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Short Communication

Production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) under photoautotrophy and heterotrophy by non-heterocystous N_2-fixing cyanobacterium

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HIGHLIGHTS

- Photoautotrophic PHBV biosynthesis exists in nature.
- PHBV production using carbon dioxide and solar energy.
- PHBV has increases in elasticity and elongation ability, relative to those of PHB.

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ABSTRACT

The photoautotrophically grown cyanobacterium *Oscillatoria okeni* TISTR 8549 was found to produce bioplastic poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV). This PHBV production occurred under nitrogen deprivation (–N) that yielded PHBV accumulation of 14 ± 4% (w/w DW) in which 3-hydroxyvalerate accounted for 5.5 mol%. The heterotrophically grown (–N condition with acetate supplementation) cells under light showed no increase of PHBV storage, but under dark condition these cells increased PHBV accumulation to 42 ± 8% (w/w DW) with 6.5 mol% of 3-hydroxyvalerate. Compared to poly-3-hydroxybutyrate (PHB), the PHBV from *O. okeni* had a lower melting temperature by 5–7 °C, a higher % elongation at break by 4–7 times and a greater Young's elastic modulus by 2.3–2.5 times.

1. Introduction

The microbial bioplastic poly-3-hydroxybutyrate (PHB) has tensile strength and thermal properties comparable to the petroleum-based plastic polypropylene; however, PHB has a much lower elongation of less than 6% length/length (Lee, 1996; Verlinden et al., 2007).

Superior levels of elongation and elasticity compared to those of PHB can be obtained from the co-polymer poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV hereafter), which comprises 3-hydroxybutyrate (HB) and 3-hydroxyvalerate (HV) monomers (Balaji et al., 2013; Verlinden et al., 2007). PHBV also melts at a lower temperature and exhibits less crystallinity relative to PHB (Lee, 1996; Verlinden et al., 2007). A wide variety of mole proportion (mol%) between HB and HV in PHBV resulted in diverse material properties suitable for various applications (Chanprateep, 2010; Lee, 1996).

PHBV has been commercially produced using heterotrophic bacteria that consume costly composite organic substrates (Chanprateep, 2010). Thus, a new PHBV producer that does not require organic substrates such as photosynthetic microbes is desirable. Still, PHBV production by phototrophic microbes has yet to be demonstrated.

Under photoautotrophy, several species of cyanobacteria produced PHB (Drosg et al., 2015; Kaewbai-Ngam et al., 2016; Koller and Maršálek, 2015). Increased PHB accumulation in cyanobacteria has been observed in cells deprived of combined inorganic nitrogen (–N) or phosphorus (–P) (Drosg et al., 2015;

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Kaewbai-Ngam et al., 2016; Koller and Maršálek, 2015). Previously, the photoautotrophic cyanobacterium *Oscillatoria limosa* was found to produce poly-3-hydroxyvalerate (PHV) (Stal et al., 1990). Nevertheless, production of the co-polymer PHBV by photoautotrophic cyanobacteria has yet to be described.

In this study, whether or not there is a cyanobacterial species capable of producing PHBV under photoautotrophy was investigated. The 137 strains of cyanobacteria were screened for bioplastic production under photoautotrophy (Kaewbai-Ngam et al., 2016). The selected *Oscillatoria okeni* TISTR 8549 (hereafter *O. okeni*) was found to produce PHBV. Here, PHBV production by *O. okeni* was monitored. PHBV was analyzed for chemical identity and material properties.

2. Materials and methods

2.1. Strain and culture conditions

The axenic *Oscillatoria okeni* TISTR 8549 was obtained from Thailand Institute of Scientific and Technological Research. The strain was isolated using ampicillin treatment (Sena et al., 2011). Approximately 5% (v/v) of a 12-d old culture was inoculated into BG-11 medium with the omission of citrate and supplemented with 20 mM HEPES-NaOH (pH 7.5) as described (Monshupanee et al., 2016). The composition of the BG11 medium is given in Supplementary information Table S1. For photoautotrophy, cells were grown under a continuous white light of 75 μmol/m^2/s at 32 °C supplied by atmospheric CO_2. The deprivation of combined nitrogen or phosphorus was obtained by omitting such nutrient as described (Monshupanee and Incharoensakdi, 2014). For heterotrophy, sodium acetate was supplied to the BG11 medium.

