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Synthetic biology of microbes synthesizing polyhydroxyalkanoates (PHA)

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ABSTRACT

Microbial polyhydroxyalkanoates (PHA) have been produced as bioplastics for various purposes. Under the support of China National Basic Research 973 Project, we developed synthetic biology methods to diversify the PHA structures into homo-, random, block polymers with improved properties to better meet various application requirements. At the same time, various pathways were assembled to produce various PHA from glucose as a simple carbon source. At the end, *Halomonas* bacteria were reconstructed to produce PHA in changing morphology for low cost production under unsterile and continuous conditions. The synthetic biology will advance the PHA into a bio- and material industry.

Contents

1. Introduction

Polyhydroxyalkanoates (PHA), polylactic acid (PLA), poly(-butylene succinate) (PBS), polyethylene (PE), poly(trimethylene terephthalate) (PTT), polypropylene (PPP), polyethylene terephthalate (PET) and poly(propylene carbonate) (PPC) are biodegradable or biobased polymers with at least one monomers produced by microbial conversions or microbial industrial biotechnology [1]. The weaknesses of microbial or enzymatic processes compared with the chemical processing make industrial biotech products less competitive with the chemical ones. However, taking advantages of the molecular biology and synthetic biology methods as well as changing process patterns, bioprocesses could be developed as competitive as chemical ones, these

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including the minimized cells, open and continuous fermentation processes et al. [2] This review aims to report progresses made by the China National Basic Science Research Project 973 during 2012–2016 on synthetic biology of PHA.

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. However, the weakening of beta-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 (Fig. 1). The PHA diversity is further widened by the endless possibility of controllable homopolymerization, random copolymerization, block copolymerization and grafting on functional PHA site chains(Fig. 1) [3].

PHA diversity is generated by engineering the three basic synthesis pathways including the acetoacetyl-CoA pathway (pathway I), in situ fatty acid synthesis (pathway II), and/or beta-oxidation cycles (pathway III), as well as PHA synthase specificity and process control. It is now possible to tailor the PHA structures via genome editing or process engineering. The increasing PHA diversity and maturing PHA production technology should lead to more focused research into their low-cost and/or high-value applications [4] (see Fig. 2).

Similarly to the genome, transcriptome, and proteome, the PHA spectrum exhibits diverse and dynamic modifications – the term 'PHAome' has been created to reflect not only the diversity of monomers, homopolymers, random and block copolymers, functional and graft polymers, molecular weights, and combinations of the above, but also the ranges of PHAs with various molecular weights and monomer ratios that are present at a particular time point in a bacterial cell. It has become very important to understand the PHAome and ensuring an ample supply of PHAs to promote the discovery of new properties and applications of this family of advanced materials [5].

2. The development of new technology for pathway cloning and assembly

PHA synthesis involves a lot of pathways. However, construction of large gene clusters containing DNA fragments is still a difficult and expensive task. To tackle this problem for complicate PHA synthesis pathway assembly, we developed a gene cluster extraction method based on *in vitro* single-strand overlapping annealing (SSOA). It starts with digesting the target gene cluster in an existing genome, followed by recovering digested chromosome fragments. Subsequently, the single-strand DNA overhangs formed from the digestion process would be specifically annealed and covalently joined together with a circular and a linear vector, respectively. The method could clone a 18 kb DNA fragment encoding NADH: ubiquinone oxidoreductase. Combined with genetic information from KEGG and the KEIO strain collection, this method will be useful to clone any specific region of an *E. coli* genome at sizes less than similar to 28 kb. The method provides a cost-effective way for genome assembly, alternative to expensive chemically synthesized gene clusters [6].

Anaerobic metabolic pathways dedicated to co-production of hydrogen and PHB were established in *E. coli* due to the advantages of directing fluxes away from toxic compounds such as formate and acetate to useful products. *E. coli* over-expressing very large hydrogenase 3 cloned using the above SSOA method and/or acetyl-CoA synthase showed improved poly-3-hydroxybutyrate (PHB) and hydrogen production when grown with or without acetate as a carbon source [7].

A method was developed to generate single-stranded DNA

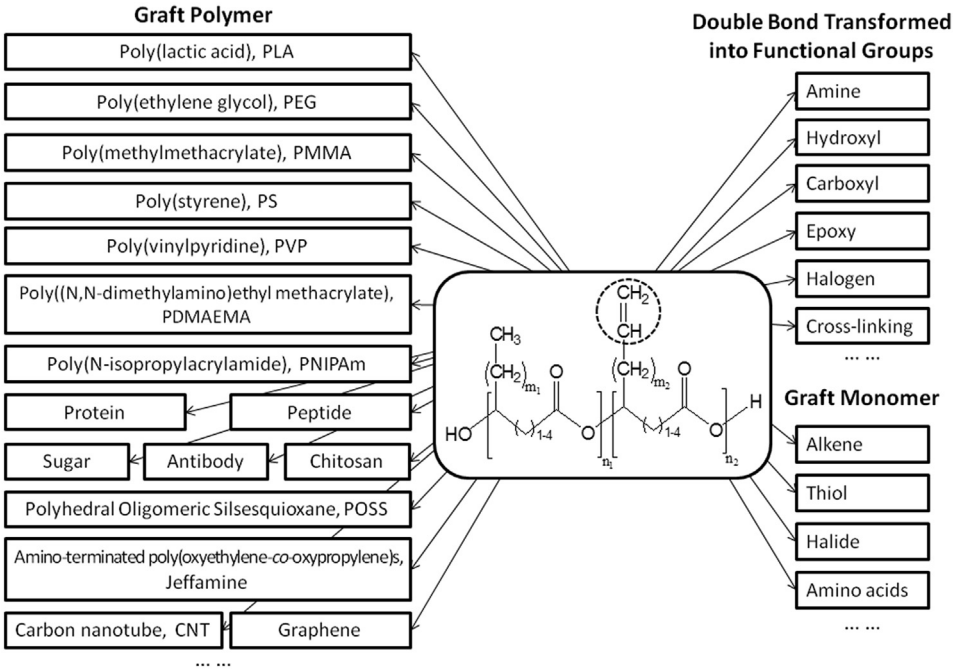

Fig. 1. Introduction of functional groups into PHA polymer chains in predefined proportions has become a reality provided fatty acids containing the functional groups are taken up by the bacteria for PHA synthesis. The PHA diversity is further widened by the endless possibility of controllable homopolymerization, random copolymerization, block copolymerization and grafting on functional PHA site chains [3].