenuation of carbon flux in tricarboxylic acid (TCA) cycle can enhance pyruvate content of cell and facilitate PHB accumulation in *Alcaligenes eutrophus* [4]. During the excess of external carbon substrate, carbon uptake is mainly driven to PHB storage and, to a lesser extent, to biomass growth. After substrate exhaustion, PHB degradation starts with the depolymerization to beta-hydroxybutyrate monomers, which can then further be used as an energy and carbon source [5].

PHB-degrading microorganisms are ubiquitous in the environment and several extra cellular PHB depolymerases from *Alcaligenes* [6], *Bacillus* [7-8], *Rhizobium* [9] and *Pseudomonas* [10-11] have been isolated, purified and characterized. However, little information is PHB production from lactic acid bacteria [2-12]. Recent research has focused on the use of alternative substrates, novel extraction methods, genetically enhanced species and mixed cultures with a view to make PHB more commercially attractive.

The aim of the present research was to determine PHB production by different genera of lactic acid bacteria and to tested for further PHB production in different nitrogen and carbon sources.

MATERIALS AND METHODS

Bacterial strains, media and growth conditions

The species of *Lactobacillus*, *Lactococcus* and *Streptococcus* used in this study were obtained from the culture collection of the Biotechnology Laboratory of Gazi University, Department of Biology Faculty of Arts and Science, in Turkey. Lactobacilli were inoculated in MRS broth, while streptococci and lactococci were inoculated in M17 broth (all media provided by Merck). All of the strains were stored at –80°C in MRS/M17 broth with 10% glycerol, and regenerated twice before use in the manipulations.

Determination of PHB

Determination of the amount of PHB was performed chemically. Bacterium was grown on appropriate broth medium at appropriate temperature for 48 h on a shaker and biomass was obtained by centrifugation (6000 rpm, 45 min). Cells were lysed by ultrasonication and PHB was converted to crotonic acid by using sulfuric acid. The amount of PHB was determined on a UV/VIS spectrophotometer (Hitachi U- 1800), wavelength 235 nm [2-9].

Effect of Production of PHB in Different Carbon and Nitrogen Sources

PHB productions of strain showing the highest PHB production were determined in different carbon and nitrogen sources. Yeast extract was taken out, and carbon sources (glucose, lactose, sucrose and pyruvate, 1% w/v) were added into M17 broth medium. Peptone in M17 broth medium was taken out, and nitrogen sources (Protease peptone, L-glycine, L-cysteine, and $(NH_4)_2SO_4$, 1% w/v) were added. Nitrogen and carbon sources were sterilized by Millipore filter with a pore size of 0.45 μm.

Statistical analysis

Statistical analysis was performed by SPSS (Version, 10.0). The correlation between bacterial cell dry weight (g/L) and PHB production (g/L) of the bacteria was determined according to Spearman's correlation.

RESULTS

A total of 28 strains of lactobacilli, lactococci and streptococci strains were determined for the production of PHB in MRS/M17 medium. PHB produced by the strains is exhibited in Table 1.

The highest PHB production and productivity percentage were found in *S. thermophilus* Ba21S (0.092 g/L, 21.15% w/v, respectively) strain. The lowest PHB production was obtained in *Lb. brevis* B-40 (0.036 g/L) strain, while the low PHB productivity percentage *Lb. lactis* Ll2 (0.93% w/v) strain. Also, it was investigated whether any relationship between the dry cell weight and PHB production existed. Statistical analysis showed that there was no correlation between cell dry weight (g/L) and PHB (g/L) content of the cultures (q = 0.099).

PHB production of *Streptococcus thermophilus* Ba21S showing the highest PHB production was determined in different carbon and nitrogen sources. The results are indicated in Table 2. The strain produced PHB in M17 containing each of the sugars tested: pyruvate was the poorest carbon sources (2.95%), and sucrose was by far the most efficient carbon source (32.56%). The amount of PHB produced by this strain in different nitrogen sources was lower than the amount of PHB production in M17 broth medium (control) (Table 2).

