BIOSYNTHESIS OF POLYHYDROXYALKANOATE BIOPLASTIC FROM GLYCEROL BY ENGINEERED *ESCHERICHIA COLI*

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ABSTRACT

Polyhydroxyalkanoates (PHAs) are potentially used as bio-plastics. However, the high cost of PHAs limits their use in the commodity market. Crude glycerol is an alternative carbon source for PHAs production. Engineered *Escherichia coli* strains were constructed to investigate their ability to synthesize poly(3-hydroxybutyrate) [P(3HB)] and Short-Chain-Length-*co*-Medium-Chain-Length PHAs (SCL-MCL-PHAs) from pure and crude glycerol. PHA biosynthesis-related genes: ß-ketothiolase (*phaA*), acetoacetyl-CoA reductase (*phaB*) and PHA synthase (*phaC*), were cloned into pETDuet-*ABC*s and co-expressed in *E. coli* for P(3HB) biosynthesis. The *phaA* and *phaB* genes were derived from *Ralstonia eutropha* whereas 3 different *phaCs* from 3 different bacteria (*Aeromonas hydrophila, R. eutropha and Pseudomonas putida*) were used. The results showed that the amount of P(3HB) is affected by the type of PhaCs (30 wt%, 24 wt%, and 2 wt% of cell dry weight (CDW), respectively). The stage of cell and the carbon: nitrogen ratio were also found to be the key parameters influencing the amount of P(3HB). To produce SCL-MCL-PHAs, three (*R*)-specific enoyl-CoA hydratase genes from *P. putida* (*phaJ1*$_{Pp}$ and *phaJ4*$_{Pp}$) and from *A. hydrophila* (*phaJ*$_{Ah}$) were cloned into pCDFDuet-*J*s and co-transformed with pETDuet-*ABC*s. Co-expression of PhaAB with each of PhaCs and PhaJs caused *E. coli* to produce SCL-MCL-PHAs with different monomer compositions, varying from C4 to C10 from pure glycerol supplemented with dodecanoate. The fractions of MCL-unit were in correlation with the concentration of dodecanoate. When 1% crude glycerol was used as a carbon source, *E. coli*-ABC$_{Ah}$ produced P(3HB) at 14 wt% of CDW; whereas, *E. coli*-ABC$_{Ah}$J$_{Ah}$ produced P(3HB-1 mol% 3HHx) at 3 wt% of CDW when dodecanoate was included in the culture media. The molecular weight of PHAs produced was in the range of 110 to 260 kDa and the thermal properties of P(3HB-*co*-3HHx) were superior to P(3HB).

KEY WORDS: POLYHYDROXYALKANOATE / GLYCEROL / *ESCHERICHIA COLI* / COPOLYMER / HYBRID PATHWAY

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