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Polyhydroxyalkanoate: a biodegradable polymer (a mini review)

A. B. Akinmulewo¹ and O. C. Nwinyi¹

¹Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria.
Corresponding author; akinmulewoadebola@yahoo.co.uk

Abstract-

The current synthetic plastic menace has driven researchers to sort sustainable alternatives. Polyhydroxyalkanoate (PHA) has been proven to be sustainable, biodegradable, biocompatible and hence could serve as suitable alternative. PHAs are biodegradable polyester produced by microorganisms that can be produced from renewable substrates such as starch and plant oils. These biopolyesters are accumulated in the intracellular granules and serve as carbon reserve for bacteria. Current studies show that there exists about 150 different monomers of PHA with shared properties similar to synthetic plastics which makes their application wide. This review is focused on giving a background study on polyhydroxyalkanoate, with special considerations on their physicochemical properties, its applications, the pathways that leads to its synthesis and the various applications.

Keywords: Biopolymers, Bioplastics, Plastics, Polyhydroxyalkanoate

1. Introduction

One of the major focal points constituted in United Nation's Sustainable Development Goals (SDG) is to create the access to clean water and the maintenance of the environment [1] for the general populace in the world. Globally, petroleum-based polymer level as recorded by the Watch world Institute in 2015, has been on the increase within a span of 5 decades, leading to a gross value of 299 tons of waste needing efficient disposal methods [2]. However, plastic produced mainly from petroleum extracts are generally not easily biodegradable, thus creating pyramids of waste causing major environmental havocs [3], [4]. These petroleum-based plastics pose the biggest threat to the sustenance of the environment by their accumulation in the environment; majorly in the marine environment [5], [6].

Several methods have been discovered by international organizations, researchers and government bodies to efficiently manage plastic wastes created [7]. One of the managerial strategies adopted by governmental incentives was to support recycling activities, illegal dumping and litter prevention [7]. Although this solution has greatly reduced environmental impact of these plastic wastes especially in Europe [7] this process tends not to be sustainable in developing economy such as Africa where little or minimal recycling is done [8]. To solve the environmental problem, there is a need for a method that can be widely employed across all sectors, economies and society. This solution is to produce biodegradable plastics that are not only environmentally friendly [9], [10], but also cost-effective compared to conventional plastics [11].

Polyhydroxyalkanoate (PHA) is one of the bio-polyesters that have gained recent attraction as a viable bioplastic, that passes the required properties of a safety engineering practice, being ethical and environmental-friendly [5], [11].



2. Polyhydroxyalkanoate

The French scientist Maurice Limoges first discovered PHA in *Bacillus megaterium* in the form of poly (3-hydroxybutyrate) (PHB) in 1926 [5], [6], [12]. Polyhydroxyalkanoate (PHA) are thermoplastic polyesters with diverse structures and are produced by microorganisms (Table 1) when there is a limited nutrient supply such as nitrogen, oxygen, phosphorus, sulphur, in the presence of excess carbon [5], [9]. Under such stringent conditions, the organism is able to assimilate the carbon source and store as hydroxyalkanoates (HA) which is further polymerized into PHA [12], [13]. PHA accumulates intracellularly as polymer granules in the inclusion bodies and are secondary metabolites [14]. Asides from being part of the intracellular component of the cell, PHAs are storage compounds and source of energy for the organism when the carbon source in the environment is depleted [5], [14], [15], [16], [17]. Organisms that accumulate PHAs are able to withstand stress conditions such as heat, osmotic shock, ultraviolet irradiation [18].

Table 1: Production of PHA by microorganisms

Microorganism	Carbon source	PHA	References
<i>Cupriavidus necator</i> H16	Glucose,	P3HB	[6]
	fructose, acetic acid, valeric acid Acetate, butyrate, lactic acid, propionic acid	PHV	[18]
<i>Burkholderia</i> sp. DSMZ 9243	Sucrose or gluconate	PHB P(3HPE)	[6]
<i>Burkholderia cepacia</i> ATCC 17759	Xylose	P(3HB-co-3HV)	[6]
	Glycerol	P3HB	[18]
<i>Burkholderia sacchari</i> IPT189	Sucrose: propionic acid	P(3HB-co-3HV)	[6]
<i>Pseudomonas</i> sp. DSY-82		Both SCL-PHA and MCL-PHA	[19]
<i>Pseudomonas stutzeri</i> A1501			
ARCHAEA			
<i>Haloferax Mediterraneus</i>	Vinasse	P3HB-co-3HV	[18]
	Crude glycerol	P3HB-co-3HV	

Macrae and Wilkinson first reported the biodegradability of PHB produced by *Bacillus cereus* and *Bacillus megaterium* in 1958 [14]. The major interests on PHAs are because they are biodegradable, recyclable, biocompatible, non-toxic and are environmentally friendly upon degradation.

