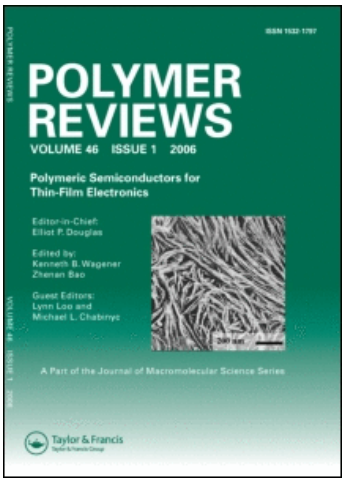

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Mini-Review: Biosynthesis of Poly(hydroxyalkanoates)

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Polyhydroxyalkanoates (PHAs) are biologically produced polyesters which can consist of a diverse set of repeating unit structures. These biologically produced polyesters have many attractive properties and have been produced for use as bulk commodity plastics, fishing lines, and medical uses. PHAs have also attracted much attention as biodegradable polymers that can be produced from biorenewable resources. The cellular factories that produce these polymers offer the ability to produce or incorporate monomers that may not be available via typical chemical synthesis. In addition, 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 organism is grown on, the types of metabolic pathways available to that organism to convert those carbon sources into PHA monomers, and the substrate specificity of the enzymes involved in PHA synthesis. This review covers known biosynthetic pathways for the production of PHAs.

Keywords polyhydroxyalkanoates, PHA synthase, genetic engineering, *in vitro* evolution, PHA monomer-supplying enzymes

1. Introduction

1.1. Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are polyesters that can be produced by some native bacterial strains, recombinant bacterial strains, and recombinant eukaryotes.[1,2] These biopolyesters are formed via metabolic transformation of various carbon sources.[1,2] In native PHA-producing organisms, these polyesters are produced as intracellular carbon storage compounds and energy reserves. Many PHA polymers also have interesting properties, such as biodegradability, and have a wide array of uses ranging from single-use bulk, commodity plastics, to specialized medical applications.[1,3–5] Recent studies have demonstrated the use of PHAs in the production of stents and in the tissue engineering of heart valves.[6–12] PHA polymers can be made from a number of different related and unrelated carbon sources derived from agricultural and forest-based industries.[13–18] The ability to produce PHAs in photosynthetic organisms such as plants, offers the opportunity to produce these

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bioproducts from CO_2.[19] Recently, these biopolyesters have been produced in a number of different plants.[20,21] Table 1 shows some of the many organisms that have been used to biosynthesize PHA to date. Because of the promising potential of these materials, there has been an increasing level of interest in developing new methods for their production. This review will present a brief overview on PHA monomer types, current biosynthetic pathways, and methods for the production of this diverse set of biopolyesters.

