Effects of even and odd number fatty acids cofeeding on PHA production and composition in \textit{Pseudomonas putida} Bet001 isolated from palm oil mill effluent

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Abstract

The biosynthesis of medium-chain-length poly-3-hydroxyalkanoates by \textit{Pseudomonas putida} Bet001 cultivated on mixed carbon sources was investigated. The mixed carbon sources consisted of heptanoic acid (HA) and oleic acid (OA). A relatively low PHA content at 1.2\% (w/w) and 11.4\% (w/w) was obtained when HA or OA was used as the sole carbon source. When these fatty acids were supplied as a mixture, PHA content increased threefold. Interestingly, the mixture-derived PHA composed of both odd and even monomer units, namely, 3-hydroxyheptanoate, 3-hydroxyoctanoate, 3-hydroxydecanoate, and 3-hydroxydodecanoate and no unsaturated monomer was detected. It is hypothesized that the even-numbered monomers were derived primarily from OA, whereas the odd-numbered monomer was derived from HA. This also points out to an efficient and yet distinct fatty acids metabolism that fed the PHA biosynthesis machinery of this particular microorganism. PHA obtained was elastomeric because melting temperature ($T_m$) and crystallinity were absent. It showed good thermal stability with degradation temperature ($T_d$) ranging from 275.96 to 283.05 °C. © 2015 International Union of Biochemistry and Molecular Biology, Inc. Volume 63, Number 1, Pages 92–100, 2016

Keywords: heptanoic acid, oleic acid, odd and even monomers, poly-3-hydroxyalkanoates, \textit{Pseudomonas putida}

Abbreviations: CaCl$_2$·2H$_2$O, calcium chloride dehydrate; CDCl$_3$, deuterated chloroform; CDW, cell dry weight; CoCl$_2$·6H$_2$O, cobalt(III) chloride hexahydrate; CuCl$_2$·2H$_2$O, copper(II) chloride dehydrate; DCM, dichloromethane; DSC, differential scanning calorimetry; FeSO$_4$·7H$_2$O, iron(II) sulfate heptahydrate; FTIR, Fourier transform infrared spectroscopy; GC, gas chromatography; GC-MS/MS, gas chromatography-mass spectrometry; GPC, gel permeation chromatography; ΔH, enthalpy changes; HA, heptanoic acid; 3HB, 3-hydroxybutyrate; HCl, hydrochloric acid; 3HD, 3-hydroxydecanoate; 3HDDe, 3-hydroxydodecanoate; 3HDD, 3-hydroxydodecanoate; 3HHp, 3-hydroxyheptanoate; 3HHx, 3-hydroxyhexanoate; 3HN, 3-hydroxynonanoate; 3HO, 3-hydroxyoctanoate; 3HTD, 3-hydroxytetradecanoate; 3HV, 3-hydroxyvalerate; kDa, kilodalton; KH$_2$PO$_4$, monopotassium phosphate; K$_2$HPO$_4$, dipotassium phosphate; mcl-PHA, medium chain length-polyhydroxyalkanoate; MgSO$_4$·7H$_2$O, magnesium sulfate heptahydrate; $M_n$, number-average molecular mass; $M_w$, mass-average molecular mass; mM, millimolar; MnCl$_2$·4H$_2$O, manganese(II) chloride tetrahydrate; NaCl, sodium chloride; NH$_4$Cl, ammonium chloride; NMR, nuclear magnetic resonance; OA, oleic acid; PHA, polyhydroxyalkanoate; POME, palm oil mill effluent; PTFE, polytetrafluoroethylene; $T_d$, degradation temperature; $T_m$, melting temperature; TGA, thermogravimetric analysis; THF, tetrahydrofuran; TMS, tetramethylsilane; UV/Vis, ultraviolet – visible spectrophotometer; XRD, X-ray diffraction; ZnSO$_4$·7H$_2$O, zinc sulfate heptahydrate.

1. Introduction

Polymers are used in the manufacturing industry all over the world because of its strength, lightness, and durability [1]. Unfortunately, its nondegradable characteristic after disposal caused negative impacts to the environment [2]. In comparison with petroleum-based polymers [3–6], biodegradable polymers are receiving much attention because of their biodegradability [7, 8], compostability, and resorbability [9]. Among the biodegradable polymers, polyhydroxyalkanoates (PHA) have been shown to be biocompatible for biomedical applications [8, 10–14].

PHA is synthesized under limited essential nutrient condition [1, 15, 16] and under excess carbon source [17, 18]. The PHA composition depends on cultivation conditions as well as carbon substrate supplied [13, 19]. \textit{Pseudomonas} sp. is among