2.1. General properties and structure of PHAs

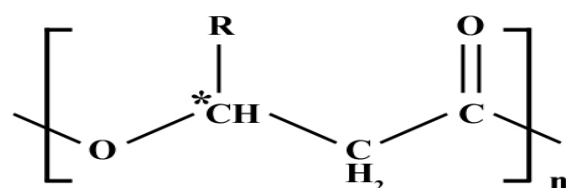
PHAs are flexible, crystalline, elastic and have thermoplastic properties similar to synthetic plastics when extracted from the cell [9], [14]. The mechanical properties of PHA like the tensile strength (40MPa), Young's modulus (3.5 GPA) are similar to synthetic plastics. PHAs are soluble in chloroforms and other chlorinated hydrocarbons but insoluble in water. They are non-toxic which makes them biocompatible.

The diverse structures of PHA is dependent on the carbon source [10] and the microorganism involved (Table 1). Majority of PHA identified consists of (R)-3-hydroxy fatty acid monomers linked by ester bonds. The R-configuration is due to their chirality and stereo-specificity of the enzymes involved in biosynthesis. The carbon atoms present in the HA monomer unit determines the length of PHAs [14]. PHAs are grouped into four based on the number carbon atoms present in each HA monomer unit. The four groups include: the short chain length PHA, Medium chain length PHA, Long chain length PHA and the co-polymers consisting of short chain and medium chain length polyhydroxyalkanoate.

Short chain length PHAs (PHA_{SCL}) have less than or equal to five carbon atoms in their HA monomer unit [6]. They are the most common, highly crystallinity (55-80%) which makes them brittle and possess low melting temperature (173 – 180°C) [20]. Examples include: poly (3-hydroxybutyrate) P(3HB), poly(4-hydroxybutyrate) P(4HB) and poly(3-hydroxyvalerate) P(3HV) or the copolymer P(3HB-co-3HV) as seen in Fig. 1 [20].

Medium chain length (MCL) monomers have up to six to 14 carbon atoms present in their HA monomer unit and are called Medium-chain lengths PHAs (PHA_{MCL}) [21]. Their structure could also include functional groups belonging to the halogens, olefins, cyano as well as alkyl groups [4], [14]. Examples include poly (3-hydroxyhexanoate) P(3HHx), poly(3-hydroxyoctanoate) P(3HO) and copolymers such as P(3HHx-co-3HO). According to the reports of [20], PHA_{MCL} are most desirable because they possess superior thermo mechanical properties. PHA_{MCL} have lower melting temperature (39- 61°C), are more flexible and have more elasticity than PHA_{SCL}.

Long chain length PHAs (PHA_{LCL}) are uncommon and have more than 14 carbon atoms present in their HA monomers [7], [15]. Examples include Poly(3-hydroxypentadecanoate), Poly(3-hydroxhexadecanoate).



Poly(3-hydroxyalkanoate)

<i>R</i> group	Carbon no.	PHA polymer
methyl	C ₄	Poly(3-hydroxybutyrate)
ethyl	C ₅	Poly(3-hydroxyvalerate)
propyl	C ₆	Poly(3-hydroxyhexanoate)
butyl	C ₇	Poly(3-hydroxyheptanoate)
pentyl	C ₈	Poly(3-hydroxyoctanoate)
hexyl	C ₉	Poly(3-hydroxynonanoate)
heptyl	C ₁₀	Poly(3-hydroxydecanoate)
octyl	C ₁₁	Poly(3-hydroxyundecanoate)
nonyl	C ₁₂	Poly(3-hydroxydodecanoate)
decyl	C ₁₃	Poly(3-hydroxytridecanoate)
undecyl	C ₁₄	Poly(3-hydroxytetradecanoate)
dodecyl	C ₁₅	Poly(3-hydroxypentadecanoate)
tridecyl	C ₁₆	Poly(3-hydroxyhexadecanoate)

