

### **Biosynthesis of Polyhydroxyalkanoate by Recombinant Bacteria: A Review**

#### **Abstract**

Polyhydroxyalkanoates (PHAs), is a biodegradable polymer, has many industrial and medical applications such as heart valves, scaffold, suture and drug delivery. Because of their structural diversity and close analogy to plastics, PHAs have gained major interest over the world. There are different sources including natural isolates, recombinant bacteria, and plants are being reported to improve the quality, quantity and economics production of PHAs. Their biodegradability makes them particularly desirable alternative source for the synthetic plastic. The biosynthetic genes of PHB: *phbA*, *phbB* and *phbC* are clustered together and systematized in one *phbCAB* operon. The PHB pathway is highly deviating in the bacterial genera with respect to alignment and clustering of the involved genes. Regarding of this, the enzymes appear high degree of sequence preservation.

The structural studies further improve the mechanism of action of these enzymes and helped in improving and choosing the good candidates for increased production of PHB.

**Keywords:** bioplastics; PHA; polyhydroxyalkanoates; recombinant bacteria

#### **1. INTRODUCTION**

Plastics are used in most of industries ranging from cars to medicine. Plastics has many of advantageous because of its synthetic polymers, their structure have a wide range of shapes and strengths with molecular weights ranging from 50,000 to 1,000,000 Da [1,2]. Many forms of synthetic polymers including polyethylene, polyvinyl chloride and polystyrene are largely used in plastics manufacturing. Plastics can be easily folded into any favorite shape as fibers and thin films with high chemical resistance and less elastic, therefore widespread in many disposal goods, durable, and as packaging materials [3].

Recently, the replacement of the non-biodegradable by degradable plastic is of more focusing for the decision-makers and for investors in the plastic industries [4]. The manufacturing of eco-friendly bioplastics can help us to solve the pollution problems caused by non-degradable

plastics. PHA is a bioplastic that have gained recent interests due to its properties which are safety engineering practice, being ethical and environmental-friendly [5, 6]. The previous studies reported that, the discovery of bioplastics as PHAs from microbial sources had important role on decreasing of the environmental pollutions [7]. Moreover, PHAs with the same properties of synthetic plastics have also wide range of biomedical field applications such as tissue engineering, drug delivery system, cardiac stents, and medical packaging purposes [8]. The currently research studies are being executed to improve the PHAs productivity, decreases the production costs, and produce PHA with specific functions. The using recombinant *E. coli* strains for the biopolymers production has many of advantages, including growing fast, using wide range of carbon sources, the genetics and metabolic pathways are well understood, and less costly downstream processing lines [9].

The common PHA producing bacteria includes *Ralstonia eutropha*, *Alcaligenes latus*, *Azotobacter beijerinckii*, *Cupriavidus necator*, *Bacillus megaterium*, and *Pseudomonas oleovorans* (Table 1).

Recently approach is to use metabolic engineering methods to create recombinant strains of commonly used bacteria. These recombinant bacterial strains have modified metabolic pathways to produce high yields of PHA [10, 11]. The resulted recombinant strains will be having many of different advantages than the wild one, includes their high efficiency in utilizing inexpensive and different sources of renewable carbon with minimal fermentative supplies for giving rapid growth and high PHA accumulation.

Table 1: Different Wild Bacterial Strains for PHA Production

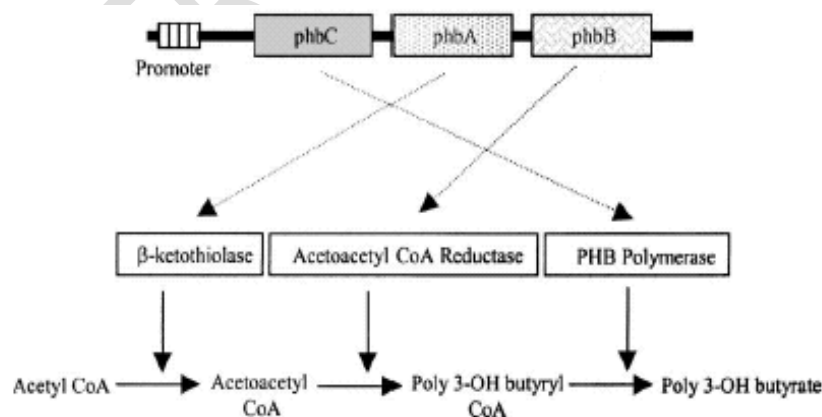
PHA producing bacteria	Carbon Source	PHA Yield (wt %)	References
<i>Bacillus megaterium</i>	Glycerol	73%	[12]
<i>Azotobacter vinelandii</i>	Sucrose	62%	[13]
<i>Cupriavidus necator</i>	Jatropha oil	24%	[14]
<i>Haloferax mediterrane</i>	Glucose	61%	[15]
<i>Pseudomonas oleovorans</i>	n-alkanes	60%	[16]
<i>Ralstonia eutropha</i>	Glucose	69%	[17]

