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Original Article

# Effect of Various Carboxylic Acids on the Biosynthesis of Polyhydroxyalkanoate in *Pseudomonas aeruginosa* PA01

Kambiz Akbari Noghabi*, Hossein Shahbani Zahiri, Abbas Sahebghadam Lotfi, Mitra Shourian, Gholamreza Karbalaei, Iman Rad, Solmaz Ahadi, Mahmood Arabnezhad

*National Institute of Genetic Engineering and Biotechnology, Tehran, Iran*

**Abstract**

In the present study, four carbon sources were tested for the polyester synthesis of *Pseudomonas aeruginosa* PA01 which is a ubiquitous environmental bacterium and is one of the top three causes of opportunistic human infections. These included linear $C_8$ to $C_{11}$ carboxylic acids. Octanoic acid, nonanoic acid, decanoic acid and undecanoic acid were added to M1 minimal medium to final concentrations of 60, 40, 30 and 20 mM, respectively. When *P. aeruginosa* was cultivated in M1 minimal medium with decanoate and nonanoate as the sole carbon sources, polyhydroxyalkanoate (PHA) was achieved up to 55.75% (w/w) and 24.53% (w/w), respectively. The maximum PHA yield at 30 ºC was obtained when decanoic acid was used as sole carbon source. Use of nonanoic acid as carbon source was resulted in a minimum PHA yield at 30 ºC. An increase in the cultivation temperature from 30 ºC to 37 ºC resulted in decrease of PHA content and cell dried weight as well. Based on the type of carbon source different monomers at the level higher than 1 mol% to 62 mol% were detected which included 3-hydroxyoheptanoic acid ($C_7$), 3-hydroxyoctanoic acid ($C_8$), 3-hydroxynonanoic acid ($C_9$), 3-hydroxydecanoic acid ($C_{10}$), and 3-hydroxyundecanoic acid ($C_{11}$).

*Keywords:* Biosynthesis; Carboxylic acid; Polyhydroxyalkanoate; *Pseudomonas aeruginosa*.
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## 1. Introduction

Polyhydroxyalkanoates (PHAs) are a class of natural and environment friendly biodegradable plastics which can be produced by a wide range of microorganisms as an energy resource in the cells. These kinds of naturally occurring biopolymers have attracted much interest because of their potential applications in various areas of biotechnology [1]. The commercialization of PHA production dates back several years. In 1980, the British company, Imperial Chemical Industries (ICI), developed a commercial process to produce poly(3HB), and a related copolymer known as poly-R-3-hydroxybutyrate-co-R-3-hydroxyvalerate, poly(3HB-co-3HV). These polymers were produced under the trade name of

*Corresponding author: Kambiz Akbari Noghabi, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Tel (+98)21-44580352, Fax (+98)21- 44580399
E-mail: Akbari@nigeb.ac.ir

Biopol®, and were developed primarily as renewable and biodegradable substitutes for petroleum-derived plastics. As a result of these activities and others both polymers became widely available, which in turn provided opportunities for their evaluation as medical biomaterials [2]. On the other hand, more recent interest in the use of PHA polymers for medical applications has arisen primarily in response to the needs of the emerging field of tissue engineering, where a much wider range of absorbable polymers are used as tissue scaffolds. In fact, in the past seven years, PHAs have become one of the leading classes of biomaterials under investigation for the development of tissue-engineered cardiovascular products since they can offer properties not available in accessible synthetic absorbable polymers.

Certain Pseudomonads are capable of accumulating high levels of medium-chain-length polyhydroxyalkanates (MCL-PHA) when grown with carbohydrates and carboxylic acids as the main carbon sources [3]. The composition of the PHA is dependent mainly on the substrate specificity of the PHA synthase and on the routes which provide precursors from various carbon sources. MCL monomers are derived from acyl carrier protein (ACP) intermediates of the fatty acid *de novo* synthesis pathway and the substrate of MCL-PHA synthase is (R)-3-hydroxyacyl-coenzyme A (CoA) [4-8]. Thus, to serve as a substrate for the PHA synthase, (R)-3-hydroxyacyl-ACP must be converted to the corresponding CoA derivative. The present research shows the ability of pathogenic bacterium *Pseudomonas aeruginosa* PA01 to utilize a variety of different carboxylic acids as the sole carbon sources. Considering what types of PHA are synthesized in the pathogenic strain. Further, all the polymers produced have been characterized.