Figure 1: General Structure of PHAs. The term R refers to the length of the side chain while the asterisk denotes the chiral centre of PHA- building block. R determines the type of HA monomer unit [18]

2.2. Identification of intracellular PHA granules

Methods of screening for the presence of PHA polymers in microbial cells are grouped into 2; genotypic and phenotypic screening. Genotypic screening is rapid and specific for the identification of microbes capable of producing PHAs using molecular techniques such as the Polymerase Chain Reaction (PCR) [15]. The genes responsible for PHA synthesis are embedded in an operon called *phaCAB* operon which codes for the following; β -Ketothiolase (*phaA* gene), Acetoacetyl-CoA reductase (*phaB* gene) and PHA-polymerase (also called PHA synthase) (*phaC* gene). The synthase gene (*phaC*) is most studied of all the associated genes of PHA and is classified (Table 2) based on the substrate specificity of the PHA synthase enzymes and the gene locus [23], [24]. Various primers have been designed from characterised PHA producers to rapidly identify these genes present in PHA producing microbes (Table 3).

Phenotypic screening involves identification based on microscopy by using distinct stains such as the Nile red, Nile blue A or the Sudan black stain, under the phase contrast or fluorescent microscope [25]. This traditional method is often times more laborious and fails to distinguish the polymer type [15]. Hence, molecular identification is the most effective and rapid method for identification.

Table 2: Well-studied classes of PHA synthase genes and examples of organisms that bear these genes [16]

Gene class	Gene	Organism
Class I	<i>phaC</i>	<i>Cupriavidus necator</i>
Class II	<i>phaC1</i> and <i>phaC2</i>	<i>Pseudomonas aeruginosa</i>
Class III	<i>phaC</i> and <i>phaE</i>	<i>Allochromatium vinosum</i>
		<i>Chromatium vinosum</i>
		<i>Thiocystis violace</i>
Class IV	<i>phaC</i> and <i>phaR</i>	<i>Bacillus megaterium</i>

Table 3 List of primers proven to screen PHA producing microbes

Primer ID	Primer sequence	Positive isolates	References
B1F	5'-AACTCCTGGGCTTGAAGACA-3'	<i>Bacillus sphaericus</i> ,	[25]
B1R	5'-TCGCAATATGATCACGGCTA-3'	<i>Bacillus circulans</i> ,	
B2R	5'-ACGGTCCACCAACGTTACAT-3'	<i>Bacillus brevis</i> , <i>Bacillus sphaericus</i> , <i>Bacillus sphaericus</i>	
E1-D	5'GGAGCGTCGTAGATGAGTAACAAGA A3'	<i>Burkholderia cepacia</i> ,	[26]
E1- R	5'AGGTTGGCGCCGA TGCCGTTGAA3'	<i>Pseudomonas spp.</i>	
E2-D	5'TGCTGGCCTGGCGCA TTCCCA A3'		
E2-R	5'AAGTGGTAGTAGAGGTTGCC3		
G-D	5' GTG CCG CC(GC) (CT)(AG)(GC) ATC AAC AAG T-3'	<i>Comamonas sp</i> ,	[20], [27]
G-IR	5'-GTT CCA G(AT) ACA G(GC) A (GT)(AG) T CGA A-3')	<i>Bacillus sp.</i> , <i>Aeromonas sp.</i> , <i>Caulobacter sp</i>	

<i>phaCF1</i>	5'TGATSSAGCTGATCCAGTAC3'	<i>Bacillus megaterium</i> ,	[24]
<i>phaCF3</i>	5'CCGCTGCTGATCGTBCCGCC3'	<i>Bacillus cereus</i> ,	
<i>phaCR1</i>	5' GTGCCGCCGAYGCAGTAGCC3'	<i>Cupriavidus necator</i>	
<i>phaC1F1</i>	5'-TGGARCTGATCCAGTAC-3'	<i>Halomonas</i> spp.,	[28]
<i>phaC1F2</i>	5'-SATCAACCTGATGACCGA-3'	<i>Staphylococcus</i> spp.,	
<i>phaC1R1</i>	5'-CGGGTTGAGRATGCTCTG-3'	<i>Paracoccus</i> spp.	
<i>phaC1R1</i>	5'-TGGTGTCTGTTGTTCCAG-3'		

3. BIOSYNTHESIS OF POLYHYDROXYALKANOATE AND APPLICATIONS

PHAs are synthesised by microbes (bacteria and archaea) from various carbon sources which includes; saccharides, alkanes, alkanolic acids, alcohols and gases (Table 1). Majority of PHA accumulating microbes are Gram negative while few are Gram positive [9]. PHA accumulating organisms belonging to the archaea are majorly limited to the Haloarchaeal species [14]. Carbon sources could also include wastes serving as renewable sources such as acetate, waste frying or cooking oil, crude glycerol, molasses, wastewater [9], [20].