1.2. Material Properties of PHAs are dictated by Monomer Composition

Polyhydroxyalkanoates (PHAs) have physical properties that are based on the number of carbon atoms in the individual monomer units as well as on the physical structure of these monomers following their incorporation into polymer chains by bacterial enzymes. There are many different monomer units that can be incorporated into PHA polymers[22,23] and a sampling of the structures of these monomers is shown in Fig. 1. There have been examples of other PHA polymers made with fluorinated side chains derived from synthesized nonanoic acid and fluorinated acid cosubstrates.[24] Monomers with conjugated side chains, once incorporated into a PHA polymer chain, can also be chemically modified to increase the functionality and number of potential applications of the polymer.[25] In general, PHA monomers may be divided up into three main classes: (i) short-chain-length (SCL) PHAs, which consist of monomers with chain lengths of 3–5 carbon units; (ii) medium-chain-length (MCL) PHAs, which consist of monomers with chain lengths between 6 and 14 carbon units; and (iii) long-chain-length (LCL) PHAs, which are composed of monomers with carbon chain lengths greater than 14 units.[5] These monomers can be incorporated to form homopolymers or copolymers with various physical properties. Polymers composed solely of SCL monomer units generally have thermoplastic properties, while polymers composed of MCL subunits generally have elastomeric properties. PHA copolymers with a relatively high mol% of SCL monomers and low mol% of MCL monomers have properties similar to the bulk commodity plastic polypropylene.[26] A comparison of properties of some PHA polymers to petroleum-based polymers is shown in Table 2. A study by Ouyang et al. demonstrated that PHA copolymers composed of increasing mol% of 3-hydroxydodecanoate (3HDD) monomers had higher crystallinity and tensile strength compared to MCL PHA copolymers with low 3HDD mol% compositions.[27] Perhaps the most significant news in the PHA field in recent years is the report by Taguchi et al. of the development of a novel biosynthetic pathway, using an engineered PHA synthase enzyme and metabolic pathway engineering, that can be used to produce lactyl-CoA for the production of a poly(lactic acid-*co*-3-hydroxybutyrate) copolymer.[28] Polylactic acid (PLA) is another class of biopolyesters that are traditionally chemically synthesized by the ring-opening polymerization of a cyclic lactide diester derived from microbially produced lactic acid.[29] This study has opened the door for the biosynthetic production of new classes of PHA copolymers from renewable resources that will likely have interesting and useful material properties.

The monomeric composition of PHA polymers can be influenced by several factors, including the organism producing the PHA polymer, the carbon source on which cells are grown, how that carbon source is metabolized in the cells, the types of monomer-supplying enzymes used, and the type of PHA synthase used to synthesize the polymer. The rest of this review will focus on the biosynthetic pathways and enzymes involved in the production of various PHA polymers.

Table 1
Organisms shown to produce PHAs

Organism	Reference	Organism	Reference
Gram-positive bacteria			
Actinomycetes	67	*Micrococcus*	68
Bacillus	69	*Microcystis*	70
Caryophanon	71	*Nocardia*	72
Corynebacterium	73	*Rhodococcus*	74
Clostridium	75	*Staphylococcus*	76
Micrococcus	68	*Streptomyces*	77
Gram-negative bacteria			
Acinetobacter	78	*Methylomonas*	79
Alcaligenes	80	*Methylosinus*	81
Aphanocapsa	82	*Methylovibrio*	81
Aphanothece	83	*Microcoleus*	84
Aquaspirillum	85	*Moraxella*	86
Asticcaulus	87	*Mycoplana*	88
Azomonas	87	*Nitrobacter*	86
Azospirillum	89–91	*Nitrococcus*	92
Azotobacter	93	*Oceanospirillum*	87
Beggiatoa	87	*Paracoccus*	94
Beijerinckia	87	*Photobacterium*	95
Beneckea	87	*Protomonas*	87
Caulobacter	96,97	*Pseudomonas*	95
Chloroflexus	87	*Rhizobium*	98
Chlorogloea	99	*Rhodobacter*	100
Chromatium	101,102	*Rhodopseudomonas*	103
Chromobacterium	104,105	*Rhodospirillum*	106
Derxia	107	*Sphaerotilus*	108
Ectothiorhodospira	109	*Spirillum*	85
Escherichia	110	*Spirulina*	111
Ferrobacillus	112	*Stella*	81
Gloeothece	70	*Syntrophomonas*	87
Haemophilus	87	*Tetrahymena*	113
Halobacterium	114	*Thiobacillus*	115
Haloferax	114	*Thiocapsa*	116
Hyphomicrobium	117	*Thiocystis*	118
Lamprocystis	118	*Thiodicotyon*	81
Lampropaedia	119	*Thiopedia*	81
Leptothrix	120	*Thiosphaera*	121
Methanomonas	122	*Vibrio*	123
Methylobacterium	124	*Xanthobacter*	125
Methylocystis	79	*Zoogloea*	126
Methylomicrobium	79		
Eukaryotes			
Arabidopsis thaliana	127	*Saccharornyces cerevisiae*	128

(*Continued on next page*)

Table 1
Organisms shown to produce PHAs (*Continued*)