## 2. Materials and methods

### *2.1. Microorganism and culture media*

*P. aerugionosa* PA01 was used throughout the experiments. Nutrient rich medium containing 1% yeast extract, 1.5% nutrient broth and 1% ammonium sulfate was used in the seeding, maintenance and storage of the strain. The modified M1 minimal salt medium

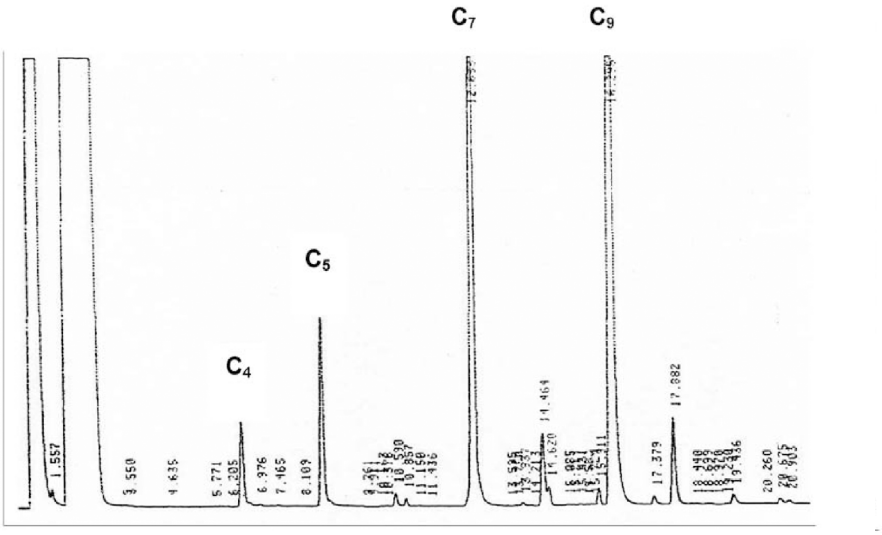


**Figure 1.** PHA monomers profile of *P. aeruginosa* PA01 grown on M1 minimal medium with nonanoic acid as sole carbon source.

**Table 1**. The effect of various carboxylic acids on the biosynthesis of PHA in *P. aeruginosa* PA01 grown on M1 minimal medium.

| Carbon source* | Culture time (h) | Culture temp (ºC) | DCW (g/l) | PHA Content (wt%) |
|---|---|---|---|---|
| Octanoic acid | 72 | 30 | 2.71 | 37.26 |
| Nonanoic acid | 72 | 30 | 1.89 | 31.74 |
| Nonanoic acid | 72 | 37 | 1.22 | 15.65 |
| Decanoic acid | 72 | 30 | 2.25 | 55.75 |
| Undecanoic acid | 72 | 37 | 1.80 | 24.05 |
| Undecanoic acid | 72 | 30 | 2.16 | 24.53 |

*Octanoic acid, nonanoic acid, decanoic acid and undecanoic acid were added to M1 minimal medium to final concentration of 60 mM, 40 mM, 30 mM and 30 mM, respectively.

containing an appropriate amount of a carbon source and 1.0 g/l ammonium sulfate, in a 2 l flask was cultivated to maximal growth. The cells were then harvested, washed and dried under vacuum at room temperature. The carbon sources including octanoic acid, nonanoic acid, decanoic acid and undecanoic acid were purchased from Sigma and were added to M1 minimal medium to final concentrations of 60, 40, 30 and 20 mM, respectively. The cell growth was monitored by measurement of OD at 600 nm in a Spectronic 20 spectrophotometer.

### *2.2. Isolation of PHA and characterization of monomer composition*

PHA was extracted from an appropriate amount of cells which had been dried overnight under vacuum at 50 ºC with hot chloroform in a Pyrex Soxhlet apparatus for 6 h. For the analysis of PHA in cells, 10 mg of dried cells were reacted with a mixture containing 1 ml of chloroform, 0.85 ml of methanol and 0.15 ml of concentrated sulfuric acid. The reaction mixture in a closed screw-capped tube was incubated at 100 ºC for 3 h and organic layer containing the reaction products was separated, dried over $Na_2SO_4$ and analyzed by gas chromatography (Hewlett Packard 5890A and HP-1 column). Each peak was standardized against standard 3-hydroxy-methylesters which were obtained by methanolysis of purified PHA with known compositions, determined by quantitative NMR analysis. For isolation of PHA, hot chloroform in a pyrex soxhlet apparatus for 6 h was used. The isolated PHA was dried overnight under vacuum and then weighed.

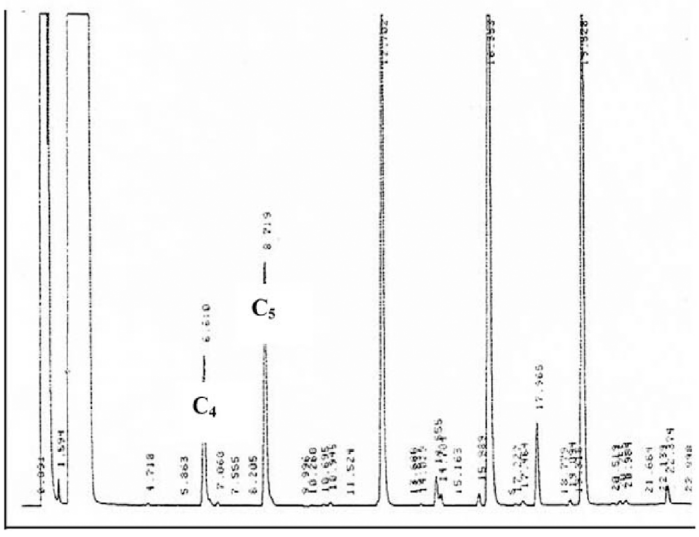


**Figure 2.** PHA monomers profile of *P. aeruginosa* PA01 grown on M1 minimal medium with undecanoic acid as sole carbon source.