The previous studies reported that, the recombinant *E. coli* has commonly used as PHA production due to its suitability for genetic handling, high rate of growth, high cell density cultivation and ability to used inexpensive sources of carbon. The *E. coli* strain has been reported to produce different types of short-chain length (scl) polyesters having C4 or C5 monomers, such

as poly(4-hydroxybutyrate) P(4HB) homopolymer, P (3HB), poly(3-hydroxyvalerate) P(3HV), P(3HB-co-3HV) copolymer and P(3HB-co- 4HB) [11]. However, the recombinant strain of *E. coli*, having the *phaC1* gene from *Pseudomonas aeruginosa*, was capable to produce medium chain- length (mcl) of PHAs, which having C6 to C14 monomers such as homopolymers of 3-hydroxydodecanoate (3HDD), 3-hydroxyhexanoate (3HHx), and terpolymer of P(3HB-co-3HV-co-3HHx), poly (3-hydroxybutyrate-co-3- hydroxyvalerate-co-hydroxyhexanoate) [18]. Furthermore, the hybrid polymers which containing both of the scl- and mcl-monomer units such as P(3HB-co-3HHx), poly(3-hydroxybutyrate-co-hydroxyhexanoate), naturally produced by *Aeromonas caviae*, can be also synthesized in the recombinant *E. coli* by genetic manipulation [19].

## 2. PHA SYNTHESIS AND PRODUCTION BY RECOMBINANT BACTERIA

The biosynthetic pathway of P(3HB) consists of three enzymatic reactions catalyzed by three different enzymes (Fig. 1). The first reaction consists of the condensation of two acetyl coenzyme A (Acetyl-CoA) molecules into acetoacetyl-CoA by  $\beta$ -ketoacyl CoA thiolase (encoded by *phbA*). The second reaction is the reduction of acetoacetyl-CoA to (R)-3-hydroxybutyryl-CoA by an NADPH-dependent acetoacetyl-CoA dehydrogenase (encoded by *phbB*). Lastly, the (R)-3-hydroxybutyryl- CoA monomers are polymerized into PHB by P(3HB) polymerase, encoded by *phbC* [20].



**Fig. 1.** Biosynthetic pathway of poly(3-hydroxybutyrate). P(3HB) is synthesized by the successive action of  $\beta$ -ketoacyl-CoA thiolase (*phbA*), acetoacetyl-CoA reductase (*phbB*) and PHB polymerase (*phbC*) in a three-step pathway. The genes of the *phbCAB* operon encode the three enzymes. The promoter (P) upstream of *phbC* transcribes the complete operon (*phbCAB*) [21].

The comparative analysis between the natural and recombinant strains of PHA production clearly indicated that the wild strains of PHA producers synthesized high amounts of PHA with definite restrictions which includes low rate of growth, cell lysis difficulty for more extraction and purification and low genetic manipulations, while, the recombinant strains of PHA producers solved all these restrictions [22]. The bacterial strain *Cupriavidus necator* showed over expressed *phaCAB* gene from a plasmid showed increase in P3HB production by 40% of cell dry weight and reduction in the time of fermentation by 20 % with the decrease in the production cost compared with the wild strain [23]. The interest now is the reducing of the production cost of PHA produced by the recombinant DNA technology. To achieve this, the PHA synthesizing gene isolated from a natural PHA producing bacteria as *Ralstonia eutropha* is inserted into a host bacterium of PHA producing as *Pseudomonas* to enhance the PHA biosynthesis or insert into a non-PHA producing bacterial strains as *E. coli* to get the capability biosynthesis of the PHA.

The metabolically engineered of *Ralstonia eutropha* strain gain the capability to expression of the L-arabinose hydrolyzing enzyme which let the recombinant strain to metabolize L-arabinose as carbon source to give high amounts of intracellular PHB accumulation [24]. Earlier studies reported that, different natural PHA producing bacteria such as *Pseudomonas oleovorans*, *Methylobacterium*, *Bacillus megaterium*, and *Ralstonia eutropha* were used mainly for production of PHA at commercial scales. Nowadays, many of recombinant bacterial strains are replaced to produce of PHAs at the industrial scales because the recombinant strains are found with many advantageous than the wild one due to its high yield of production, fast production, easy manipulation, ability to use broad range of inexpensive carbon source and the low production costs [12]. The bacterial strains as *E. coli*, *Bacillus subtilis* and *Pseudomonas putida* are the common used as recombinant bacterial strains for PHA production at the industrial scales [22].

