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Engineering bacteria for enhanced polyhydroxyalkanoates (PHA) biosynthesis

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ABSTRACT

Polyhydroxyalkanoates (PHA) have been produced by some bacteria as bioplastics for many years. Yet their commercialization is still on the way. A few issues are related to the difficulty of PHA commercialization: namely, high cost and instabilities on molecular weights (Mw) and structures, thus instability on thermo-mechanical properties. The high cost is the result of complicated bioprocessing associated with sterilization, low conversion of carbon substrates to PHA products, and slow growth of microorganisms as well as difficulty of downstream separation. Future engineering on PHA producing microorganisms should be focused on contamination resistant bacteria especially extremophiles, developments of engineering approaches for the extremophiles, increase on carbon substrates to PHA conversion and controlling Mw of PHA. The concept proof studies could still be conducted on *E. coli* or *Pseudomonas* spp. that are easily used for molecular manipulations. In this review, we will use *E. coli* and halophiles as examples to show how to engineer bacteria for enhanced PHA biosynthesis and for increasing PHA competitiveness.

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1. Introduction

Polyhydroxyalkanoates (PHA) have been produced since the 1980s with limited market success [1–3]. Many challenges are related to the limited PHA commercialization (Table 1), especially the high production cost and instability on thermo-mechanical

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Table 1
Challenges for producing cost competitive PHA.

Problems	Reasons	Solutions	Reference
High energy demands	Sterilization and intensive aeration	Unsterile and micro-aerobic processes	[17]
Low substrates to PHA conversions	Substrates are consumed for other purposes	Deletion or weakening PHA unrelated pathways	[18,19]
Unstable PHA structures	Multiple pathways consuming PHA precursors	Deletion or weakening PHA unrelated pathways	[19,20]
Unstable batch Mw	Unstable PHA synthase activity	Controlling PHA synthase activity	[8,10]
Slow growth	Binary fission et al.	Multiple fission et al.	[21]
Discontinuous processes	Avoid possible contamination	Use contamination resistant strains	[16,17,22]
Expensive downstream	Complexity to extract and purify products	Morphology engineering	[23,24]

properties resulted from unstable molecular weights (Mw) and structures, that are also associated with unstable PHA synthase activity and monomer supplies [4–10]. Efforts have been made to meet these challenges [5,11–13].

The high cost is the result of high energy demand related to complicated sterilization and intensive aeration, low conversion of carbon substrates (S) to PHA products (P), slow growth of microorganisms, discontinuous processes and expensive downstream processing et al. (Table 1) [14,15]. The use of extremophilic bacteria combined with metabolic engineering and synthetic biology could fully address these issues [16,17].

Future engineering on PHA producing microorganisms should be focused on contamination resistant bacteria especially extremophiles, developments of engineering approaches for the extremophiles (which is called “Next Generation Industry Biotechnology” or “NGIB”, which will be discussed in section 6 in this paper), increase on carbon substrates to PHA conversion and controlling Mw of PHA (Table 1). The concept proof studies could still be conducted on *E. coli* or *Pseudomonas* spp. that are easily used for molecular manipulations. In this review, we will use *E. coli, Pseudomonas* spp., and halophiles as examples to show how to engineer bacteria for better PHA biosynthesis and for increased PHA application competitiveness.

2. Redirecting substrates to PHA conversion pathways

In many cases, substrates are the most important factor for high production cost. This is especially true for PHA production [11]. For example, the production of PHA containing non 3-hydroxubutyrate (3HB) monomers requires fatty acid(s) as substrate for formation of other non 3HB short-chain-length (scl) or medium-chain-length (mcl) monomers [25–29]. Since most fatty acids will be beta-oxidized to acetyl-CoA for the uses of many other biosynthesis pathways other than for PHA synthesis, it wastes a lot of expensive fatty acids for generating acetyl-CoA (Fig. 1) [20], which can be formed from low-cost glucose [18,30]. Due to the beta-oxidation, fatty acid substrates conversion to PHA products are very low, resulting in high cost of PHA production.

