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Can bio-plastics replace non-biodegradable plastics?

Abstract

Poly-3-hydroxybutyrate (PHB) is widely studied and best-characterized bioplastic within polyhydroxyalkanoates family, which can be used to produce a wide range of household and packaging products as well as medical products. Although biodegradable PHB is environmental friendly and not dependent on fossil resources, its production cost has been traditionally very expensive by bacterial fermentation techniques using recombinant *E. coli*. The recombinant diatoms and transgenic plants have also been evaluated for efficient PHB production. But, it has proved extremely difficult to increase the PHB yield that prohibits its production at the industrial scale. To address these problems, this paper has focused on the metabolic pathway manipulations in recombinant *E. coli* since they lack PHB degradation pathways unlike native producers. Another advantage of using recombinant *E. coli* is their ability to use a wide range cheap carbon sources, accumulate large amounts of polymers with higher productivity, maintain the high-cell density fermentation and recover the PHB easily. Since no single strategy has been proved to be sufficient enough to produce PHB industrially until today, the advanced and integrated approaches have to be considered for its efficient production in order to compete with non-biodegradable petrochemical plastics.

Keywords: *E.coli, phb* genes, transgenic plant, bacterial fermentation, non-biodegradable, ecosystems

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Abbreviations: PHB, poly-3-hydroxybutyrate; PHAs, polyhydroxyalkanoates; EMP, embden-meyerhof-parnas; PP, pentose phosphate; ED, entner doudoroff; SD, serine deaminase

Introduction

Polyhydroxyalkanoates (PHAs) represent a diverse group of polyesters known as bioplastics that are synthesized by many microorganisms as intracellular carbon and energy storage compounds under stress conditions.[1] Since PHAs are completely biodegradable and have thermoplastic or elastomeric properties, they could offer an excellent alternative to non-biodegradable petrochemical based plastics.[2] However, the production cost of biodegradable plastics is comparatively very high that limits its industrial production. As a result, the current world is heavily dependent on non-biodegradable petrochemical plastics that are very cheap. Consequently, millions of tons of non-biodegradable waste plastics are usually burned and/or dumped in landfills worldwide every year, which basically increase carbon footprint in the environment leading to global warming and natural calamities through affecting natural ecosystems. Poly-3-hydroxybutyrate (PHB) is widely studied and best-characterized thermoplastic polyester within PHA family, which can be used to produce a wide range of household and packaging products such as grocery bags, soda bottles, disposable razors, flatware, etc. When they are discarded after use, the soil bacteria degrade the bioplastics naturally into water and carbon dioxide within a very short period of time. In addition, PHB and its derivatives are currently used in the medical procedures such as internal suture, vehicle for drug delivery, etc. Since bioplastics are nontoxic, biodegradable and biocompatible, they do not have to be removed after patient's recovery. PHB occurs naturally in bacteria such as *Ralstonia eutropha* and *Bacillus megaterium* that has become a model polymer for research on PHA, and for establishing and optimizing biotechnological production processes. PHB synthetic pathway genes from *Ralstonia eutropha* have been expressed in *E. coli* in order to maximize the PHB production since the bacteria *E. coli* do not possess PHB degradation pathways unlike native producers.[3–5] Consequently, many different fermentation strategies and downstream methods have been developed for its efficient production.[6,7]

The bioplastic PHB can also be produced from plants using modern molecular biology techniques. Bacterial enzymes for PHB synthesis have been introduced into the model plant *Arabidopsis thaliana*, but the PHB yield was extremely low.[8] The main barriers of using plants are their slow growth and requirement of valuable large land area. However, biotechnological advances might improve the yield of bioplastics significantly in near future that would ultimately lead the companies to turning acres of weeds into plastic factories. To reduce the carbon footprint and the dependence on foreign oil, this new green alternative might provide an additional cash crop for farmers. The PHB encoding genes have also been expressed in diatom (*Phaeodactylum tricornutum*) resulting PHB accumulation into cytoplasm. The PHB yield in diatom was about 10% of the dried cell weight after seven days.[9] Moreover, a cyanobacterium, *Synechocystis* has been reported to produce PHB when nutrients such as nitrogen become limited, which basically helps the cyanobacteria to survive under adverse conditions. However, the cyanobacteria do not naturally produce sufficient PHB. To address the limitations and opportunities for efficient PHB production, this paper has reviewed the metabolic pathway manipulations in recombinant *E. coli* since they lack PHB degradation pathways. As a result, PHB granules are not degraded in recombinant *E. coli* once synthesized. Moreover, the bacteria *E. coli* possess several advantages such as the ability to use a wide range cheap carbon sources, accumulating large amounts of polymers with higher productivity, maintaining the high-cell density fermentation and recovering PHB easily. This paper also urges to conduct more research on developing an advanced and integrated system for efficient PHB production in order to explore its feasibility at commercial scale production.