Organism	Reference	Organism	Reference
Brassica napus	129	*Solanum tuberosum*	130
Gossypium hirsutum	131	*Spodoptera frugiperda*	132
Nicotiana tabacum	133	*Zea mays*	134
Pichia pastoris	135		

2. Key Biosynthetic Pathways for PHA Production

PHA polymers are produced via a series of enzymatic reactions in both native and recombinant organisms. The properties of PHA polymers are dependent on the starting carbon feedstocks, the metabolic pathways for the conversion of those feedstocks into precursors for PHAs, and the specific activities and substrate specificities of the enzymes involved in the process. In native PHA producing organisms, PHAs are accumulated as granules that are surrounded by specific lipids and proteins.[30–32] It has been proposed by Uchino et al. that the granules in native polyhydroxybutyrate (PHB)-producing organisms act as "organelles" that are involved in a process of simultaneous production and degradation via biosynthetic activity of PHA synthases and the thiolytic activities of PHA depolymerases.[30] Recombinant, non-native, PHA-producing organisms that express genes for PHA monomer supply and PHA synthesis are also capable of forming inclusion bodies composed of PHA. However, recombinant, non-native, PHA-producing organisms are not subject to the same types of metabolic regulation as native PHA-producing organisms and may be better suited for large-scale production. Further research on these metabolic pathways and enzymes for the production of PHAs will allow researchers and engineers to optimize the production of tailor-made PHA polymers.

2.1. Production of PHAs from Related Carbon Sources

Many of the monomers in Fig.1 can be incorporated into PHA polymers by supplementing the growth media of the microorganism with feedstocks of the related monomer precursor. These related precursors are generally various fatty acid variants that can be processed into PHA monomers through the enzymatic activity of the β-oxidation pathway. The physiological role of the β-oxidation pathway is to catabolize fatty acids for the production of reducing equivalents to produce energy from the respiratory electron transport chain. A fatty acid is activated by an acyl-CoA synthase and ATP to produce a substrate that will pass through a series of enzymes to produce acetyl-CoA and reduce the number of carbons in the fatty acid by two in a cyclic nature (Fig. 2A). For pseudomonads, the β-oxidation pathway has been implicated as an important metabolic route for the production of MCL PHA polymers.[23] Researchers have engineered bacterial strains to improve MCL PHA production through gene knockouts of FadAB of the β-oxidation pathway.[33]

It has been demonstrated that overflow of intermediates from the β-oxidation pathway can be shunted towards PHA production via enzymes such as enoyl-CoA hydratases like PhaJ[34,35] and MaoC[36] or FadB homologs such as YfcX,[37] PaaG, PaaF, and YdbU[38] in FadB-deficient strains to produce 3-hydroxyacyl-CoA (3-HA-CoA) precursors (Fig. 2B). 3-HA monomers can also be supplied by the conversion of 3-ketoacyl-CoA to 3-HA-CoA by the 3-ketoacyl-reductases FabG[39,40] or RhlG[41] (Fig. 2C). Overexpression of these

