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Poly (3-hydroxybutyrate) production from glycerol by marine microorganisms

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Abstract

Poly(3-hydroxybutyrate) (PHB)-producing bacteria generally store the PHB material as a response to nutrients imbalance i.e., the limiting of nitrogen, sulfur, phosphorus or oxygen, with an excessive carbon source. PHB production cost is relatively high due to the raw material cost. Therefore, using glycerol, a by-product from biodiesel production plant, is an economical way to solve this problem. The purpose of this study was to use glycerol as carbon source to produce PHB by marine microorganisms (M26, M27, S3, S6, and S16) that were screened and isolated from marine sediment at Samut Sakhon and Samut Prakan province, Thailand. The capability of these strains to synthesize PHB from glycerol concentration ranging from 0.0% to 6.0% (v/v) was studied. Strain M27 shows the maximum PHB content (15.87%) at 6% (v/v) glycerol concentration after 36 h shaking at 120 rpm at 37 °C. The extracted polymers were identified as PHB using ^{1}H NMR and FTIR analysis. The results from this study can be further investigated to reduce cost of PHB production.

Keywords: Poly-ß-hydroxybutyrate (PHB), production, marine microorganism

1. Introduction

Petrochemical-derived plastics have become an integral part of contemporary life because of their many desirable properties including durability and resistance to degradation. Recently, issues concerning the global environment and solid waste management have created much interest in the development of biodegradable plastics such as poly-β-hydroxybutyrate (PHB) (1). PHB is a biopolyester that can be accumulated as an intracellular carbon-energy storage source by various bacterial strains under conditions of nutrient shortage such as nitrogen, phosphate, sulphur or oxygen (2). Of all bacterial diversities in the marine environment, *Vibrio natriegens* possesses a rapid growth with the shortest generation time (9.8 min) and be able to accumulate PHB (3). However, PHB production cost is high which limit their industrial application (2). Inexpensive fermentable raw materials such as glycerol, an abundant by-product of the biodiesel industry, have emerged to be promising

carbon sources for industrial fermentations (4). The production of PHB using various bacteria with glycerol as substrate is presented in Table 1. In this study, we used glycerol as carbon source to produce PHB by marine microorganisms. The extracted polymers from marine microorganism were identified as PHB using ^{1}H NMR and FTIR analysis (Table 1).

Table 1. Production of PHA by various bacteria

Microorganism	Carbon source	% (v/v)	%PHB	Reference
strain M26	glycerol	4.5	15.57	This study
strain M27	glycerol	6.0	15.87	This study
strain S3	glycerol	3.0	10.87	This study
strain S6	glycerol	1.5	7.38	This study
strain S16	glycerol	0.0	10.34	This study
Cupriavidus necator DSM 545	commercial glycerol	-	62	(5)
Osmophilic organism	waste glycerol	-	49.6	(6)
Methylobacterium rhodesianum MB 126	commercial glycerol	-	50	(7)
Ralstonia eutropha DMS 11348	commercial glycerol	-	65	(7)
Cupriavidus necator JPM134	commercial glycerol	-	70	(8)
Cupriavidus necator JPM134	waste glycerol	-	48	(8)
E. coli CT1061	commercial glycerol	-	51	(9)
E. coli (ATCC:PTA–1579)	commercial glycerol	-	60	(10)
Bacillus thuringiensis R1	Glycerol	1	64.10	(11)
Vibrio spp.	glycerol	1.0	24-43	(3)
Vibrio natriegens M11	glycerol	1.0	41	(3)
Mixed microbial consortia	crude glycerol	-	45-62	(12)

2. Materials and Methods

2.1 Microorganisms

Throughout this work, the marine microorganisms (M26, M27, S3, S6, and S16) were screened and isolated from Samut Sakhon and Samut Prakan province, Thailand. Their ability to synthesize PHB was determined using marine basal agar medium containing Nile red (0.5 μg ml^{-1}) and directly examined for fluorescence under ultraviolet light in order to detect the accumulation of lipid storage compounds including PHB (13).

2.2 System culture conditions

Seed culture was prepared in marine basal medium (MB) containing glycerol (3%, v/v), sea salts (28 g /l), tryptone (2.5 g/l) and yeast extract (1 g/l). The pH of media was neutralized with NaOH or HCl and the media were sterilized before use (3). The cultures were incubated at 37 °C with shaking at 120 rpm for 24 h.

2.3 Effects of different carbon sources on bacterial growth and PHB production

Seed culture was inoculated to 250 ml Erlenmeyer flask containing 50 ml of marine basal medium (MB) at 10% (v/v) (OD_{600} = 0.5). The production medium for the system was the same as described above with glycerol concentration ranging from 0.0% to 6.0% (v/v). The cultures were incubated at 37 °C with shaking at 120 rpm for 36 h.

2.4 Determination of cell dry weight and PHB concentration

Fermentation broth was centrifuged at 4,000 rpm for 15 min. The cell pellets were collected and washed twice with distilled water. The cells were dried at 80 °C until a constant weight was obtained.

PHB concentration was determined by the method developed by Shrivastav et al. (14).

