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Engineering the diversity of polyesters

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Many bacteria have been found to produce various polyhydroxyalkanoates (PHA) biopolyesters. In many cases, it is not easy to control the structures of PHA including homopolymers, random copolymers and block copolymers as well as ratios of monomers in the copolymers. It has become possible to engineer bacteria for controllable synthesis of PHA with the desirable structures by creating new PHA synthesis pathways. Remarkably, the weakening of β-oxidation cycle in *Pseudomonas putida* and *Pseudomonas entomophila* led to controllable synthesis of all kinds of PHA structures including monomer ratios in random and/or block copolymers when fatty acids are used as PHA precursors. Introduction of functional groups into PHA polymer chains in predefined proportions has become a reality provided fatty acids containing the functional groups are taken up by the bacteria for PHA synthesis. This allows the formation of functional PHA for further grafting. The PHA diversity is further widened by the endless possibility of controllable homopolymerization, random copolymerization, block copolymerization and grafting on functional PHA site chains.

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Introduction

Microbial polyhydroxyalkanoates (PHA), a family of biodegradable and biocompatible polyesters, have been produced as a source of chemicals, materials and biofuels [1••,2,3]. Compared with other well known biodegradable or biobased polymers with less CO_2 emission such as PBS, PLA and PTT [4], PHA have much wider diversity in monomers with over 150 structural variations reported [5,6].

In many cases, however, it is not easy to achieve a precise control of the structure. For example, random copolymers consisting of 3-hydroxyhexanoate (3HHx or C6), 3-hydroxyoctanoate (3HO or C8), 3-hydroxydecanoate (3HD or C10) and 3-hydroxydodecanoate (3HDD or C12) are always formed when a fatty acid is added to cultures of *Pseudomonads* belonging to the rRNA homology group I [7], as β-oxidation in *Pseudomonas* spp. will always shorten the C12 to C10, C8 and C6, among others [8]. On the other hand, the *in situ* fatty acid synthesis pathway, although lower in fatty acid synthesis rate for supplying PHA monomers than the β-oxidation one does, will also supply various monomers for PHA synthesis [9], leading to PHA consisting of various monomers in random copolymers [6].

In many cases, precursors such as fatty acids, alcohols or functional monomers are expensive, new pathways are established to synthesize PHA monomers *in vivo* from low cost glucose [9,10]. This approach is very important if the PHA is to be produced in an industrial scale [4]. Recent advances in systems biology have improved the amount of information that can be collected [11], synthetic biology tools are melding modeling and molecular implementation, promising to move microbial engineering from the iterative approach to a design-oriented paradigm [12].

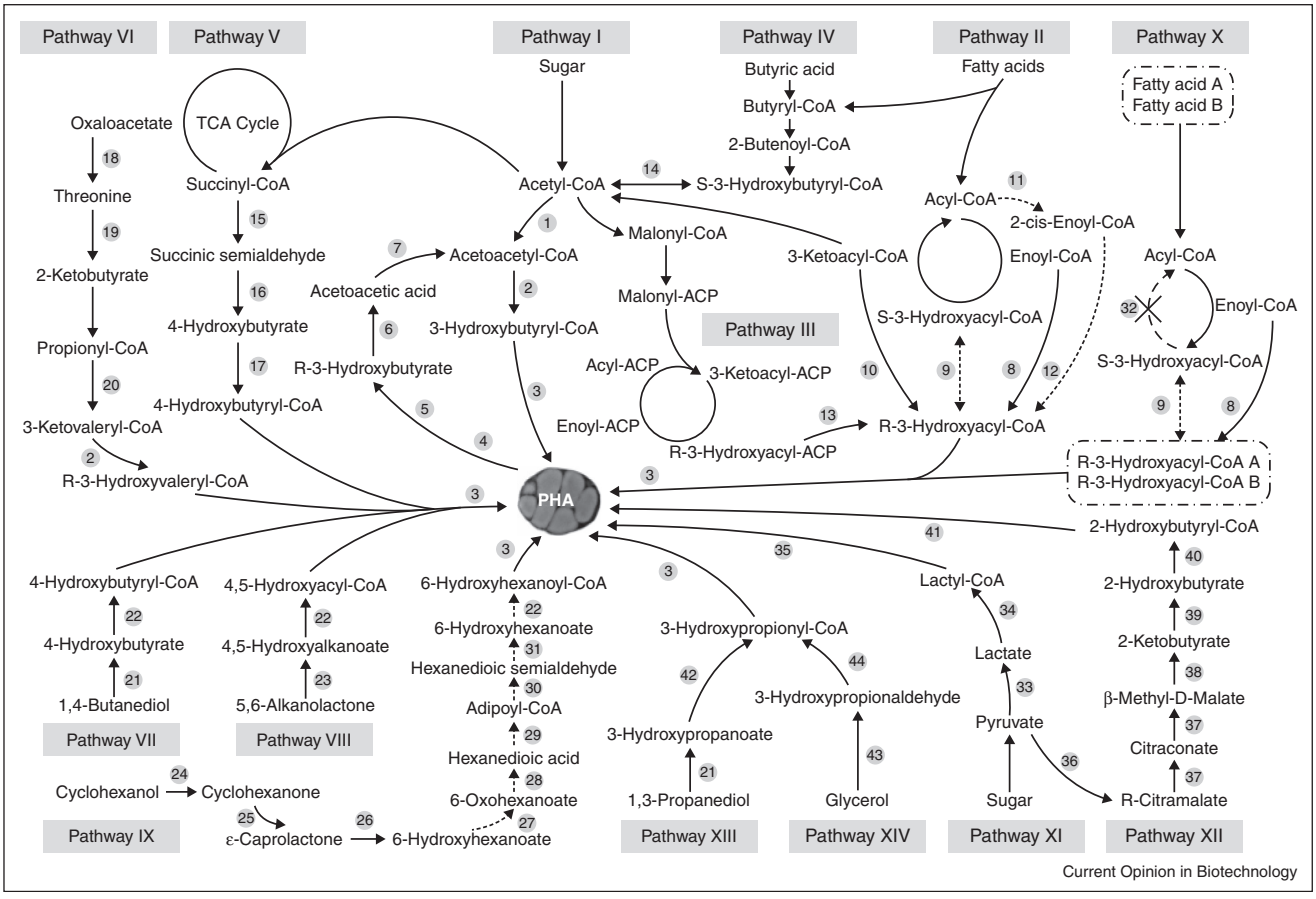
Recent developments showed that it is possible to establish a microbial platform for producing not only random copolymers with controllable monomers and their ratios but also structurally defined homopolymers and block copolymers [3,13,14•]. This was achieved by engineering the genome of *Pseudomonas putida* or *Pseudomonas entomophila* to weaken the β-oxidation and *in situ* fatty acid synthesis pathways, so that a fatty acid fed to the bacteria will maintain its original chain length when incorporated into the PHA chains [15•,16••]. Most importantly, the engineered bacterium will allow functional groups in a fatty acid be introduced into PHA, forming functional PHA, which grafting will generate endless functional PHA.