Discussion

In native microorganisms, PHB is synthesized from acetyl-CoA by three enzymatic reactions that are catalyzed by the enzymes β-ketothiolase, acetoacetyl-CoA reductase and PHB synthase encoded by *phaA*, *phaB* and *phaC* genes, respectively.[10] Since the whole genome and metabolic pathways of *E. coli* are known, a range of studies have been conducted to evaluate PHB production in recombinant *E. coli*. It has been found that increasing level of the precursor acetyl-CoA and the cofactor NADPH enhances the synthesis of PHB in recombinant *E. coli*.[3,5] The principle metabolic pathways and PHB synthetic pathways in *E. coli* have been illustrated in Figure 1. In *E. coli*, most of the acetyl-CoA is synthesized from pyruvate through Embden-Meyerhof-Parnas (EMP) pathway whereas the cofactor NADPH is mainly synthesized via pentose phosphate (PP) pathway (Figure 1). Modulating the EMP pathway by over expressing the *fbaA* gene encoding fructose bisphosphate aldolase and *tpi* gene encoding triosephosphate isomerase in recombinant *E. coli* had resulted higher PHB accumulation compared to the reference strains because of increased carbon flux towards the intermediary metabolite acetyl-CoA.[7] It has been found that inactivation of *pgi* encoding enzyme phosphoglucose isomerase, which catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate, increased the pool of NADPH because of reduced carbon flux to the EMP pathway. As a consequence, most the carbon flux was channeled to PP pathway that eventually improved the PHB yield in recombinant *E. coli* since the NADPH is required in the synthesis of PHB.[3,5] The most critical enzyme trans-ketolase (encoded by *tktA*) in the non-oxidative PP pathway was transformed into *E. coli* containing *phbCAB* operon to reinforce the metabolic flux of carbohydrate towards PHB accumulation (Figure 1). It was observed that biosynthesis of PHB was significantly increased due to carbon flux redistribution to supplement the precursor molecules acetyl-CoA.[11] Moreover, the *talA* gene encoding trans-aldolase, another important enzyme in the non-oxidative PP pathway, was amplified in *E. coli* having the *phb* genes to modulate the metabolic flux towards the PHB biosynthetic pathway. The study showed that the PHB content in recombinant *E. coli* co-harboring the *phb* and *talA* genes was increased from 28% to 52%.[12] The reason was due to the abundant supply of both NADPH and acetyl-CoA, which were basically supplemented from the activated PP pathway through the *talA* gene encoding enzyme trans-aldolase mediated modulation. Over expression of *zwf* gene encoding enzyme glucose-6-phosphate dehydrogenase and *gnd* gene encoding 6-phosphogluconate dehydrogenase in oxidative PP pathway increased the pool of NADPH that ultimately enhanced the PHB synthesis in recombinant *E. coli*.[13] Moreover, the NADP-dependent glyceraldehyde-3-phosphate dehydrogenase (encoded by *gapN*) from a mutant of *Streptococcus*, which catalyzes the conversion of glyceraldehyde-3-phosphate to glycerate-1, 3-bisphosphate, was over expressed in *E. coli* having *phb* genes.[14] The result showed that the PHB accumulation was increased due to the excess supply of NADPH. Furthermore, deletion of the main acetate pathway genes (*ackA* and *pta*) and the lactate pathway gene (*ldh*) in recombinant *E. coli* abolished acetate and lactate production leading to increased pool of acetyl-CoA, which eventually enhanced the PHB synthesis.[14,15]

In *E. coli*, acetyl-CoA is mostly synthesized from pyruvate catalyzed by the pyruvate dehydrogenase (PDH) complex, which is an important intermediate metabolite in both catabolic and anabolic reactions (Figure 1). On the other hand, pyruvate is mainly synthesized through the coupled reaction of glucose uptake mediated by the phosphotransferase transport system, and through the EMP pathway and the Entner Doudoroff (ED) pathway.[16] Moreover, pyruvate can also be synthesized in *E. coli* from L-serine via serine deaminase (SD) pathway.[17] Therefore, L-serine deaminases (encoded by the gene *sdaA*), catalyze the reactions to converting L-serine to pyruvate, were over expressed in recombinant *E. coli*.[18] It was found that PHB accumulation was enhanced due to increased supply of pyruvate towards the PHB synthetic pathway via the precursor metabolite acetyl-CoA. The expression of SD and ED pathway genes together with PDH complex encoded genes (*aceE*, *aceF* and *lpdA*) have also been cloned in recombinant *E. coli* to evaluate the PHB production (see Figure 1 for pathways). Co-over expression of *sdaA* gene encoding L-serine deaminase that catalyzes the conversion of L-serine to pyruvate and L-serine biosynthesis genes (*SerA, SerB* and *SerC*), and *pgk* gene encoding phosphoglycerate kinase activated the SD pathway in recombinant *E. coli* harboring *phb* genes from *Ralstonia eutropha*.[5] The result showed that the PHB yield was increased by 2. 3-fold compared to the reference strain when glucose was used as the sole carbon source. Moreover, activating the ED pathway coupled with over expression of PDH complex encoded genes further enhanced the PHB production in recombinant *E. coli* due to the increased level of both pyruvate and acetyl-CoA.[5]

