Construction of Recombinant *Escherichia coli* Strains for Production of Poly-(3-hydroxybutyrate-co-3-hydroxyvalerate)

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**Abstract**

Plastic wastes constitute a worldwide environmental problem, and the demand for biodegradable plastics has become high. One of the most important characteristics of microbial polyesters is that they are thermoplastic with environmentally degradable properties. In this study, pUC19/PHA was cloned and transformed into three different *Escherichia coli* strains. Among the three strains that were successfully expressed in the production of polyhydroxyalkanoates (PHA), *E. coli* HMS174 had the highest yield in the production of poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (P[HB-HV]). The cell dry weight and PHA content of recombinant HMS174 reached as high as 10.27 g/L and 43% (w/w), respectively, in fed-batch fermentor culture. The copolymer of PHA, P(HB-HV), was found in the cells, and the biopolymers accumulated were identified and analyzed by gas chromatography, proton nuclear magnetic resonance spectroscopy, and differential scanning calorimetry. We demonstrated clearly that the *E. coli* host for PHA production has to be carefully selected to obtain a high yield. The results obtained indicated that a superior *E. coli* with high PHA production can be constructed with a desirable ratio of P(HB-HV), which has potential applications in industry and medicine.

**Index Entries:** *Escherichia coli*; polyhydroxyalkanoates; fed-batch fermentation; nuclear magnetic resonance; differential scanning calorimetry.

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Introduction

Plastics have become integral to our lives, and the generation of plastic wastes has increased dramatically. From 1986 to 1998, about 15% of the total domestic, commercial, and industrial waste in Hong Kong was plastic (1). The most immediate advantage of making biodegradable plastics is to address the problems of litter and marine pollution resulting from plastics disposal, which are difficult to solve any other way.

Polyhydroxyalkanoate (PHA) is a polyester of hydroxyalkanoates synthesized by numerous bacteria as an intracellular carbon and energy storage compound and accumulated as granules in the cytoplasm of cells (2). PHA has been attracting much attention because it is a biodegradable, biocompatible, microbial thermoplastic that is regarded as a potentially useful polyester to replace petroleum-derived thermoplastics (3).

The copolymer poly-(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHB-co-PHV) produced by A. eutrophus has generated more interest than poly-(R)-3-hydroxybutyrate (PHB) homopolymer. Since these bacterial polyesters are biodegradable thermoplastics, their mechanical and physical properties have received much attention. PHB is a relatively stiff and brittle material because of its high crystallinity. However, the physiochemical and mechanical properties of [P(HB-HV)] vary widely and depend on the molar percentage of 3-hydroxyvalerate (HV) in the copolymer (4,5) as shown in Table 1. Propionic acid is converted by a synthetase to propionyl-CoA, and the biosynthetic β-ketothiolase catalyzes the condensation of propionyl-CoA with acetyl-CoA to 3-ketovaleryl-CoA by the acetoacetyl-CoA reductase. The hydroxyvaleryl moiety is finally covalently linked to the polyester by the PHA synthase (6).

However, the price of PHB-co-PHV exceeds US $10/kg, which is much higher than the cost of conventional oil-derived plastic (7). PHA yield is another major factor of the economic production of PHA. The bacterium Escherichia coli has proven to be a powerful microbial species in the microbial synthesis of bioproducts using molecular biology techniques.

One of the major factors affecting the overall production cost is bioreactor productivity, which can be defined as the amount of PHA accumulated per unit volume per unit time. To increase PHA productivity, not

Table 1
Thermal and Mechanical Properties of Different Polymer Samples (6,7)*

<table>
<thead>
<tr>
<th>Sample</th>
<th>(T_m) (°C)</th>
<th>(T_g) (°C)</th>
<th>Tensile strength (Mpa)</th>
<th>Elongation to break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P(3HB)</td>
<td>177</td>
<td>4</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>P(HB-HV) 10% HV</td>
<td>150</td>
<td>ND</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>P(HB-HV) 20% HV</td>
<td>135</td>
<td>ND</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

*ND, not determined; \(T_g\), glass transition temperature; \(T_m\), melting temperature