Quantitative determination of the monomer units in the PHA was performed by GC as mentioned above.

## 3. Result and discussion

It has been reported that PHA composition depends on the PHA syntheses percent, the carbon sources and the path by which the provided carbon sources are metabolized [6]. There are various types of PHA with chemically different monomer compositions, exhibiting many useful physicochemical properties [9]. It is possible to design PHA using many techniques such as homologous PHA synthesis-related gene insertion, combination of different precursor carbon sources, multistep cultures [10] and pathway routing by inhibitors [11] to improve the material properties such as melting point, glass transition temperature, crystallinity, degradation rate, mechanical strength, etc. [12].

Two types of polyesters, short-chain-length polyhydroxyalkanoates (SCL PHAs) and medium-chain-length (MCL) PHAs, are generally synthesized with common carbon sources [13]. The SCL monomer units include 3-hydroxypropionate, 3-hydroxybutyrate, 4-hydroxybutyrate, 3-hydroxyvalerate (3HV), and 4-hydroxyvalerate [14]. The MCL monomer units have three to nine more carbon atoms than the SCL monomer units. *Alcaligenes eutrophus* [15], *Rhodospirillum rubrum* [16] and *P. pseudofiava* [17] are known to accumulate copolyesters composed of SCL monomer units only, while *P. oleovorans* [18] *P. putida* [19] and other fluorescent *Pseudomonas* strains [20] biosynthesize copolyesters principally composed of MCL monomer units.

In a previous study, *P. putida* KT2442 grown on octanoate and decanoate synthesized a PHA composed of 3-hydroxy-octanoic acid (3HO) as the major constituent and two other monomer units, 3-hydroxyun-decanoic acid (3HC) and 3-hydroxydecanoic acid (3HD), and containing none of the other four closely related monomer units (3-hydrox-ydodecanoate, C12:1, 3-hydroxytetra decanoate, and C14:1) [21]. However, no detailed study on the characteristics of PHA synthesis involving pathogenic *Pseudomonas* strains especially *P. aeruginosa* PA01 has been reported yet. In the present study, we

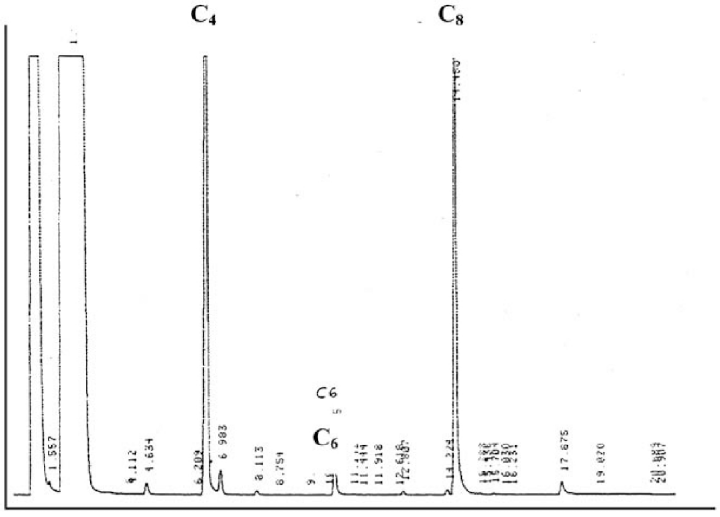


**Figure 3.** PHA monomers profile of *P. aeruginosa* PA01 grown on M1 minimal medium with octanoic acid as sole carbon source.

found that *P. aeruginosa* PA01 was able to synthesize various PHAs including 3-hydroxyoheptanoic (3HH), 3HO, 3-hydroxynonanoic acid (3HN), 3HD, and 3HC from structurally related carbon sources such as octanoic acid, nonanoic acid, decanoic acid and undecanoic acid in the M1 minimal medium. The results obtained from this study also showed that, when *P. aeruginosa* PA01 was cultivated in M1 minimal medium with decanoate and nonanoate as the sole carbon sources, PHA yield up to 55.75 (w/w)% and 24.53 (w/w)% were achieved respectively (Table 1). The maximum PHA yield at 30 ºC was obtained when decanoic acid was used as sole carbon source. Using of nonanoic acid as carbon source was resulted in a minimum PHA yield at 30 ºC. An increase in the cultivation temperature from 30 ºC to 37 ºC resulted in decreasing of PHA content and cell dried weight (DCW) as well. Based on the type of carbon source, different monomers at the levels higher than 1 mol% to 62 mol% were detected which included 3-hydroxyoheptanoic ($C_7$), 3-hydroxyoctanoic acid ($C_8$), 3-hydroxynonanoic acid ($C_9$), 3-hydroxydecanoic acid ($C_{10}$), and 3-hydroxyundecanoic acid ($C_{11}$) (Figures 1, 2 and 3). As a conclusion, though a lot of reports are available in literature about PHA synthesis by various *Pseudomonas* groups, but to the best of our knowledge, this is the first report on the PHA synthesis from different carboxylic acids by a pathogenic strain namely *P. aeruginosa* PA01.

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