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Review

Biotechnological production of (*R*)-3-hydroxybutyric acid monomer

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Abstract

The escalating problems regarding the treatment of plastic waste materials have led to development of biodegradable plastics. At present, a number of aliphatic polyesters; such as poly[(*R*)-3-hydroxybutyrate] (PHB), poly(L-lactide), polycaplolactone, poly(ethylene succinate) and poly(butylene succinate) have been developed. Among these aliphatic polyesters, PHB is one of the most attractive since it can undergo biodegradation at various environmental conditions and has properties similar to polypropylene. Although much effort has been made to produce PHB and its copolyesters from renewable resources or through microbial processes, their commercialization and widespread application are still not economically attractive compared to conventional non-biodegradable plastic. Moreover, wide application of PHB and its copolyesters as biodegradable plastic have not only been limited by the cost of production but also by their stinky smell during industrial processing. However, (*R*)-3-hydroxybutyric acid, a monomer of PHB has wide industrial and medical applications. (*R*)-3-hydroxybutyric acid can also serve as chiral precursor for synthesis of pure biodegradable PHB and its copolyesters. A number of options are available for production of (*R*)-3-hydroxybutyric acid. This review discusses each of these options to assess the alternatives that exist for production of pure biodegradable PHB and its copolyesters with good properties.

Keywords: Degradation; Depolymerase; Poly[(*R*)-3-hydroxybutyrate]; (*R*)-3-Hydroxybutyric acid; *Streptomyces* sp. MG

Contents

1. Introduction

Currently, most petroleum-derived plastics that are widely used by humans in daily lives are non-biodegradable. However, with the increase in population and industrialization, there is now increasing awareness about the impact of these non-biodegradable plastics on the environment. Thus, a lot of efforts are now geared towards developing various biodegradable plastics. Biodegradable polymers can be broadly classified under polynucleotides, polyamides, polysaccharides, polyoxoesters, polythioesters, polyphosphates, polyisoprenoides and polyphenols (Steinbüchel, 2001; Steinbüchel and Lüke-Eversloh, 2003).

Poly[(*R*)-3-hydroxybutyrate] (PHB), the most widely studied member of PHA (polyoxoesters) is very promising as a biodegradable plastic because of its material properties which are comparable to those of the polypropylene. Furthermore,

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PHB has attracted much ecological interests since it can undergo rapid degradation under various environmental conditions. For instance, a number of reports have clearly demonstrated that PHB can be degraded under environmental conditions, such as aerobic and anaerobic (Tokiwa et al., 1992; Nishida and Tokiwa, 1993a) and thermophilic conditions (Tansengco and Tokiwa, 1998; Calabia and Tokiwa, 2006). It was also reported that rapid biodegradability of PHB under aerobic and anaerobic conditions would help in solving the problem of vanishing landfill space, which is one of the fastest and least expensive ways to deal with the disposal of polymers (Tokiwa and Calabia, 2004).

PHB can be readily produced from renewable resources such as sugars, fatty acids and plant oil. Studies have also shown that some bacteria can accumulate high level of PHB per cellular dry mass (Bryom, 1987; Brandl et al., 1990; Doi, 1990; Steinbüchel and Lüke-Eversloh, 2003). Some groups of bacteria, including *Ralstonia eutropha* (now renamed, *Cupriavidus necator*), *Protomonas extorquens*, *Protomonas oleovorans*, require limitation of essential nutrients (such as nitrogen, magnesium, phosphorous or sulphur), and thus two-step fermentation processes are required. During the first stage, the cells are supplied with sufficient nutrients (to raise the cell concentration to a considerable level) while in the second stage, the nutrient limitation is applied for PHB production in the cells. On the other hand, *Alcaligenes latus* and *Azotobacter vinelandii* do not require nutrient limitation or two-step fermentation since they are growth-associated PHB producers. Among these groups of bacteria, *R. eutropha* and *A. latus* are the most widely studied (Braunegg et al., 1978; Kawaguchi and Doi, 1992; Hahn et al., 1995), and very high PHB contents, up to 76% (w/w) have been reported (Palleroni and Palleroni, 1978; Kim et al., 1994). It is not within the scope of this article to give a detailed review of PHB biosynthesis and their biotechnological strategies. However, it is worthy to mention that despite the considerable work on production of PHB and its copolyesters, only few commercial plants have been established in the past few decades. These drawbacks in commercialization of PHB production could be attributed to high cost of production, limited microbial strains, difficulty in recovering the polymer, presence of impurities and stinky smell of PHB during industrial processing.