The condition required for PHA accumulation differ in bacteria. Some bacteria genera accumulate PHA in a limiting nutrient culture with excess carbon source (*Cupriavidus necator*, *Protomonas extorquens*) while others accumulate PHA during the growth phase without requiring the limitation of an essential nutrient (Recombinant *Escherichia coli*, *Alcaligenes latus*). These attributes should be placed under consideration during PHA production [14]. Other characteristics to be considered during PHA accumulation is the type of PHA polymer to be produced. The substrate or carbon source utilised determines the type of PHA produced [9] because of the substrate specificity of the enzymes involved, hence, the metabolic pathway would differ [6], [10]. For example, *Cupriavidus necator* (formerly called *Ralstonia eutropha*) produced a copolymer of 3-hydroxybutyric acid and 3-hydroxyvaleric acid P(HB-HV) in a medium containing glucose when propionic acid was added to it [4], [14]. *Rhodococcus* sp. produced a PHA_{SCL} while utilizing hexanoate [14].

The pathway leading to the synthesis of PHA is linked to existing metabolic pathway (such as cycle (or TCA cycle), *de novo* fatty acid and the β -oxidation pathway) of the microorganism by shared intermediates (Fig 2); most common intermediate being acetyl-CoA from Krebs cycle [18]. High amounts of coenzyme A produced from the Krebs cycle inhibits the enzymatic activity of 3-ketothiolase (PhaA) and channels the acetyl CoA back to the Krebs cycle which in turn inhibits PHA synthesis. This occurs in a growth medium with high level of rich nutrients (nitrogen, phosphorus). Alternatively, in a limiting nutrient growth medium (with excess carbon), the levels of coenzyme A produced is low and are non-inhibitory towards the Pha A enzyme, thereby allowing acetyl CoA to be channelled for PHA synthesis [18].

3.1. Biosynthesis of Short-chain-length Polyhydroxyalkanoate (PHA_{SCL})

Polyhydroxybutyrate (PHB), the most characterised PHA and the most common PHA_{SCL} is synthesized by three major enzymatic reactions from acetyl CoA [20]. The enzymes involved are: β -Ketothiolase (*phbA* gene), Acetoacetyl-CoA reductase (*phbB* gene) and PHB-polymerase (*phbC* gene). The organism *Cupriavidus necator* uses the pathway to synthesise PHB in a limiting essential nutrient medium containing glucose.

- Condensation: the enzyme β -Ketothiolase condenses 2 moles of acetyl CoA into acetoacetyl-CoA
- Reduction: the acetoacetyl CoA is reduced to (R)-3-hydroxybutyryl-CoA by the NADPH-dependent enzyme, acetoacetyl-CoA reductase [22]
- Polymerization: finally, the enzyme P(3HB) polymerase (PHA synthase) polymerizes (R)-3-hydroxybutyryl-CoA to PHB

3.2. Biosynthesis of medium-chain-length Polyhydroxyalkanoate (PHA_{MCL})

PHA_{MCL} are majorly synthesised through the *de novo* fatty acid pathway or the fatty acid β -oxidation cycle by converting intermediates of the fatty acid metabolism to (R)-3-hydroxyacyl-CoA. The intermediates such as 3-Keto-acyl-CoA are used as the substrates by the PHA synthase enzyme for further polymerisation into PHA_{MCL} (Figure 2). *Pseudomonas* spp is an example of organism that utilizes this pathway for the synthesis of 3-hydroxyacyl moieties which is further polymerized to PHA_{MCL} [29].