The mechanisms of biosynthesis of PHA inside the bacterial strains is the conversion of different carbon sources into PHAs. The use of recombinant strain, can be activated to produce PHAs without convincing stressed conditions, such as phosphorus or nitrogen starvation, which lead to decrease the production cost. In addition, the recombinant strains are well-defined and thus could be further engineered for optimization for PHA production. In addition, *E. coli* is the common organism used as recombinant strain for producing PHAs. It utilizes high lactose containing dairy whey as a carbon source with the PHA-producing genes (the *pha* operon) from *C. necator*.

The traditional strains of *E. coli* available in molecular biology labs include DH5 $\alpha$ , JM, or XL1-Blue often lack the capability to use lactose as a nutrient carbon source. When nine different strains derived from wild-type cells were tested for their ability to produce PHA using lactose as a sole carbon source, it was reported that the strains GCSC6576 and GCSC4401 are transformed with a high-copy-number plasmid, while pSYL107 containing the *A. eutrophus* PHA biosynthesis operon, were the greatest able to PHAs producing [25]. The *E. coli* strains GCSC6576 (pSYL107) was grown on a high concentration of whey as a carbon substrate, yields 87 g/L of dry cell weight with 79% content of PHB [26]. As the same, recombinant *E. coli* is the excellent PHAs producer when using the *pha* operon from *C. necator* bacterial strain [27]. Several of *E. coli* strains were known to be able to apply lactose when transformed with the plasmid pJC4 and *A. latus pha* genes. Also, the strain CGSC 4401 was the best strain for PHB production with 119.5 g/L dry cell weight and 80.5% PHB content [28].

Two other previous studies were used the recombinant *E. coli* in production of PHAs from whey. Pais et al. [29] used proton suicide methodology to select the recombinant strain of *E. coli* that synthesized low amount of organic acids after transformation with operon of the *C. necator pha*. The study results obtained the low production of organic acid resulted in slow growth of the bacterial strain with high production of PHB accounted as 18.88 g PHB/L for the recombinant strain vs 7.8 g PHB/L for the wild strain [29]. The maximum cell density and PHB concentrations were 70.1 g/L and 73% [30].

Another study successfully cloned of the *pha* operon from *C. necator* into *E. coli* XL1-Blue with utilizing soy waste for PHB production, the soy waste was hydrolyzed by NaOH for 8h and 27.83% of the accumulated PHB was observed [31]. Furthermore, when this recombinant strain grown in glucose, about 43% dry cell weight of PHAs are produced [32]. This confirm that, the waste of soy and malt are good sources of carbon for PHA production.

Another study used restaurant waste that use the anaerobically digestion to produce lactic as a carbon source for the recombinant *E. coli* pNDTM2, the PHB accumulation content was 44% [33]. In addition to *E. coli*, another *Aeromonas* sp. (strain KC007-1) has also been used and accumulate 32.7% PHA [34]. With increasing of the genetic tools and techniques, it helps in optimizing of the synthesis pathways for non-native bacterial hosts to utilize and consume the substrates for PHAs production [35].

Table 2. Different Recombinant Bacterial Strains for PHA Production

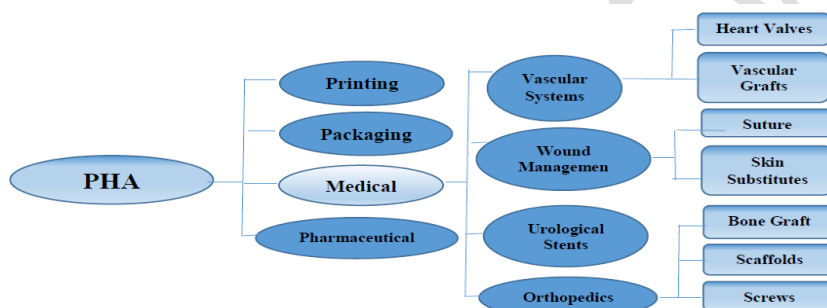
Strain	Plasmid	PHA operon origin	Dry cell weight (g/L)	PHA production (g PHA/g dcw)	Reference
<i>E. coli</i> GCSC 657	pSYL107	<i>C. necator</i>	6.6	82%	[25]
<i>E. coli</i> GCSC 657	pSYL107	<i>C. necator</i>	88	81%	[26]
<i>E. coli</i>	pSYL107	<i>C. necator</i>	34	81%	[27]
<i>E. coli</i> CML3-1	pMAB26	<i>C. necator</i>	33	28%	[29]
<i>E. coli</i> CGSC 4401	pJC4	<i>Alcaligenes latus</i>	119	80%	[28]
<i>E. coli</i> CGSC 4401	pJC4	<i>Alcaligenes latus</i>	192	86%	[36]
<i>E. coli</i> K24K	pJP24K	<i>Azotobacter</i> sp. FA8	NA	72%	[30]
<i>E. coli</i>	pUC19/PHA	<i>C. necator</i>	71	24%	[32]
<i>E. coli</i> XL1-Blue	pKS	<i>C. necator</i>	3	27%	[31]
<i>Aeromonas</i> KC007-R1	pRK415H16	<i>C. necator</i>	1.86	32%	[34]

