Metabolic Engineering of *Lactococcus lactis* to Enhance Biopolymer Bead Production

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Abstract

Polyhydroxyalkanoates (PHAs) are a group of biopolyesters that are synthesized by polyester synthases in a wide range of Gram-positive and Gram-negative bacteria, and are stored in bacterial cells as intracellular inclusions. Recently, these inclusions have been considered for biotechnological and biomedical applications as surface-functionalized micro-/nanobeads. The production of functionalized poly[(*R*)-3-hydroxybutyrate] (PHB; a biopolyester) beads in the food-grade host *Lactococcus lactis* has recently been established, however the levels of PHB production are low in comparison to levels produced by recombinant *E. coli*. In an attempt to improve PHB production in *L. lactis*, the metabolic flux of carbon from pyruvate was engineered to redirect the flux towards acetyl Co-A, one of the precursors for PHB. This involved knocking out two enzymes involved in conversion of acetyl Co-A to acetate and ethanol (acetaldehyde dehydrogenase and phosphate acetyltransferase, respectively). PHB production using a strain deficient in acetaldehyde dehydrogenase (*adhE*) was not assessed due to difficulties encountered in creating the knockout strain. This study showed the successful construction and phenotypic characterisation of a phosphate acetyltransferase (*eutD*) deficient strain of *L. lactis*. Production of acetate was substantially reduced in this mutant, and growth of the strain was improved when PHB production was established. However, rather than increasing, levels of PHB production by the *eutD* knockout were comparable to WT. Additionally, complementation of the mutant strain still needs to be achieved to confirm that the observed acetate-production phenotype is attributable to the lack of the *eutD* gene.

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“A little more persistence, a little more effort, and what seemed hopeless failure may turn to glorious success”

Abbreviations

°C	Degree Celsius
3HB	3-hydroxybutyrate
AGE	Agarose Gel Electrophoresis
BSA	Bovine serum albumin
DMSO	Dimethyl sulfoxide
GC/MS	Gas chromatography mass spectrometry
LDH	Lactate dehydrogenase
PHA	Polyhydroxyalkanoate
PhaC	PHA synthase
PHB	Polyhydroxybutyrate
RE	Restriction endonuclease
WT	Wildtype

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