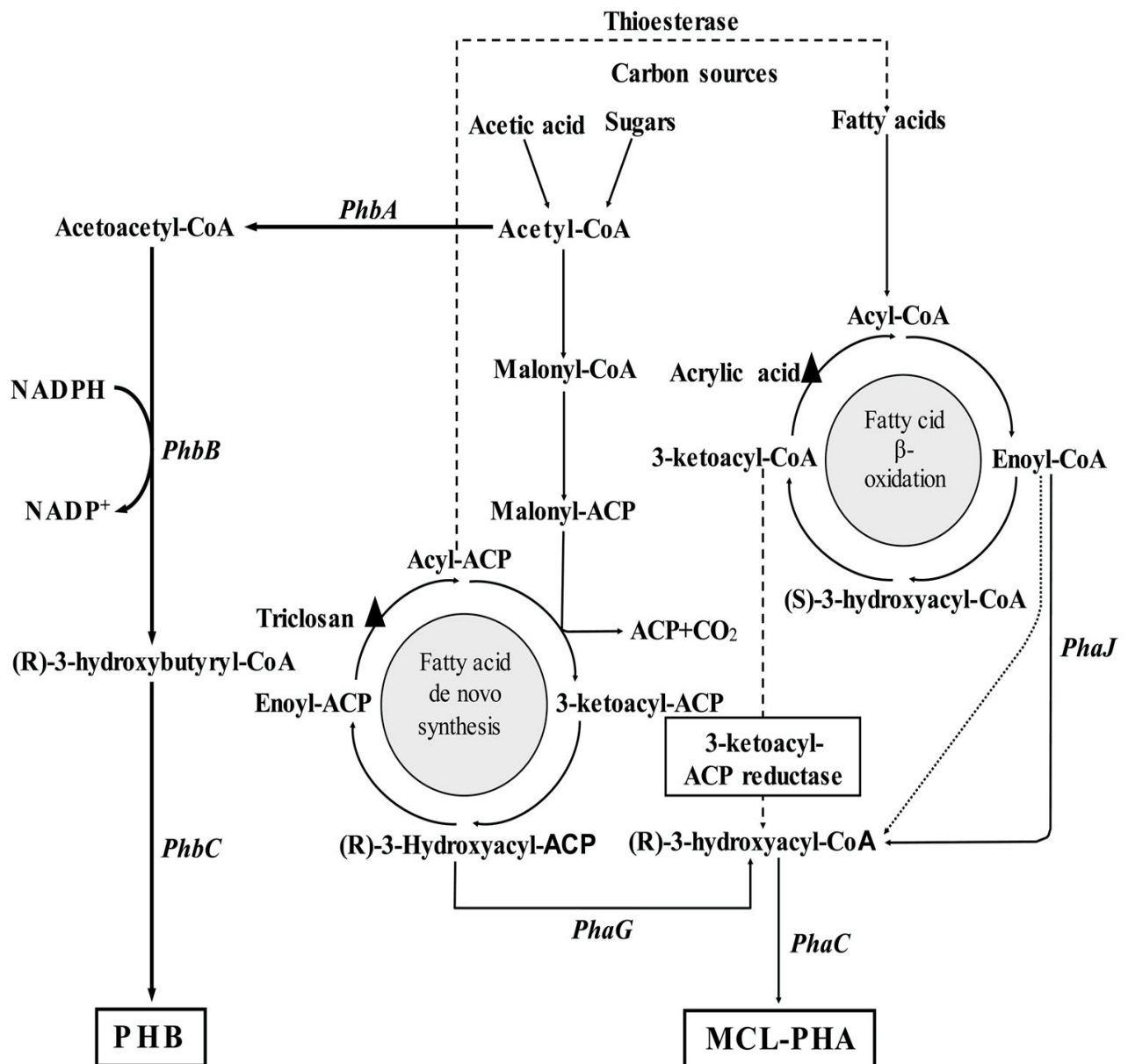


Fig 2: Synthesis of PHA_{SCL} and PHA_{MCL} via existing pathways [14]

3.3. Key enzymes involved in biosynthesis of PHAs

The major enzyme involved in the biosynthesis of PHA is the synthase enzyme (PhaC) encoded on the *phaC* gene [23]. It is most studied because it is directly involved in catalysing the committed steps to leading to PHA synthesis [29]. The gene that encodes this enzyme is also the most characterised as seen in table 2. Besides the well characterised PhaC enzyme, there are other enzymes associated with the biosynthesis of PHA (Table 3).

