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Engineering microorganisms for improving polyhydroxyalkanoate biosynthesis

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Biosynthesis of polyhydroxyalkanoates (PHA) has been studied since the 1920s. The biosynthesis pathways have been well understood and various attempts have been made to improve the PHA biosynthesis efficiency. Recent progresses have been focused on systematic improvements on PHA biosynthesis including changing growth pattern for rapid proliferation, engineering to enlarge cell sizes for more PHA accumulation space, reprogramming the PHA synthesis pathways using optimized RBS and promoter, redirecting metabolic flux to PHA synthesis using CRISPR/Cas9 tools, and very importantly, the employment of non-traditional host such as halophiles for reduced complexity on PHA production. All of the efforts should lead to ultrahigh PHA accumulation, controllable PHA compositions and molecular weights, open and continuous PHA production with gravity separation processes, resulting in competitive PHA production cost.

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Introduction

Microbial synthesis of polyhydroxyalkanoates (PHA) has been studied as biodegradable materials for many years with a hope to at least partially replace the non-degradable petroleum-based plastics [1–3]. Only limited successes have been achieved. Many efforts have been made to improve the biosynthesis efficiency [4,5•,6–8]. Challenges are still to be met so that PHA can be synthesized more efficient [7,9,10,11••,12–14].

PHA biosynthesis pathways and their related enzymes have been extensively studied [15–17], especially related to the multiple pathways leading to the formations of various PHA monomers (Figure 1). A lot of studies have been conducted to improve metabolic flux to PHA synthesis [4,5•,6,7], such as essential element limitation such as nitrogen, phosphorus, sulfur or iron [18], oxygen limitation [19–21], the weakening of beta-oxidation cycle [22••,23,24], over-expression of NADH (or NADPH) synthesis enzymes [25,26] and new pathway construction for non-3-hydroxybutyrate (non-3HB) monomer synthesis such as 4-hydroxybutyrate (4HB) or 3-hydroxyvalerate (3HV) from glucose alone [27–31], as well as the deletion or repression on pathways competing for PHA monomers (Figure 1) [24,26,32,33•].

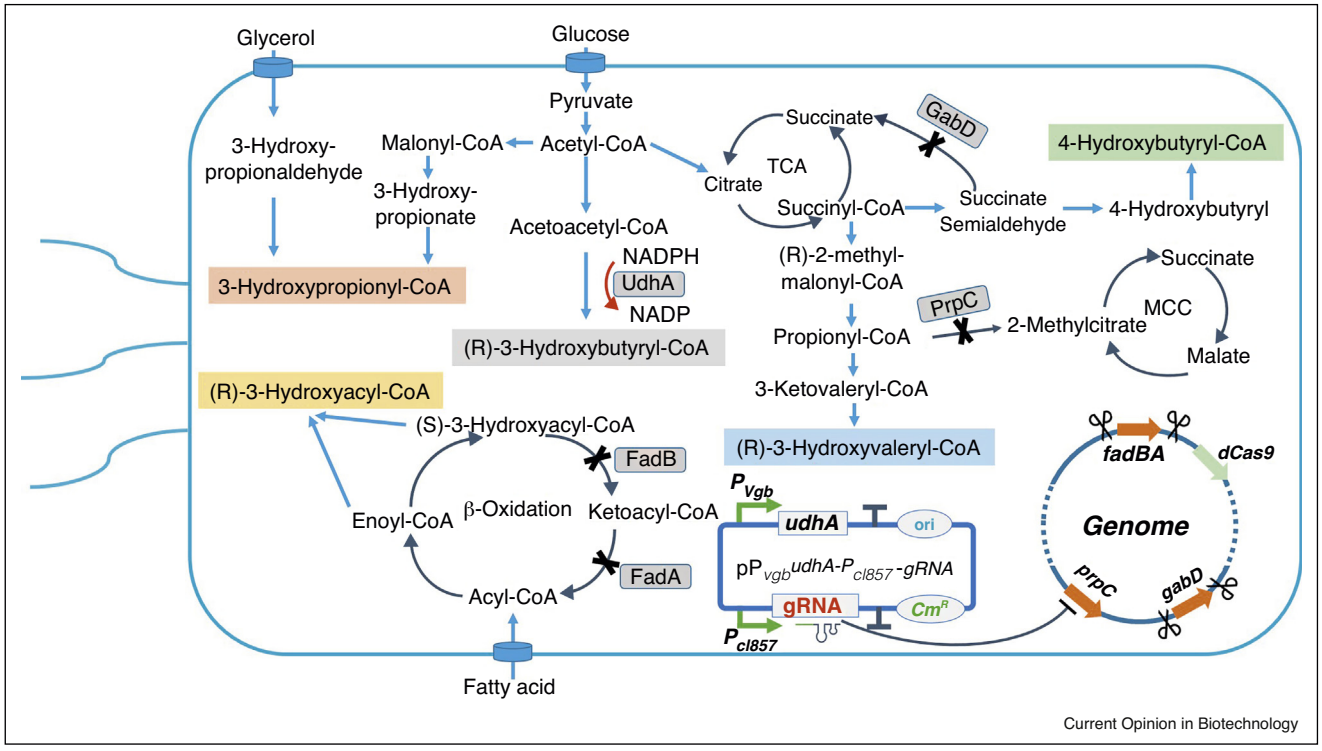
On the other hand, the DNA reprogramming technology has been developed very fast. Now the PHA synthesis can also be improved by reprogramming RBS to achieve optimized levels in the PHA synthesis operon [34••]. The CRISPR/Cas9 technology, especially the CRISPRi, has been used successfully to manipulate the PHA synthesis-related genes [13,35••] for controlling the PHA structures and molecular weights.