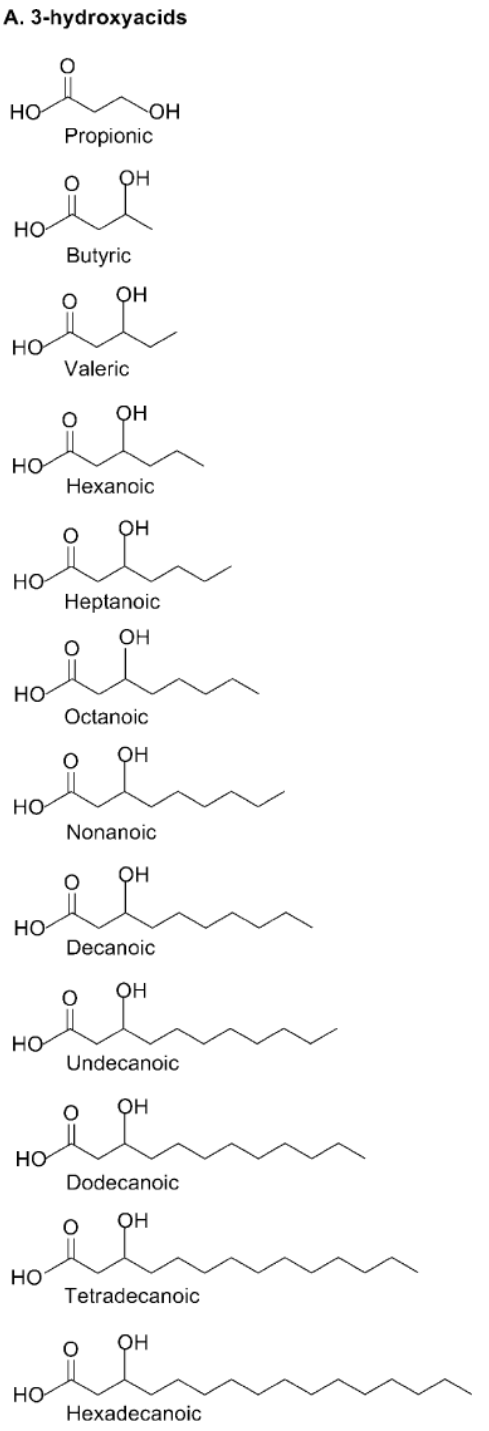


Figure 1. Structures of PHA monomer units. The structures for various monomers found in PHA and PHA comonmers.[22,138–142,28,143,144] A. Saturated 3-hydroxyacids. B. Unsaturated 3-hydroxyacids. C. Branched 3-hydroxyacids. D. 3-hydroxyacids with substituted side chains. E. Monomers other than 3-hydroxyacids that can be incorporated into PHAs. (*Continued*)