Table 3: Enzymes involved in the biosynthesis of PHA [18]

No.	ENZYME	ABBREVIATION	SPECIES
1	Glyceraldehyde-3-phosphate dehydrogenase	-	<i>Cupriavidus necator</i>
2	Pyruvate dehydrogenase complex	-	<i>Cupriavidus necator</i> and <i>Burkholderia cepacia</i>
3	3-Ketothiolase	PhaA	<i>Cupriavidus necator</i>
4	NADPH-dependent acetoacetyl-CoA reductase	PhaB	<i>Cupriavidus necator</i>
			<i>Rhizobium (Cicer) sp. CC 1192</i>
5	PHA synthase	PhaC	<i>Cupriavidus necator</i>
6	Acetyl-CoA carboxylase	ACC	<i>Escherichia coli</i> K-12MG1655
7	Malonyl-CoA:ACP transacylase	FabD	<i>Escherichia coli</i> K-12MG1655
8	3-Ketoacyl carrier protein synthase	FabH	<i>Escherichia coli</i> K-12MG1655
9	NADPH-dependent 3-Ketoacyl reductase	FabG	<i>Pseudomonas aeruginosa</i>
10	Succinic semialdehyde dehydrogenase	SucD	<i>Clostridium kluyveri</i>
11	4-Hydroxybutyrate dehydrogenase	4HbD	<i>Clostridium kluyveri</i>
12	4-Hydroxybutyrate-CoA: CoA transferase	OrfZ	<i>Clostridium kluyveri</i>
13	Alcohol dehydrogenase, putative	-	<i>Aeromonas hydrophila</i> 4AK4
14	Hydroxyacyl-CoA synthase, putative	-	Mutants and recombinants of <i>Cupriavidus necator</i>
15	Methylmalonyl-CoA mutase	Sbm	<i>Escherichia coli</i> W3110
16	Methylmalonyl-CoA racemase	-	<i>Nocardia corallina</i>
17	Methylmalonyl-CoA decarboxylase	YgfG	<i>Escherichia coli</i> W3110
18	Acyl-CoA synthetase	FadD	<i>Pseudomonas putida</i> CA-3 and <i>Escherichia coli</i> MG1655
19	(R)-Enoyl-CoA hydratase	PhaJ	<i>Pseudomonas putida</i> KT2440
20	3-Ketoacyl-CoA thiolase	FadA	<i>Pseudomonas putida</i> KT2442
21	3-Hydroxyacyl-ACP:CoA transacylase	PhaG	<i>Pseudomonas mendocina</i>
22	Cyclohexanol dehydrogenase	ChnA	<i>Acinetobacter sp. SE19</i> and <i>Brevibacterium epidermidis</i> HCU

23	Cyclohexanone monooxygenases	ChnB	<i>Acinetobacter</i> sp. SE19 and <i>Brevibacterium epidermidis</i> HCU
24	Caprolactone hydrolase	ChnC	<i>Acinetobacter</i> sp. SE19 and <i>Brevibacterium epidermidis</i> HCU
25	6-Hydroxyhexanoate dehydrogenase	ChnD	<i>Acinetobacter</i> sp. SE19 and <i>Brevibacterium epidermidis</i> HCU
26	6-Oxohexanoate dehydrogenase	ChnE	<i>Acinetobacter</i> sp. SE19 and <i>Brevibacterium epidermidis</i> HCU

3.4. Application

The versatile application of PHA are due to their biocompatibility [4]. Several applications include: bone tissue engineering [11], [30], drug delivery or as drug itself (Albureikan, 2019), packaging, biofuels [16]. Due to the absence of inflammatory responses to PHAs when used as catalysts for drug delivery, scaffold bone tissue, they have been further applied to bio-implants for the body of humans including animals [5]. PHB showed promising results when used as a nerve graft in rats for nerve regeneration. PHA are compatible for bone tissue engineering because when they biodegrade, their degraded components which is 3-hydroxybutyrate is a component of the blood usually produced in the liver when fatty acid is broken down [30]. PHAs are recently been applied in nanotechnology for specific detection of hepatitis B virus [5].

4. CONCLUSION

Industrial production of polyhydroxyalkanoate is not yet on full scale because of high production cost as opposed to the synthetic plastics. Understanding the biosynthetic pathways and further manipulating the pathways would be key to achieving low and competitive production cost, as well as product design of the desired polymer.

5. RECOMMENDATION

More research should focus on achieving PHA blends with better mechanical strength, this would enhance PHA application in bone tissue engineering.

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