This paper reviews recent progresses on engineering the diversity of polyesters both on the microbial genomes and on introduction of foreign pathways into PHA production hosts.

Known pathways leading to PHA syntheses

The specificity of a PHA synthase (PhaC) is the most important element determining PHA monomers composition. PhaC from *Ralstonia eutropha* has been known to be able to polymerize PHA monomers consisting of three

Figure 1



Known pathways leading to PHA synthesis and diversity.

(C3) to five (C5) carbon chain lengths termed short-chain-length PHA or scl PHA [17], that include poly(3-hydroxypropionate) (P3HP) [18,19], poly(3-hydroxybutyrate) (PHB) [20], poly(4-hydroxybutyrate) (P4HB) [21,22••], poly(3-hydroxyvalerate) (PHV) [23], and copolymers of 3-hydroxypropionate and 4-hydroxybutyrate (P3HP4HB) [14•], as well as similar copolymers of P3HB4HB, P3HP3HB and PHBV [10,24•,25]. Many *Pseudomonas* spp. contain PhaCs that can polymerize monomers of six (C6) to fourteen (C14) carbon-chain-length to form medium-chain-length PHA (or mcl PHA) [7]. Only very few bacteria were found to have PhaCs that can polymerize C4 to C14 to form scl-mcl copolymers [26,27].

Totally 14 pathways have been reported leading to PHA formations (Figure 1 and Table 1). Natural PHA synthesis pathways include: pathway I starts from sugar to form acetyl-CoA, acetoacetyl-CoA to 3-hydroxybutyryl-CoA, which enters the polymerization process to form PHB [7,28]; Pathway II begins from fatty acid(s) as substrate to enter the β-oxidation cycle, leading to formation of R-3-hydroxyacyl-CoA monomers for mostly mcl PHA synthesis [7]; while pathway III directs acetyl-CoA to malonyl-CoA to 3-ketoacyl-ACP for forming R-3-hydroxyacyl-CoA monomers [29,30]. Pathway IV uses butyric acid without entering the β-oxidation cycle to form S-3-hydroxybutyryl-CoA, then to acetyl-CoA [31]. The types of PHA formed depend not only on monomer supply pathways, but also on specificity of PHA synthases [26]. Generally a low specificity of a PhaC allows formation of diverse PHA structures [5,27].

There are engineered pathways leading to unconventional PHA, these are pathways V to XIV (Figure 1). They will be discussed later.

Diversity of PHA

Diversity of PHA has always been focused more on monomer variations, less on the composition of PHA especially on PHA main chain structures. This was due to the difficulty to precisely control the PHA composition. Recently, the authors' lab succeeded in engineering the β-oxidation pathway encoded on the chromosomes of *P. putida* and *P. entomophila*, resulting in controllable PHA composition including formation of PHA homopolymers,