

SII-1-4

Size Control of Polyhydroxybutyrate (PHB) molecule by Modulation of PHA Synthase Activity

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The average molecular weight and molecular weight distribution of polyesters isolated from recombinant *E. coli* constructs were examined by multi-angle light scattering. Investigations focused on a plasmid-encoded native *A. eutrophus* operon and a synthetic operon encoding the PHA biosynthetic enzymes which was designed to yield high levels of *in vivo* PHA synthase. Fermentation of *E. coli* strains possessing the two constructs and characterization of both the molecular weight and molecular weight distribution of the isolated polyesters has suggested a role for PHA synthase activity in controlling the molecular weight of PHAs produced *in vivo* in a host organism.

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Metabolic Engineering of *Alcaligenes eutrophus* for Polyhydroxy-alkanoate Biosynthesis

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The effect of variation of enzyme activities related to poly- β -hydroxy-butyrate(PHB) biosynthesis in *A. eutrophus* on the characteristics of biosynthesis of PHB and its copolymer poly(3-hydroxybutyrate-3-hydroxyvalerate) was investigated using PHB negative mutant and several transformants in which β -ketothiolase, acetoacetyl-CoA reductase, and PHB synthase were reinforced through the transformation of the cloned *phbC*, *phbAB*, and *phbCAB* genes. The variation of β -ketothiolase and acetoacetyl-CoA reductase influenced mainly on the rate of biosynthesis of PHB and P(3HB-3HV), however, did not significantly affect on their contents and the molar fraction of 3-hydroxyvalerate(3-HV) in P(3HB-3HV). It seems that the biosynthesis rate is mainly determined by the efficiency of supplement of acetyl-CoA by β -ketothiolase. Unlikely, the PHB synthase influence only slightly on the rate of PHB and P(3HB-3HV), but influenced significantly on the molar fraction of 3-HV in P(3HB-3HV), which might be due to the accelerated condensation reaction of 3-HV to 3-hydroxybutyrate by PHB synthase. The molar fraction of 3-HV in P(3HB-3HV) biosynthesis was also critically influenced by the ratio of activity of PHB synthase to β -ketothiolase.

The granular structure of PHB and P(3HB-3HV) accumulated inside of cell was also observed. The enforced β -ketothiolase and acetoacetyl-CoA reductase tended to enlarge the the size of granules, meanwhile, PHB synthase induced the number of PHB and P(3HB-3HV) granules.