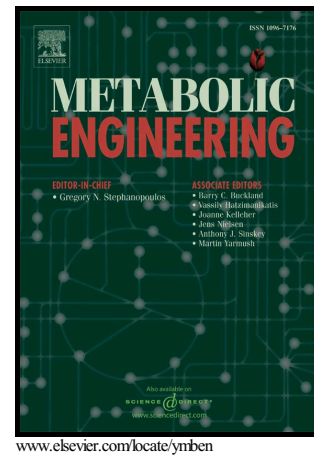


Author's Accepted Manuscript

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PII: S1096-7176(18)30018-1
DOI: <https://doi.org/10.1016/j.ymben.2018.03.013>
Reference: YMBEN1367

To appear in: *Metabolic Engineering*

Received date: 18 January 2018
Revised date: 13 March 2018
Accepted date: 14 March 2018

Cite this article as: Ye Jianwen, Hu Dingkai, Che Xuemei, Jiang Xiaoran, Li Teng, Chen Jinchun, Zhang Haoqian and Chen Guo-Qiang, Engineering of *Halomonas bluephagenesis* for Low Cost Production of Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) from Glucose, *Metabolic Engineering*, <https://doi.org/10.1016/j.ymben.2018.03.013>

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Engineering of *Halomonas bluephagenesis* for Low Cost Production of Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) from Glucose

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Abstract

Poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HB)] is one of the most promising biomaterials expected to be used in a wide range of scenarios. However, its large-scale production is still hindered by the high cost. Here we report the engineering of *Halomonas bluephagenesis* as a low-cost platform for non-sterile and continuous fermentative production of P(3HB-co-4HB) from glucose. Two interrelated 4-hydroxybutyrate (4HB) biosynthesis pathways were constructed to guarantee 4HB monomer supply for P(3HB-co-4HB) synthesis by working in concert with 3-hydroxybutyrate (3HB) pathway. Interestingly, only 0.17mol% 4HB in the copolymer was obtained during shake flask studies. Pathway debugging using structurally related carbon source located the failure as insufficient 4HB accumulation. Further whole genome sequencing and comparative genomic analysis identified multiple orthologs of succinate semialdehyde dehydrogenase (*gabD*) that may compete with 4HB synthesis flux in *H.*

¹ These authors contributed equally to this work.

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