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Polyhydroxyalkanoates (PHAs), Intracellular Pathways and Properties

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ABSTRACT

Manufacturing of hard biodegradable petroleum based plastics harmfully affect the environment. Polyhydroxyalkanoates (PHAs) naturally produced as carbon storage polymer by various Monera kingdom microorganisms, resemble synthetic polymers in many chemical and physical properties. Renewable and biodegradable features made attracted much attention to these polyester bond polymers. PHAs extraction studied in many microorganisms made it naturally or engineered, where Poly 3-hydroxybutyric acid (PHB) is the most common. Main enzymes involved in PHB synthesis by *Ralstonia eutropha* encode by phbCAB gen cluster. Production of polyester particles is induced by excess quantity of carbon sources and nitrogen or some other factors starvation. PHAs production costs are still a drawback to wide usage, the future trend should focus on more efficient and economical processes developing for PHA production.

Key words: Bioplastics, Polyhydroxyalkanoates(PHAs), Microorganisms, Pathways

INTRODUCTION

Manufacturing of plastics requires multiple amounts of petroleum; affect the environment harmfully (Bubacz and Goldsbery, 2014). The term "biomaterials" includes chemically unrelated products that are synthesized by organisms under different environmental conditions Luengo *et al*., 2003). Polyhydroxyalkanoates (PHAs) linear structure made biologically by diverse set of repeating ester bond units, where poly 3-hydroxybutyric acid (PHB) made by hydroxybutyrate units (Shakeri *et al*., 2011) is the most common (Singh and Parmar, 2011). PHAs accumulates water insoluble (Shakeri *et al*., 2011) carbon storage intracellular polymers (Sing and Parmar, 2011), have no osmotic pressure effects (Ksekdau *et al*., 2003).

PHAs naturally produced by various prokaryotic microorganisms including archaea and bacteria (Shakeri *et al*., 2011) either gram positive or gram negative (Elsayed *et al*., 2013) and some phototrophic cyanobacteria (McQualter *et al*., 2014; Schlebusch *et al*., 2013). They resembles synthetic polymers in many chemical and physical properties (Bagheriasl *et al*., 2012; Mullaney and Rehm, 2010), have been produced for use as bulk commodity plastics, fishing lines, and medical uses (Lu *et al*., 2009) such as pharmacy and drug delivery systems (Shakeri *et al*., 2011). PHAs have also attracted

much attention as biodegradable polymers that can be produced from biorenewable resources (Lu *et al.*, 2009).

PHA synthesis pathway

Our Knowledge about PHB production derived from *Ralstonia eutropha* (Jo *et al.*, 2007) also known as *Cupriavidus necator*, *Wautersia eutropha*, and *Alcaligenes eutrophus* (Kocharin, 2012). A single chain translational fusion protein comprising three enzymes is required to establish the PHB biosynthesis pathway (Mullaney and Rehm, 2010). Enzymatic activities involved in PHB synthesis codes by phbCAB operon (Jo *et al.* 2007) as phbCAB gen cluster (Peralta-Gil *et al.* 2002). These enzymes are PHA synthase, NADPH-dependent acetoacetyl-CoA reductase, and â-ketothiolase encoded by phbC, phbA, and phbB genes, respectively (Ojume and Solomon 2003). Peralta-Gil *et al.* (2002) reported, in *Azotobacter vinelandii* the *phbR* transcriptional activator gene located upstream of *phbBAC, belonging* AraC family of activators (Peralta-Gil *et al.*, 2002). The PHA synthesis can be summarized in eight pathways showed in fig. 1.

The expression PHB synthesis is post transcriptional at the level of â-ketothiolase activity, which catalyzes the first step of PHB synthesis (Peralta-Gil *et al.*, 2002). Glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase are $NADP^+$ regenerating enzymes as cosubstrate of acetoacetyl-CoA reductase, one of three key enzymes involved in the biosynthesis of PHB (Yamane, 1992). CoA inhibits â-ketothiolase activity under relaxed oxygen conditions by feeding acetyl coenzyme A into the tricarboxylic acid cycle. Under oxygen limitation and carbon excess conditions, NAD(P)H level increases, inhibits citrate synthase and isocitrate dehydrogenase. The level of acetyl-CoA and lowering the CoA has elevated by inhibiting these two enzymes, inhibition of â-ketothiolase by CoA is overcome and allows synthesis of PHB to proceed (Peralta-Gil *et al.* 2002).

Many genes encoding various enzymes directly or indirectly involved in PHA synthesis. Table 1 summarized pathways, abbreviation, and involved enzymes in PHAs synthesis.

DISCUSSION

Bioplastics are an alternative substitute for petrochemical synthetic plastics (Ismail *et al.*, 2010), preferred candidates for developing controlled release drug delivery vehicles and also can be used in biomedical implants and biofuels (Elsayed, 2013). Bioplastics are becoming increasingly prominent owing mainly to scarcity of oil, increase in the cost of petroleum based commodities, and growing environmental concerns with the dumping of non biodegradable plastics in landfills (Chen, 2014).