2.5 Characterization of PHB

^{1}H NMR spectrum was recorded at 400 MHz for protons with 45° pulse using deuterated chloroform as solvent. FTIR studies were performed using a spectrophotometer from 400 to 4000 cm^{-1}. These methods were used for the characterization of PHB compared with the commercial PHB (Sigma-aldrich).

3. Results and Discussion

3.1 Effects of different carbon sources on bacterial growth and PHB production

Strains M26, M27, S3, S6, and S16 can synthesize PHB. The cell growth as determined by dry cell weight (DCW), PHB concentration as determined by above method, and the total PHB content, determined as the proportion of the bacterial DCW and PHB concentration were calculated. Fig. 1 shows the PHB production by marine microorganisms when grown on marine basal medium for 36 h at 37 °C. DCW of these strains (M26, M27, S3, S6, and S16) are similar and the highest PHB concentrations obtained are 0.48, 0.41, 0.37, 0.22 and 0.24 g/l at 4.5, 6.0, 3.0, 1.5, and 0.0% of glycerol concentration, respectively. Therefore, M27 strain can produce the highest %PHB content (15.87%) at 6.0% glycerol concentration and was selected for further study (Figure 1).

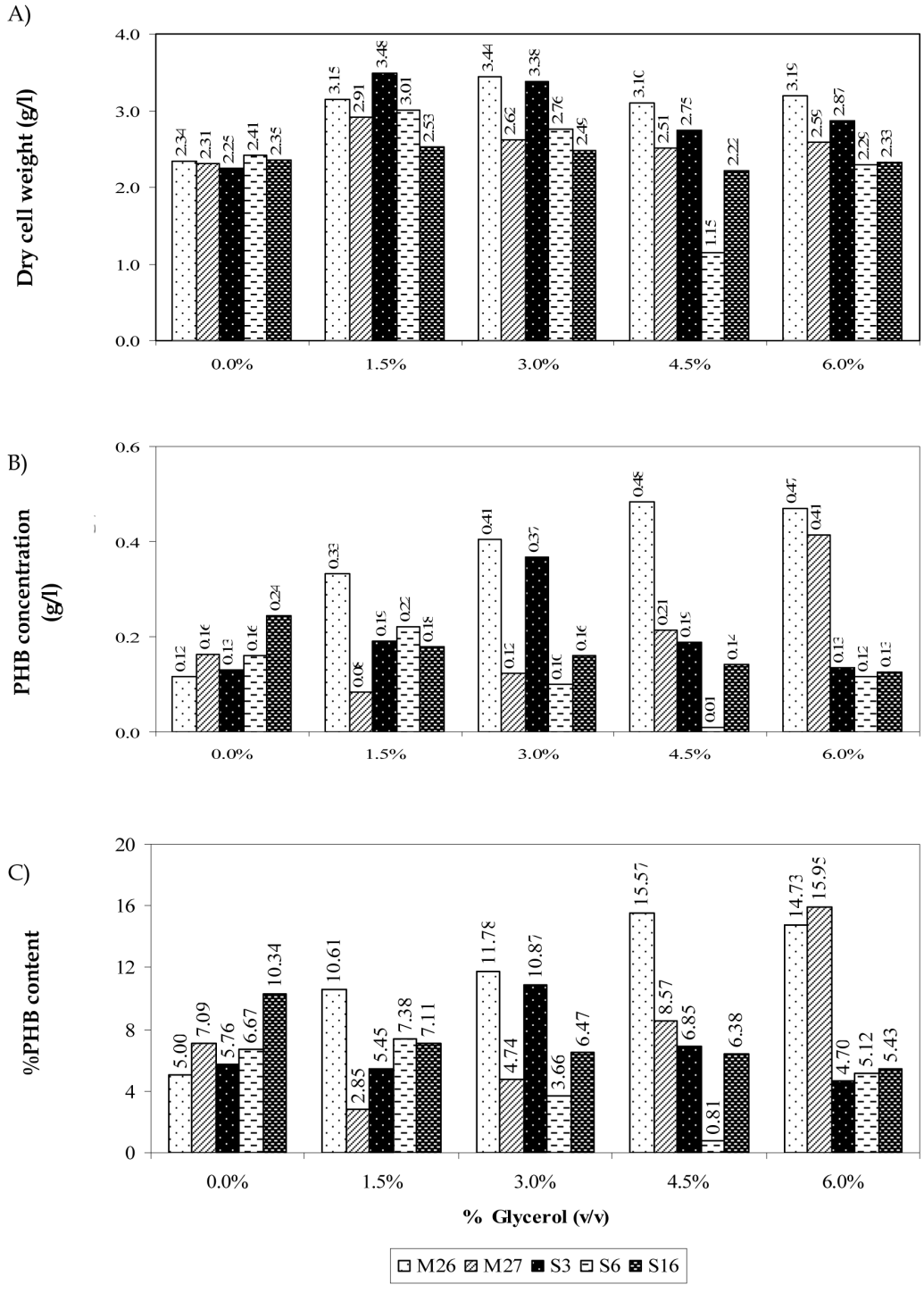

Figure 1. Production of PHB by marine microorganisms grown on marine basal medium in a rotary shaker at 120 rpm and 37 °C for 36 h. (A) Dry cell weight (DCW) (g/l), (B) PHB concentration (g/l) and (C) PHB content (%).