### 3. GENETIC BASIS OF PHA SYNTHESIS

In the synthetic pathway of the PHB formation in the recombinant strains, the biosynthetic genes including, *phbA* (for 3-ketothiolase), *phbB* (NADPH-dependent acetoacetyl-CoA reductase), and *phbC* (PHB synthase) from Acetyl-CoA are clustered and apparently systematized in only one operon named *phbCAB* [21]. The multiplicity of the P(3HB) biosynthetic pathways indicates how far the *pha* loci have differed. Meanwhile, the genes encoding enzymes of ssc-PHA (*phb*) production and the genes encoding enzymes for msc-PHA (*pha*) production are not necessarily to clustered and the gene organization differs from one species to another. In *Acinetobacter* sp.: *Pseudomonas acidophila*, *Alcaligenes latus*, and *Ralstonia eutropha*, the *phbCAB* genes are in tandem on the chromosome and also not necessarily to clustered. While, *Rhizobium meliloti*, *Paracoccus denitrificans*, and *Zoogloea ramigera*, the *phbAB* and *phbC* loci are not linked [37]. In addition to, the *Chromatium vinosum*, *Synechocystis*, and *Thiocystis violacea*, the PHA polymerase has two sub-units encoded by *phbE* and *phbC* genes, while, the *phbAB* and *phbEC* genes are located in one locus but divergently oriented [38]. The *phb* loci in *P. acidophila*, *C. vinosum*, *R. eutropha*, *T. violacea* and *R. meliloti* have an additional gene named *phbF*, its function not defined in the PHA metabolism [39], while part of the gene encoding a protein homologous to the hypothetical *E. coli* protein *yfiH* is located upstream of the *P. acidophila*, *Z. ramigera*, and *R. eutropha* P(3HB) polymerase genes. On the other hands, the *P. oleovorans* and *Pseudomonas aeruginosa* bacterial strains producing msc-PHA, the *pha* loci contain two *phaC* genes separated by *phaZ*, which encodes an intracellular PHA depolymerase [38].

### 4. APPLICATIONS

Different applications of the PHAs, because of its chemical properties such as they are inert, non-toxic and has antimicrobial activities, therefore, they are used for manufacturing of pharmaceutical items such as the adhesive films and hygiene-related baby items, including nappies [21]. As well as, they can be used in manufacture of electronic products such as mobile phones and computer tablets and due to their amphiphilic properties makes them appropriate for encapsulating seeds and fertilizers, biodegradable films and storage containers of crops. Also, PHAs have many of medical applications including orthopaedic straps and pins, adhesive bandages, surgical sutures, repair stents, drug delivery systems, patches and matrix for bone marrow. Previous study reported that, when PHA combined with hydroxyapatite, it becomes a bioactive composite proper for regeneration and replacement of bone tissue [40]. There are other commercial uses of PHAs indicated in Fig. 2.



**Fig. 2** A flow chart indicating the various uses of PHAs.

The review concluded that, the recombinant bacterial strains have numerous of advantages than the wild type of bacterial PHA producers. In addition to, the recombinant bacterial strains have the capability to give large numbers and low production cost of different types of PHAs by using different sources of cheap and renewable carbon such as the industrial waste water, agricultural waste, dairy waste products. The other advantages include high rates of growth to realize high cell density, easy intracellular recovery of PHA, simplified manufacturing methods, effective for genetic and metabolic manipulation.

## 5. CONCLUSIONS

PHAs are extremely important because they allow society to separate the economic growth from the resource tiredness and the environmental destruction. With increase in the PHAs diversity there will be a significant increase in their application. However, the cost of production of PHAs forces their large-scale commercialization and industrialization. A lower-cost can be attained by

participating different scientific fields such as synthetic biology, systematic biology, biotechnology and cultivation of high-cell-density, which will simplify the synthesis of all PHA kinds.

## **CONSENT**

It is not applicable.

## **ETHICAL APPROVAL**

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

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