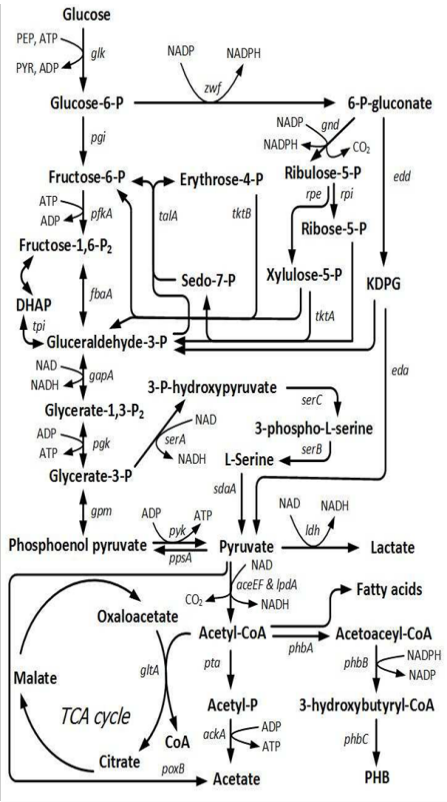

Figure 1 The main metabolic pathways and PHB synthetic pathways with their respective genes in recombinant *E. coli*.

KDPG, 2-keto-3-deoxy-6-phospho gluconate; DHAP, dihydroxyacetone phosphate; Sedo-7-P, sedoheptulose-7-phosphate; TCA, tricarboxylic acid; glk, glucokinase; pgi, phosphoglucose isomerase; pfkA, phosphofructokinse; fbaA, fructose bisphosphate aldolase; zwf, glucose-6-phosphate-1-dehydrogenase; gnd, 6-phosphogluconate dehydrogenase; tkt, transketolase; talA, transaldolase; edd, phosphogluconate dehydratase; eda, 2-keto-3-deoxygluconate-6-phosphate Aldolase; gapA, glyceraldehyde-3-phosphate dehydrogenase; pgk, phosphoglycerate kinase; gpm, phosphoglyceromutase; sera, D-3-phosphoglycerate dehydrogenase; serB, phosphoserine phosphatase; serC, 3-phosphoserine aminotransferase; sdaA, l-serine deaminase; pyk, pyruvate kinase; ppsA, phosphoenolpyruvate synthase; ldh, lactate dehydrogenase; aceE, pyruvate dehydrogenase E1; aceF, pyruvate dehydrogenase E2; lpdA, lipoamide dehydrogenase; poxB, pyruvate oxidase; pta, phosphate acetyltransferase; ackA, acetate kinase; phaA, β-ketothiolase; phaB, NADPHdependent acetoacetyl-CoA reductase; phaC, PHB synthase; gltA, citrate synthase

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Conclusion

Millions of tons of petroleum-based plastics are produced and consumed every year worldwide generating a significant amount of waste, which have been affecting our environment adversely leading to global warming. Although PHB is biodegradable and not dependent on fossil resources, its production cost has been traditionally very expensive by bacterial fermentation techniques using recombinant *E. coli*. The recombinant diatoms and transgenic plants have also been tested for PHB production. But, it has proved extremely difficult to increase the yield of bioplastics that limits its viability for industrial production. Until today, no single strategy has been shown to be effective for the production of bioplastics in order to compete with petroleum-based plastics. Therefore, the advanced and integrated approaches must be considered for production of biodegradable plastics. One such strategy would be to explore an integrated waste water treatment plant where different types of native and/or recombinant microorganism including *E. coli*, microalgae and diatom will be harvested in sequential procedures or in mixed cultures for concomitant production of bioplastics and biofuel besides clean water (Figure 2).

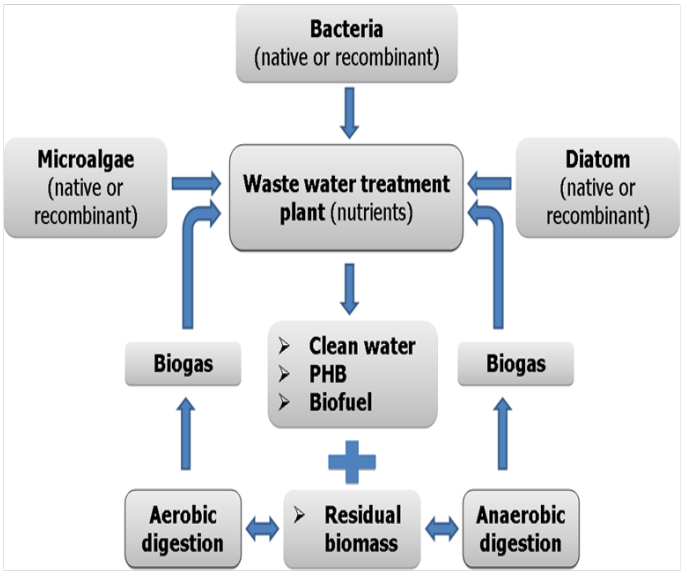

Figure 2 An advanced and integrated waste water treatment plant to produce PHB.

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Conflict of interest

The author declares no conflict of interest.

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