POLYHYDROXYALKANOATES - PRODUCTION STRATEGIES AND THEIR APPLICATION IN MEDICINE

JUSTYNA PRAJSNAR[1]*, JAKUB STAROŃ[2], ANDRZEJ BOJARSKI[2], MAŁGORZATA WITKO[1], MACIEJ GUZIK[1]

[1] JERZY HABER INSTITUTE OF CATALYSIS AND SURFACE CHEMISTRY POLISH ACADEMY OF SCIENCES, KRAKOW, POLAND
[2] MAJ INSTITUTE OF PHARMACOLOGY POLISH ACADEMY OF SCIENCES, KRAKOW, POLAND
*E-MAIL: JUSTYNA.PRAJSNAR@IKIFP.EDU.PL

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Introduction

Polyhydroxyalkanoates (PHA) are natural polyesters with unique properties, valuable for the environment, medicine, pharmacy and industry. They are a diverse group of biopolymers characterized by biodegradability and biocompatibility, and additionally, they are often made of industrial, petrochemical or municipal waste [1]. PHAs are most often produced by fermentation in the cells
of many strains of environmental bacteria. They are composed of (*R*)-3-hydroxy acids, which makes them an additional rich source of optically active compounds [2]. PHAs can be composed of over 150 different monomers, which makes them a reservoir of biopolymers with various properties. PHAs with up to five carbon atoms in their monomer molecule belong to the group of short-chain polyhydroxyalkanoates (scl-PHA). They have been used mainly in industry as biodegradable polymers for food packaging [3,4], but also as hard materials in tissue engineering, soluble sutures or bone implants. Medium chain length polyhydroxyalkanoates, mcl-PHAs (C6 – C14 carbon atoms) are biocompatible elastomers, therefore they are good materials for biomedical applications for soft tissues. They are also widely used as drug carriers [3,4]. Soft, hydrophilic aromatic polymers such as poly(3-hydroxy-5-phenylvalerate) (PHPV) have been used successfully to regenerate cortical neuronal cells [5]. In the presented research, two types of mcl-PHA polymers of medical importance are produced: aliphatic poly(3-hydroxynonanoate) (PHN) and aromatic PHPV by using two different biosynthesis strategies.

Materials and Methods

Two bacterial strains were used for the biosynthesis of polymers: *Pseudomonas putida* CA-3 (synthesis of aromatic PHA) and *Pseudomonas putida* KT2440 (synthesis of aliphatic PHA). PHPV synthesis was performed by pulse-fed flask cultures (400 ml of cultures in 2L flasks) in MSM medium supplemented with trace elements (TE), where the carbon source was the sodium salt of 5-phenylvaleric acid (40 mM). The flasks were incubated for 48 h at 30°C with shaking (250 rpm). Then, another portions of sodium salt of 5-phenylvaleric acid (40 mM) and trace elements were added to the culture and incubated under the same conditions for another 48h (limited amount of nitrogen in the medium step) [5]. The aliphatic polymer PHN was produced under fully controlled conditions in a 5L reactor (Biostat® B, Sartorius) using the *P.putida* KT2440 strain. The culture medium was also MSM with the addition of TE and $MgSO_4$ (0.2 gL^{-1}). The carbon source for the bacteria was a mixture of nonanoic acid and butyric acid (4: 1), fed automatically throughout the duration of the culture. The pH of the culture was kept constant at 6.9. Phosphate deficiency was the limiting factor in the medium. The cultivation was carried out for 30 h at 30°C with stirring and aeration. Both polymers were extracted with ethyl acetate from the resulting bacterial dry matter. Then the solvent was evaporated and the polymers were precipitated in cold methanol and dried. Biosynthesis efficiency, PHA content in bacterial dry matter and polymer purity were assessed by performing analyses using HPLC-MS/ MS (Agilent 1290 Infinity System with MS Agilent 6460 Triple Quad Detector) and GC (Varian CP-3800 with FID detector).

Results and Discussion

Two PHA polymers were obtained: aromatic PHPV and aliphatic PHN using two feeding strategies. The obtained results are shown in TABLE 1.

TABLE 1. Comparison of the pulse-fed flask reactor and fed-batch reactor feeding strategies for two bacterial strains (*P. putida* CA3 and *P. putida* KT2440) in the production of PHPV and PHN.

Strain	*P.putida* CA3	*P.putida* KT2440
Carbon source	sodium salt of 5-phenylvaleric acid	nonanoic acid/ butyric acid
Reactor type	pulse-fed flask reactor	fed-batch reactor
Limiting factor in MSM medium	nitrogen deficiency	phosphate deficiency
PHA content [%]	57%	71%
Polymer type	homopolymer	heteropolymer
Polymer composition [%]	**100%** of 3-hydroxy-5-phenyl valeric acid	**69%** of 3-hydroxy nonanoic acid, **31%** of 3-hydroxy heptanoic acid

According to the literature, *P. putida* strains, for which the carbon source during fermentation are salts of aromatic acids, produce a polymer consisting of only one type of 3-hydroxy aromatic acids [6]. Due to the nature of the degradation of aliphatic medium length chain fatty acids in the β-oxidation pathway in *P.putida* KT2440, co-polymers composed of 3-hydroxy acids and 3-hydroxy acids shorter by two carbons in acyl moiety are produced [7]. The use of both culture strategies for the strains resulted in a high PHA content in CDW.

Conclusions

The *P.putida* CA3 strain produces poly(3-hydroxy-5-phenylvalerate) homopolymer during fermentation. Pluse-fed flask culture with staged feeding allowed to obtain 57% PHPV content in CDW. *P.putida* KT2440 strain produces heteropolymer during fermentation in a fed-batch reactor, and its content in CDW is 71%.

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