Tablo 1. PHB production by some *Lactobacillus, Streptococcus* and *Lactococcus* species.

Strains	Cell dry weight (g/L)*	PHB[a] (g/L)*	PHB[b] (%)
Lb. acidophilus L-60	1.040±0.050	0.049±0.013	4.71
Lb. helveticus Hy1L	0.955±0.155	0.086±0.017	9.00
Lb. helveticus Hy2L	2.400±0.020	0.079±0.033	3.29
Lb. helveticus Hy5L	1.965±0.225	0.073±0.027	3.72
Lb. brevis B-40	1.895±0.065	0.036±0.018	1.89
Lb. brevis Hy20L	2.095±0.065	0.081±0.030	3.86
Lb. casei C-20	3.690±0.220	0.087±0.004	2.36
Lb. casei Lc6	3.790±0.160	0.066±0.016	1.74
Lb. casei Hy6L	2.200±0.020	0.050±0.010	2.27
Lb. lactis Ll2	5.365±0.535	0.050±0.007	0.93
Lb. lactis Hy17L	1.100±0.100	0.059±0.021	5.36
Lb. fermentum F-15	3.200±0.18	0.050±.0010	1.56
Lb. plantarum ATCC 20246	4.935±0.375	0.061±0.007	1.24
Lb. plantarum Lp7	4.000±0.520	0.088±0.009	2.20
Lb. plantarum Lp21	3.350±0.200	0.095±0.029	2.84
Lb. plantarum P-30	3.860±0.150	0.130±0.010	3.38
S. thermophilus Ba21S	0.435±0.075	0.092±0.029	21.15**
S. thermophilus Zm5S	0.595±0.030	0.047±0.005	7.89
S. durans Zm7S	0.895±0.020	0.049±0.000	5.47
Lac. diacetylactis P-90	0.425±0.035	0.068±0.016	16.00
Lac. cremoris Zm8S	0.575±0.015	0.087±0.018	15.13
Lac. cremoris Zm14S	0.590±0.060	0.075±0.026	12.71
Lac. cremoris Zm21S	0.560±0.040	0.074±0.010	13.21
Lac. cremoris TO(2-2)	0.620±0.050	0.044±0.004	7.09
Lac. lactis TO(1-1)	0.495±0.025	0.052±0.014	10.51
Lac. lactis Zm1S	0.565±0.045	0.058±0.018	10.27
Lac. lactis SL2	0.615±0.035	0.073±0.015	11.87
Lac. lactis SL24	0.600±0.100	0.074±0.004	12.33

* Values are the means ± standard deviations of duplicate measurements.

a Determined at dry cell weight. b According to dry cell weight.

** The highest PHB production.

Table 2. Production of PHB of the *S. thermophilus* Ba21S strain in media with different carbon and nitrogen sources

Carbon Sources	Cell dry weight (g/L)	PHB (g/L)	PHB (%)
Glucose	0.385±0.015	0.048±0.002	12.47
Lactose	0.240±0.005	0.070±0.004	29.17
Sucrose	0.215±0.000	0.050±0.006	32.56*
Pyruvate	0.880±0.060	0.026±0.009	2.95
Nitrogen sources			
Protease peptone	0.175±0.025	0.037±0.004	21.14
L-glycine	0.215±0.095	0.034±0.006	15.81
$(NH_4)_2SO_4$	0.160±0.07	0.011±0.002	6.88
L-cysteine	1.030±0.01	0.038±0.012	3.69
Control (M17)	0.435±0.075	0.092±0.029	21.15

*The highest PHB production

DISCUSSION

Species from more than 50 genera are known to be capable of synthesizing PHB [2]. The thermoplastic properties of the polymer and its biodegradability determine its importance as a substitute for petrochemical plastics [10]. Different amounts of PHB were produced by the strains studies; however, PHB levels were 0.93 to 21.15% in MRS/M17 medium. In general, the amount of PHB produced by some *Lactococcus* species was higher than that produced by *Lactobacillus* and *Streptococcus* strains (Table 1). In contrast, Aslim *et al* [2] mentioned that the amount of PHB produced by some *Lactobacillus* species was higher than that produced by *Lactococcus, Pediococcus* and *Streptococcus* strains.