Cellular production of PHAs may be more “green” as compared to the use of specific metal catalysts for the production of polymers. The biosynthetic incorporation of specific monomers into PHA polymers is dependent on many factors that include the type of carbon source that the microorganisms are grown on (Lu *et al.*, 2009). The culture conditions have main effects to induce PHA production, where different species of single genus don’t even have the same physiological response when exposed to the same culture conditions (Shakeri *et al.*, 2011).

Production of polyester particles is induced when carbon source is in excess quantity or growth conditions have been imbalanced by declining other nutrient factors (Shakeri *et al.* 2011). PHAs extraction studied in many research from variety of microorganism including *Azotobacter* spp. (Khanafari *et al.*, 2006), lactic acid bacteria (Ksekdau *et al.*, 2003), *Bacillus* spp. (Chaijamrus and Udpuay, 2008), *Ralstonia* spp. (Shakeri er al., 2011), *Azomonas* sp. (Elsayed *et al.*, 2013) *Paracoccus denitrificans (Yamane, 1992), Azotobacter vinelandii (Peralta-Gil et al., 2002), Serratia* sp. (Keshavarz and Roy, 2010), *Sinorhizobium* sp. (Shakeri *et al.*, 2011), *Enterobacter aerogenes* (Aslam *et al.*, 2013) and some engenieerd bacteria such as *Escherichia coli* (Mullaney and Rehm, 2010), *Aeromonas hydrophila* (Enan and Bashady 2004) and eukaryotic *Saccharomyces cerevisiae* (Kocharin, 2013).

The viability of microbial large scale production of PHB is dependent on the development of a low cost process. The commercial production of PHB has reported by using cheap substrates such