However (*R*)-3-hydroxybutyric acid ((*R*)-3-HB), a monomer of PHB, has been known to exhibit some antimicrobial, insecticidal, and antiviral activities (Peypoux et al., 1999; Chen and Wu, 2005; Shiraki et al., 2006). (*R*)-3-HB serve as chiral building blocks for synthesis of fine chemicals; such as antibiotics, vitamins, aromatics, and pheromones (Chiba and Nakai, 1985; Steinbüchel and Valentin, 1995; Seebach et al., 2001). Studies have shown that (*R*)-3-HB confers partial protection and stability to neurons during glucose deprivation (Holmes, 1985; Massieu et al., 2003). There are also some evidences that they could serve as energy substrate in increasing cardiac efficiency and thus, prevents brain damage (Kashiwaya et al., 2000). This article briefly reviews possible options for production of (*R*)-3-HB. Furthermore, possible methods for synthesis of pure PHB and its copolyesters are proposed.

2. Microbial production of PHB

PHB metabolism in *R. eutropha* has been extensively studied (Kawaguchi and Doi, 1992; Aneja et al., 2002). Based on PHB cycle, synthesis of PHB occurs when excess carbon in the form of acetyl-CoA is condensed via ketothiolase (encoded by the *phbA* gene) enzyme to generate acetoacetyl-CoA. Acetoacetyl-CoA is reduced to (*R*)-3-hydroxybutyrl-CoA by NADP-dependent acetoacetyl-CoA reductase (encoded by *phbB* gene). The PHB synthase enzyme (encoded by *phbC* gene) catalyzes the final polymerization of (*R*)-3-hydroxybutyrl-CoA. PHB accumulates in the granule but can be degraded by intracellular PHB depolymerase and oligomer hydrolase to form (*R*)-3-HB and oligomer (Saegusa et al., 2002; Shiraki et al., 2006).

For improved production of PHB, various fermentative and metabolic approaches have been established in some bacteria strains. Thus, by using recombinant *Escherichia coli* (harboring *R. eutropha* genes) biosynthetic genes, it was possible to produce relatively higher concentrations of PHB (up to 81% of cell dry weight) compared to those of the wild-type PHB producers (Slater et al., 1988; Kim et al., 1992; Choi et al., 1998; Kahar et al., 2004).

Overview of scientific literatures reveals a variety of methods for quantification of intracellular contents of PHB (Rijk et al., 2002). Intracellular concentration of PHB can be quantified after recovering the polymer with chloroform and drying or by gas chromatography. Gravimetric method involves extraction processes with solvents such as chloroform, sodium hydroxide, sodium hypochlorite or combination of these solvents (Hahn et al., 1995) to recover polymer from the cells. Generally, cells are freeze-dried followed by extraction with hot chloroform in a Soxhlet extractor. The extract is cooled at room temperature and passed through a filter. The filtered solution is concentrated (using rotary evaporator) and PHB is finally precipitated with solvents, such as ethanol. The ethanol–chloroform mixture is filtered, washed with ethanol twice to ensure purity of the polymer. The purified polymer is then vacuum-dried to constant weight. This method is more tedious, requires large sample volumes, and time consuming compared to gas chromatographic method. However, this ensures that PHB polymer is recovered from the cells.

With respect to gas chromatographic method, PHB is depolymerized under acidic or alkaline conditions and monomeric compounds are detected in form of crotonic acid (Slepecky and Law, 1969) or as (*R*)-3-hydroxybutyric acid (Braunegg et al., 1978). Gas chromatography analysis, described by Braunegg et al. (1978), is the widely used method for quantitative analysis of PHB. This involves direct acid methanolysis on the cells followed by gas chromatography analysis. This method is very accurate, reproducible and requires small sample volumes. In addition, gas chromatography analysis is very useful for process control.

Although a number of reports that dealt with microbial production of PHB are available, most of these works were concentrated on evaluation of PHB content (i.e., concentration of PHB with respect to the cellular dry weight) rather than the quantity of PHB produced per volume of the culture or quantity