The substrate to PHA conversion efficiency has been significantly improved with the deletions of enzymes FadA and FadB in the beta-oxidation pathway of *Pseudomonas putida* or *P. entomophila* (Fig. 1), as fatty acid substrates were mostly converted into 3-hydroxyacyl-CoA for PHA synthesis instead of being oxidized to become acetyl-CoA [20,31,32]. Beta-oxidation pathway deleted *Pseudomonas* spp. have been reported to produce PHA containing 3-hydroxyhexanoate (3HHx), 3-hydroxyoctanoate (3HO), 3-hydroxydecanoate (3HD) and 3-hydroxydodecanoate (3HDD) in the forms of homopolymers, block- or random copolymers [33].

A metabolically engineered *Escherichia coli* has been constructed by co-expressing genes involved in succinate degradation in *Clostridium kluyveri* and P(3HB) accumulation pathway of *Ralstonia eutropha*. This engineered *E. coli* can produce poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] from glucose. Also, *E. coli* native succinate semialdehyde dehydrogenase genes *sad* and *gabD* were both deleted to enhance the carbon flux to 4HB biosynthesis [18]. Povolo et al. [34] reported that the production of P(3HB-co-3HV-co-4HB) terpolymer can be obtained directly by the use of lactose or waste raw materials such as cheese whey as carbon sources. Cerrone et al. [35] demonstrated the use of a mannitol rich ensiled grass press juice (EGPJ) as a renewable carbon substrate for polyhydroxyalkanoates (PHA) production. Fed-batch cultivations of *Burkholderia sacchari* IPT101 using EGPJ as sole carbon source produced 44.5 g/L CDW containing 33% poly-3-hydroxybutyrate (PHB) in 36 h. Park et al. [36] constructed a sucrose utilization pathway in *Ralstonia eutropha* NCIMB11599 and *R. eutropha* 437–540 by introducing the *Mannheimia succiniciproducens* MBEL55E *sacC* gene that encodes β-fructofuranosidase. β-fructofuranosidase excreted into the culture medium could hydrolyze sucrose to glucose and fructose, which efficiently used sucrose as carbon sources by recombinant *R. eutropha* strains. A high P(3HB) content of 73.2 wt% was obtained when *R. eutropha* NCIMB11599 was cultured in nitrogen-free chemically defined medium containing 20 g/L of sucrose. Qi group [37] synthesized mcl-PHAs in *E. coli* directly from glucose by engineering the reversed fatty acid β-oxidation cycle. After deletion of the major thioesterases and expression of a low-substrate-specificity PHA synthase from *Pseudomonas stutzeri* 1317, the engineered *E. coli* produced 12.10 wt% of cell dry weight scl-mcl PHA copolymers, of which 21.18 mol% was 3-hydroxybutyrate and 78.82 mol% was medium-chain-length monomers.

3. Stabilization of PHA monomer ratios and molecular weights (Mw)

Due to the fluctuation of PHA synthase activity and monomer supplies, monomer ratios in copolymers and PHA Mw vary from batch to batch, this is not desirable by any consumer. Therefore, a lot of efforts have been made to stabilize the PHA monomer structures and Mw. Tripathi et al. [19] used the above beta-oxidation deleted *Pseudomonas putida* KT2442 as a platform for the biosynthesis of polyhydroxyalkanoates with adjustable monomer contents and compositions. The monomer ratios can be precisely controlled by feeding fatty acids with a predefined ratio (Fig. 2). They achieved to prepare random copolymers PHBHHx or block copolymers consisting of precisely adjustable 3-hydroxybutyrate (3HB) and 3-hydroxyhexanoate (3HHx). The materials thus showed stable properties if the monomer ratios were stable [19,38].

Similarly, Wang Ying et al. [39] succeeded in synthesizing homopolymers of C5 (3-hydroxyvalerate) to C14 (4-hydroxytetradecanoate) using beta-oxidation deleted *P. entomophila* LAC23 grown on different fatty acids as precursors, respectively. The examples clearly demonstrate that beta-oxidation deleted mutants can help control PHA monomer structures, as also evidenced by several other studies [40].

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