2.2. Analysis of bioplastic contents

Approximately 20 mg of dried biomass was subjected to methanolysis in 15% (v/v) sulfuric acid in methanol as described (Huijberts et al., 1994). The resulting 3-hydroxybutyric acid methyl ester and 3-hydroxyvaleric acid methyl ester were analyzed by gas chromatography as described (Huijberts et al., 1994). The benzoic acid was included in all samples prior to methanolysis as the internal standard. The commercial PHB and PHBV (12 mol% HV) from Sigma-Aldrich, St. Louis, MO, USA, were analyzed as samples for quantification references. PHB and PHBV contents were calculated as % weight of the polymer to cell dry weight (w/w DW). The HB/HV composition was reported as mol% of each monomer to the total mole of all monomers.

2.3. PHBV extraction, NMR and material property analyses

Dry cells were subjected to PHBV purification using chloroform extraction, followed by diethyl ether precipitation as described (Yellore and Desai, 1998). PHBV was analyzed for naturally occurring ^{1}Hydrogen and ^{13}Carbon by nuclear magnetic resonance (NMR) at 25 °C using the Bruker Advance 400 MHz spectrometer (Germany). Physical properties were examined using a material analyzing machine (Hounsfield H10KM, UK). Thermal properties were determined using differential scanning calorimetry (Netzsch DSC-204-F1, Germany). Polymer molecular weight was estimated

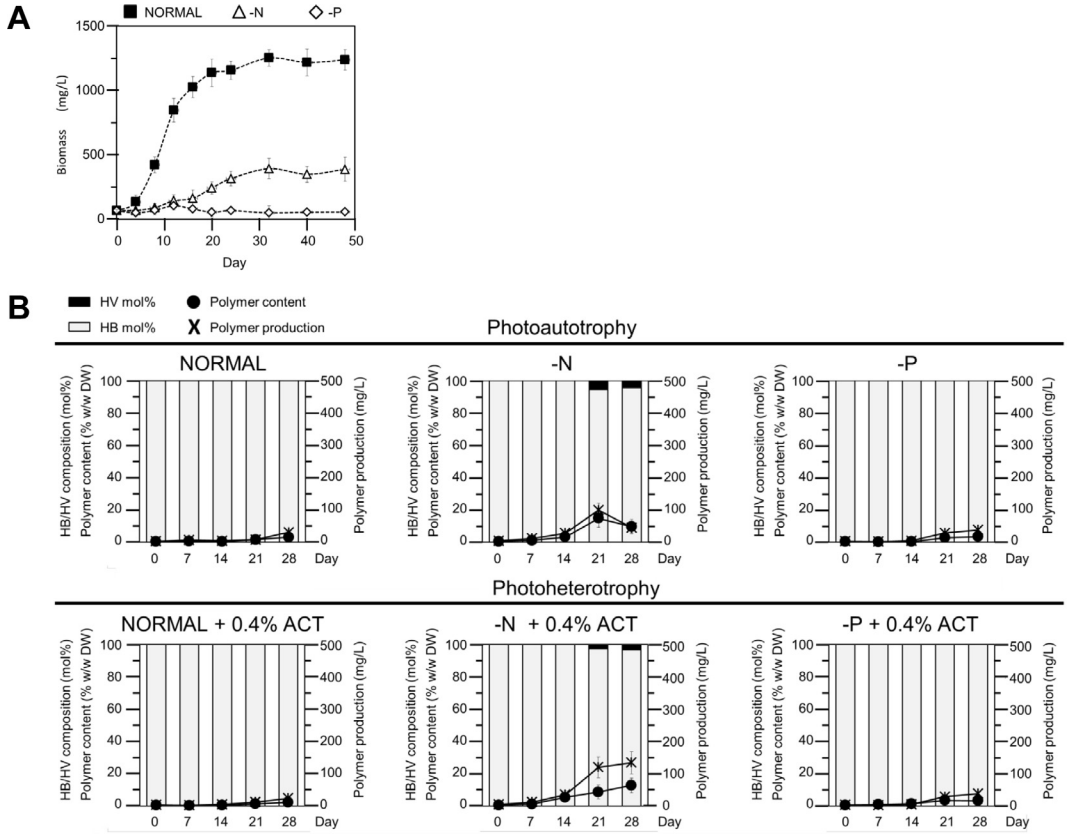

Fig. 1. (A) Photoautotrophic growth under normal nutrient condition (NORMAL), nitrogen deprivation (–N), or phosphorus deprivation (–P). (B) PHB and PHBV levels under photoautotrophy or photoheterotrophy (using 0.4% w/v acetate (ACT) supply). Twenty-day photoautotrophically-grown cells were transferred to the indicated nutrient conditions. Data are the mean ± SD derived from 3 to 5 independent experiments.