On the other hand, it is becoming very important to control cell growth rate and cell size for improving PHA synthesis. As growth rate will determine the PHA synthesis yield in terms of g/L/h, cell sizes will decide the amount of PHA accumulated intracellularly [36,37]. Last but not least, the PHA synthesis operon host organisms, namely, the chassis, is one of the most critical factors for PHA biosynthesis. It is suggested that robust microorganisms, especially bacteria that are resistant to contamination, are more useful for the above engineering to add new properties for effective PHA production and applications [38,39]. The coming Next Generation Industrial Biotechnology (NGIB), which will be discussed in other places, will rely on contamination resistant microorganisms for the open and continuous production of bulk chemicals, materials and fuels. This paper review progresses made in the past few years to improve PHA biosynthesis efficiency with an aim to economic production of PHA (Table 1).

Improving metabolic flux to PHA synthesis

Since PHA biosynthesis is competing with many other metabolites and intermediates, it is important to remove

Figure 1



Engineering metabolic pathways to channel more carbon source to PHA synthesis. The cost of PHA production was reduced by engineering the extremophiles for consuming low-cost substrates (such as glucose or glycerol) to produce various PHA monomers (highlighted by different colored boxes). At the same time, the weakening of beta-oxidation cycle, the deletion or repression (CRISPR/Cas9 tool) on pathways competing for PHA monomers and the over-expression of NADH (or NADPH) synthesis enzymes help to improve metabolic flux to PHA synthesis. *Abbreviations*: UdhA, soluble pyridine nucleotide transhydrogenase; GabD, succinate semialdehyde dehydrogenase; PrpC, 2-methylcitrate synthase; FadA, thiolase; FadB, hydroxyacyl-CoA dehydrogenase/enoyl-CoA hydratase.

Table 1

Various possibilities to improve PHA biosynthesis.

Approach	Advantages	Disadvantages	References
Substrate limitation such as N, P, S, Fe, and so on	Convenient	Limitation affects cell growth	[18]
Anaerobic PHA synthesis	High substrate to PHA conversion and saving energy	Slow growth and low biomass	[19–21]
Over-expression on PHA synthesis operon	Most convenient	Plasmid instability	[27–31]
Chromosomal over-expression of PHA synthesis genes	Convenient and stable	Low gene copy	[25,38]
Enhanced NADH or NADPH supply for PHA synthesis	Convenient	Upset other metabolic balances	[25,26]
Shutdown competing pathways: deleting β-oxidation	Improving fatty acids to PHA conversion	Fatty acids are only PHA precursors but not cell growth substrates	[24,33•]
Morphology engineering to increase cell sizes	Increase PHA content	Cell number can be low	[37,45•]
Controllable morphology engineering	Increase PHA content and biomass	Difficulty to induce morphology changes at high cell density	[47••]
Optimization of RBS or/and promoter of PHA operon	Convenient	Promoter difficult to induce at high cell density	[34••]
Engineering extremophiles	Reduce PHA process complexity	Difficulty to conduct molecular engineering	[43,58••]
CRISPR/Cas9 or/and CRISPRi to program PHA synthesis	Convenient for multiple gene deletions or repressions	Large Cas9 a burden for cell growth	[35••]