3.2 Characterization of PHB

The extracted polymer from M27 strain was identified its chemical structure as PHB using ^{1}H NMR and FTIR analysis. Figure 2 shows three peaks of the ^{1}H NMR spectra of PHB from M27 strain confirmed with the commercial PHB (data not shown). Peak positions at 1.2, 2.5 and 5.2 ppm represent the structure of the methyl (CH_3), methylene (CH_2) and methine (CH), respectively.

FTIR analysis of the polymer from M27 strain compared with the commercial PHB revealed absorption bands at 1724 cm^{-1}, corresponding to the ester carbonyl group of PHB as shown in Figure 3. The bands at 2978 and 2935 cm^{-1} were corresponded to the CH_3 and CH_2, respectively.

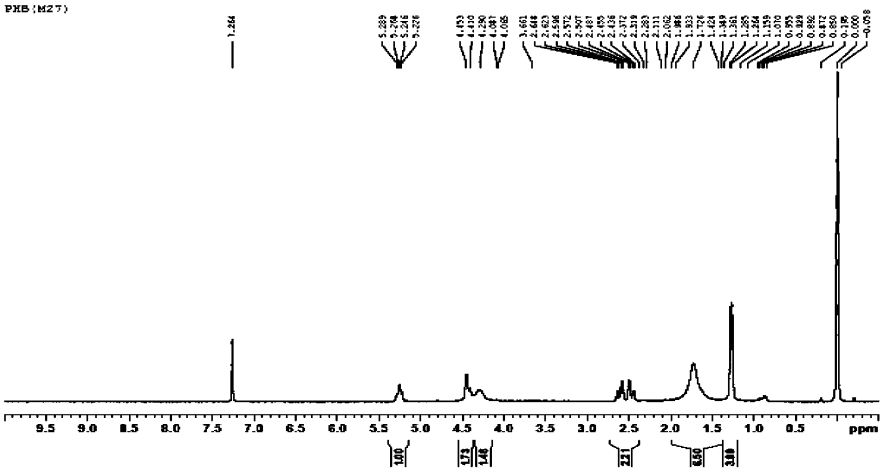

Figure 2. ^{1}H NMR spectra of the PHB synthesized by M27 strain with resonance signals of CH at 5.2 ppm, CH_2 at 2.5 ppm and CH_3 at 1.25 ppm

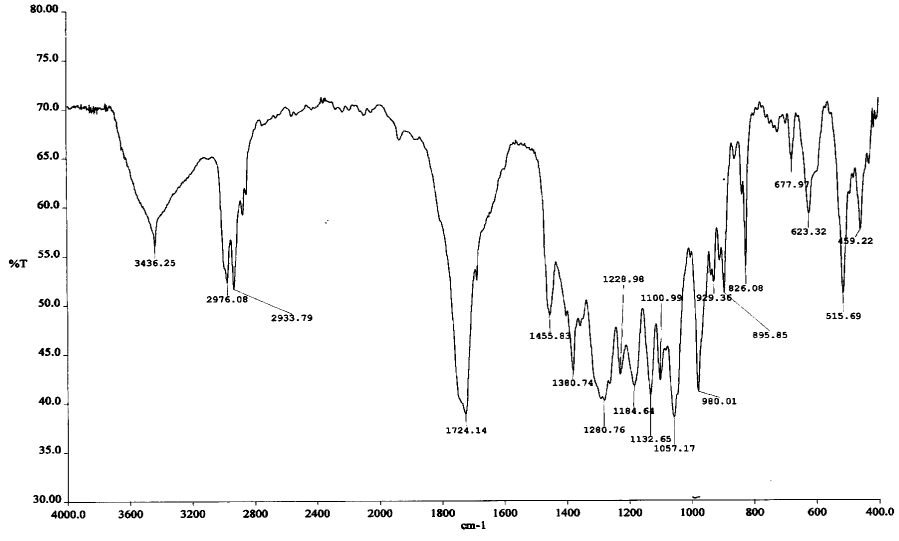

Figure 3. FTIR spectrum in the region 4000–400 cm^{-1} of the PHB produced by M27 strain.

4. Conclusion

The above results demonstrated that the five bacterium strains (M26, M27, S3, S6 and S16) were able to accumulate PHB. Strain M27 shows the maximum PHB content (15.87%) at 6% (v/v) glycerol concentration after shaking at 120 rpm, 37 °C for 36 h. Based on the characterization of the polymer produced by M27 strain through ^{1}H NMR, FTIR and comparison with the commercial PHB, it was observed that the polymer obtained from M27 strain has properties similar to that of the commercial PHB, so the polymer produced by the bacteria strain M27 is polyhydroxybutyrate (PHB). It would thus be of interest to further investigate to reduce PHB production cost by using the inexpensive carbon source for the industrial use.

5. Acknowledgment

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