Some of the *A. eutrophus* strains used for commercial PHB production have a PHB concentration which is approximately 80% (w/w) of the dry cell weight [13]. Chen *et al* [14] studied PHA in 11 different *Bacillus* spp. and found PHB consisting 50% (w/v) of dry cell weight of the bacteria. Our previous research [12] showed that PHB yields (%) accumulated in cells according to dry weight were also different: 0.52% for *Lactobacillus acidophilus* Z1L, 4.32% for *Lb. helveticus* Z2L, 10.00% for *Lb. bulgaricus* Z18L, 12.57% for *Lb. lactis* Z16L, 12.84% for *Lb. plantarum* Z11L, 4.71% for *Lb. brevis* Z20L, 15.14% for *Lactococcus lactis* SL7, 6.75% for *Lac. cremoris* Z14S and 6.52% *Streptococcus thermophilus* Z5S. In one of the studies conducted by Aslim *et al* [2], it was reported the production of PHB by some lactic acid bacteria and found the highest value of PHB was 35.80% (w/v) (for *Lb. bulgaricus* C8) for dry cell weight. When compared to related literature, our results show a lower PHB production. The differences above, we think, were resulted from different strains, types of medium and cultivation method used in individual study.

PHB production of Ba21S strain showing the highest PHB production was determined in different carbon and nitrogen sources. The type of carbon source has a huge influence on PHB productivity. When pyruvate was used as carbon sources, PHB production dramatically decreased (2.95% w/v). In *S. thermophilus* strain Ba21S, sucrose, lactose and glucose produced the yield of PHB of 32.56, 29.17, 12.47% w/v, respectively (Table 2). It can be seen that there was a clear increase in the amount of PHB of medium with sucrose and lactose as compared with M17 medium (control). Several factors influence the economics of biodegradable polymer production. Such factors include substrate cost and the ability to produce biodegradable polymers from inexpensive or renewable substrate [10]. The carbon source was always provided in excess to allow maximum PHB accumulation in the biomass. However, under non-optimized conditions, the total accumulation of PHB in *P. pseudoflava* never exceeded 22% (w/w) of the biomass dry weight. The cessation of PHB accumulation in *Ralstonia eutrophus* seems to result from physical space limitation [15]. Some researchers reported that PHB and exopolysaccharide (EPS) are competing metabolites for the carbon source utilized by bacteria [2-10-16]. Carbon sources used in this study may be utilized in EPS production by Ba21S strain and favored EPS production rather than PHB production.

The amount of PHB produced by Ba21S strain in different nitrogen sources was lower than the amount of PHB production in M17 broth medium (control) (Table 2). Similar results were obtained by Gokcen *et al* [10]. They have reported that the *Pseudomonas cepacia* G13 strain was examined for PHB production in different nitrogen sources. The amount of PHB produced by this strain in these medium were lower than the amount of PHB production in nutrient broth medium. Xi *et al* [11], determined that PHB synthesis was highly dependent on the nitrogen source. PHB synthesis by *Pseudomonas oleovorans* grown was not significantly stimulated by nitrogen limitation, but *P. resinovorans* responded to nitrogen limitation by greatly increasing the PHB production rate [17]. These observations corroborate our results.

When compared to the values reported in the literature, the amount of PHB accumulated in lactic acid bacteria was generally lower than that accumulated by the soil bacteria *Ralstonia eutropha* [15], *Bacillus* species [7], *Pseudomonas cepacia* [10] and *Rhizobium meliloti* [16]. *S. thermophilus* Ba21S may be an attractive candidate for use production of biodegradable plastics, but further research is needed to determine that Ba21S strain has the potential for commercial production of biodegradable polymers.

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