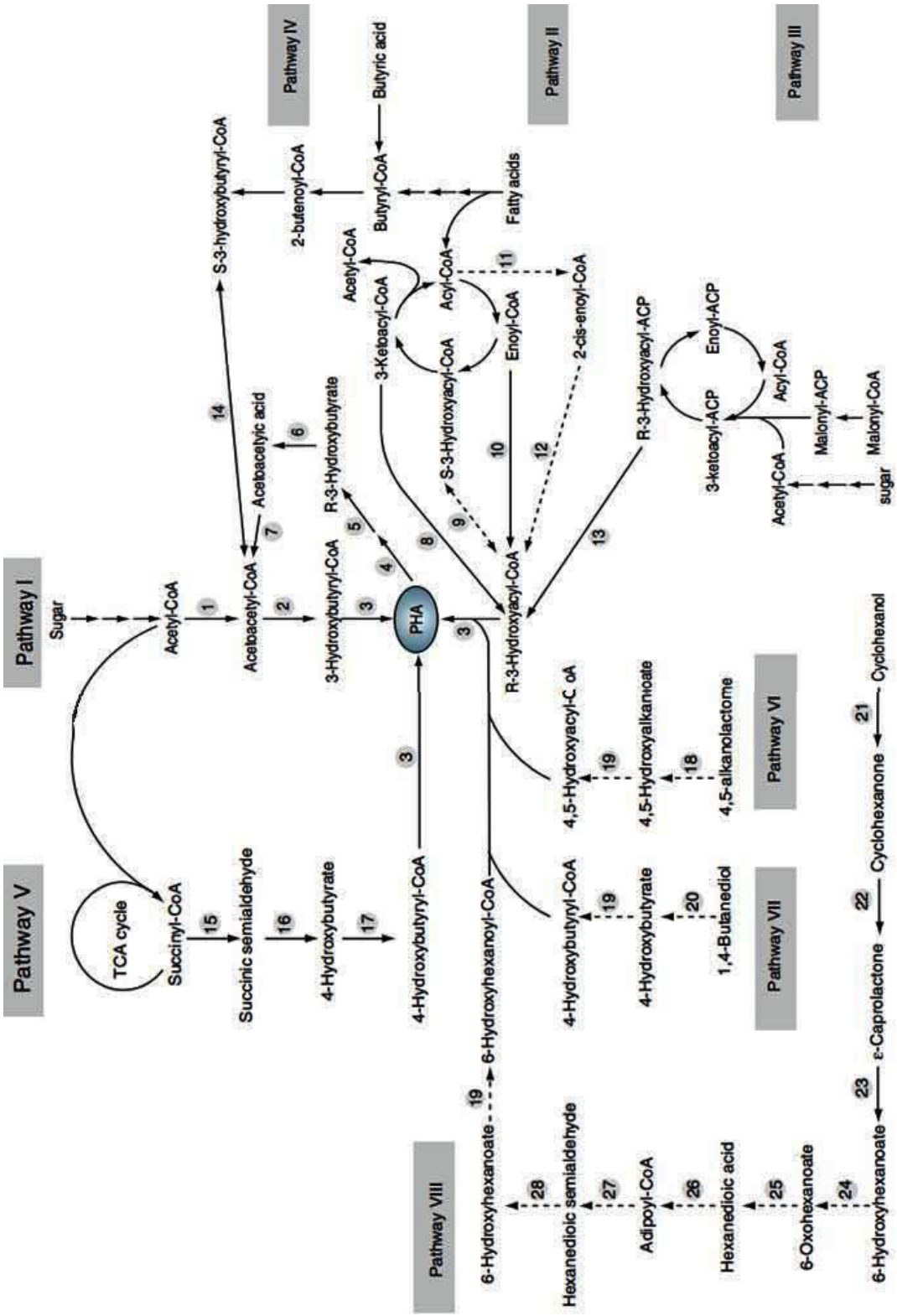

Fig. 1: Biosynthesis of PHA pathways (Chen, 2010)

Table 1: Synthesis pathways for PHAs (Chen, 2010).

No.	Pathway	Abbreviation	Enzyme
1	Pathway I	PhaA	β-ketothiolase
2		PhaB	NADPH dependent aceto-acetyl CoA reductase
3		PhaC	PHA synthase
4	Associated way	PhaZ	PHA depolymerase
5		PhaA	β-Ketothiolase
6		PhaB	-dependent acetoacetyl-CoA reductase
7		PhaC	PHA synthase
8	Pathway II	FabG	3-Ketoacyl-CoA reductase
9			Epimerase
10		PhaJ	(R)- Enoyl-CoA hydratas elenoyl-CoA hydratase I
11			Acetyl-CoA oxidase, Putative
12			Acetyl-CoA hydratase I, putative
13	Pathway III	PhaG	3-Hydroxyacyl-ACP-CoA transferase Malonyl CoA-ACP transacylase
		FabD	
14	Pathway IV		NADPH-dependent acetoacetyl-CoA reductase
15		SucD	Succinic semialdehyde dehydrogenase
16	Pathway V	4hbD	4-Hydroxybutyrate dehydrogenase
17		OrfZ	4-Hydroxybutyrate-CoA:CoA transferase
18	Pathway VI		Lactonase, putative
19			Hydroxyacyl-CoA synthase, putative
20	Pathway VII		Alcohol dehydrogenase, putative
21	Pathway VIII	ChnA	Cyclohexanoll dehydrogenase
22		ChnB	Cyclohexanone monooxygenase
23		ChnC	Caprolactione hydrolase
24		ChnD	6-Hydroxyhexanoate dehydrogenase
25		ChnE	6-Oxohexanoate dehydrogenase
26			Semialdehyde dehydrogenase, Putative
27			6-Hydroxyhexanoate dehydrogenase, putative
28			Hydroxyacyl-CoA synthase, putative

as methanol, beet molasses, ethanol, starch, whey, molasses, and soy in articles (Wei *et al.*, 2009). Khanafari *et al.* (2006) reported, PHB production by *Azotobacter chroococcum* on whey broth medium without extra salt can be was higher than other examined commercial media in their own research (Khanafari *et al.* 2006). Ksekdau *et al.* (2003) reported, *Lactobacillus* spp. produce the most PHB between lactic acid bacteria, where no significant correlation was observed between PHB production and the cell densities (Ksekdau *et al.*, 2003). Chaijamrus and Udpuay (2008) had a research on PHB production by *Bacillus megaterium* utilizing commercial nutrients, concluded the highest production was observed after 45h of growth when 4% molasses and 4% corn sleep liquor respectively as carbon and nitrogen sources were used (Chaijamrus and Udpuay, 2008).

CONCLUSION

Establishing industrial biotechnology for the production of chemical compounds from the biosynthetic pathway has received a significant boost with the implementation of metabolic engineering, produce new products with higher yield and productivities (Kocharin *et al.*, 2012). The increasing effects of non degradable plastic wastes is a growing concern environmental problems (Enan *et al.*, 2004; Suriyamongkol *et al.*, 2007). The versatility of PHAs has made them good candidates for the study of

their potential in a variety of areas from biomedical fields to food, packaging, textile and household materials (Keshavarz and Roy, 2010). Renewable PHAs biodegradable macromolecule polyesters naturally produced by many species of prokaryotic microorganisms, these features make them superior to their petroleum based plastics rival.

Depending on PHAs microbial origin, bioplastics differ in their monomer composition, macromolecular structure and physical properties. Most of them are biodegradable and biocompatible, which makes them extremely interesting from the biotechnological point of view (Luengo *et al*., 2003). PHAs production costs are still a drawback to PHAs wide usage, the future trend should focus on more efficient and economical processes developing for PHA production, isolation, purification and improvement of PHA material properties.

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