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Heterologous expression of *phaC2* gene and poly-3-hydroxyalkanoate production by recombinant *Cupriavidus necator* strains using canola oil as carbon source

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Highlights

- The functional activity of PhaC2 synthase of *Pseudomonas putida* CA-3 is first reported.
- Heterologous expression is performed in *Cupriavidus necator* strains.
- PhaC2 synthase enzyme has a broad-substrate specificity of scl-PHA to mcl-PHA monomers.
- Co-expression study produced a PHA blend-polymer.
- Use of canola oil for scl-mcl PHA production is promising

Abstract

Many heterologous transformation studies have been carried out using the ***Cupriavidus necator* PHB^{-4}** strain to investigate the expression characteristics of various polyhydroxyalkanoate (PHA) synthase enzymes. In this study, we generated a recombinant *C. necator* PHB^{-4} strain by transforming a plasmid (pMRC03) harbouring the synthetic *phaC2* gene of *Pseudomonas putida* CA-3. Under conditions favourable for expression of the *phaC2* $_{P.put\ \text{CA-3}}$ gene, canola oil was used as carbon source for the synthesis of PHAs. The expressed synthase polymerised monomers of 3-hydroxybutyrate (3-HB), 3-hydroxyvalerate (3-HV) and 3-hydroxyhexanoate (3-HHx) in the recombinant ***C. necator* PHB^{-4} (pMRC03)** strain. We then co-expressed the *phaC2*$_{P.\ put\ \text{CA-3}}$ gene with the native *phaC1*$_{C.\ ne}$ gene in wild type ***Cupriavidus necator* H16 (*C. necator* H16 (pMRC03)**). This co-expression produced a PHA blend of 3-HB, 3-HV, 3-HHx and 3-