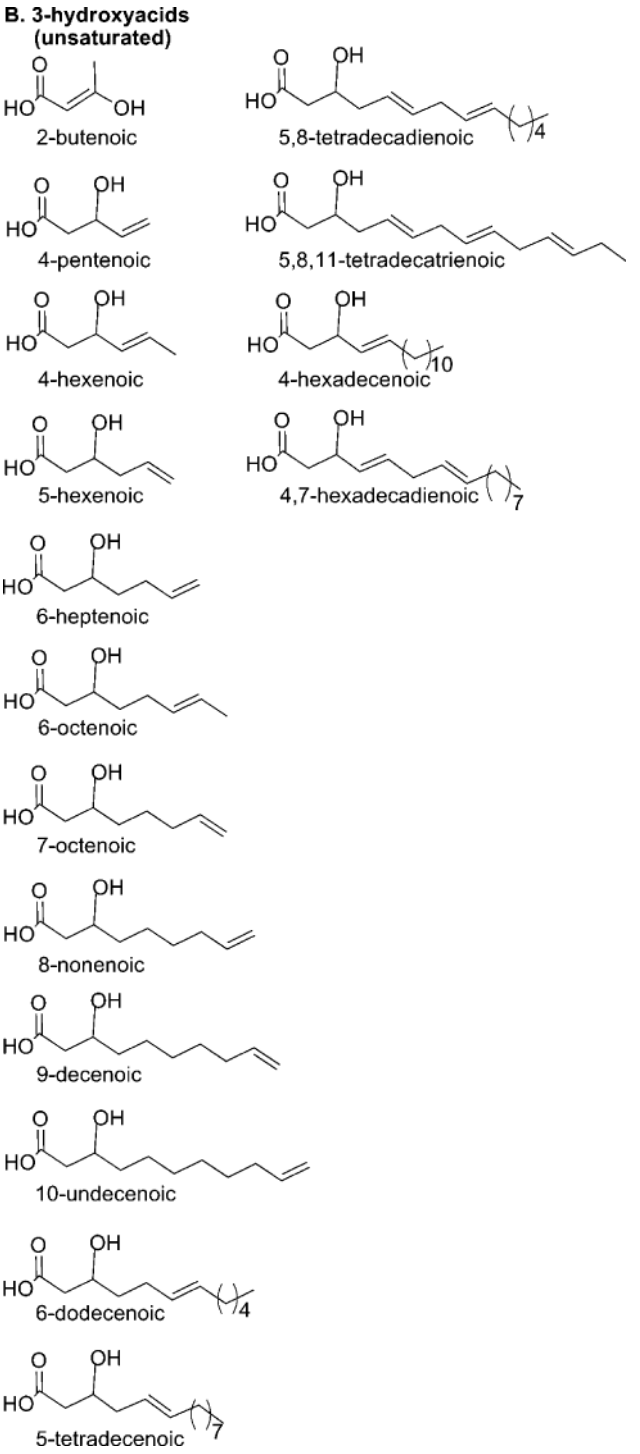
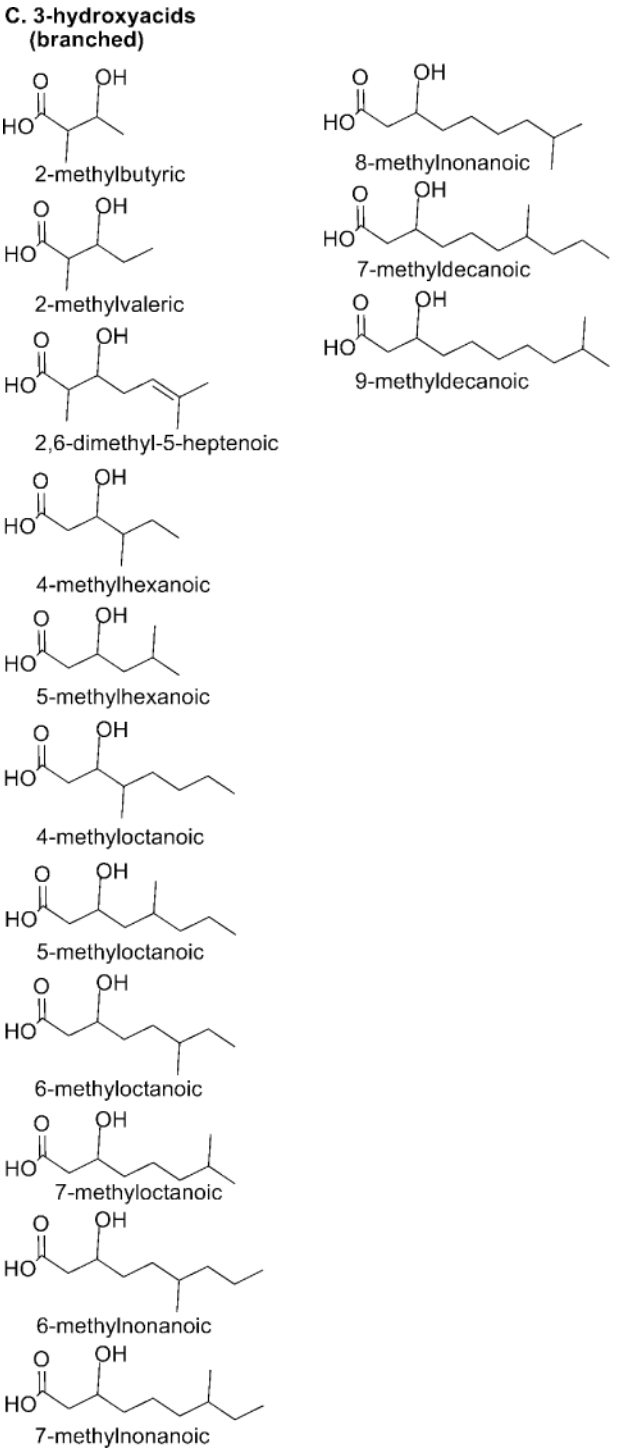


Figure 1. (*Continued*)



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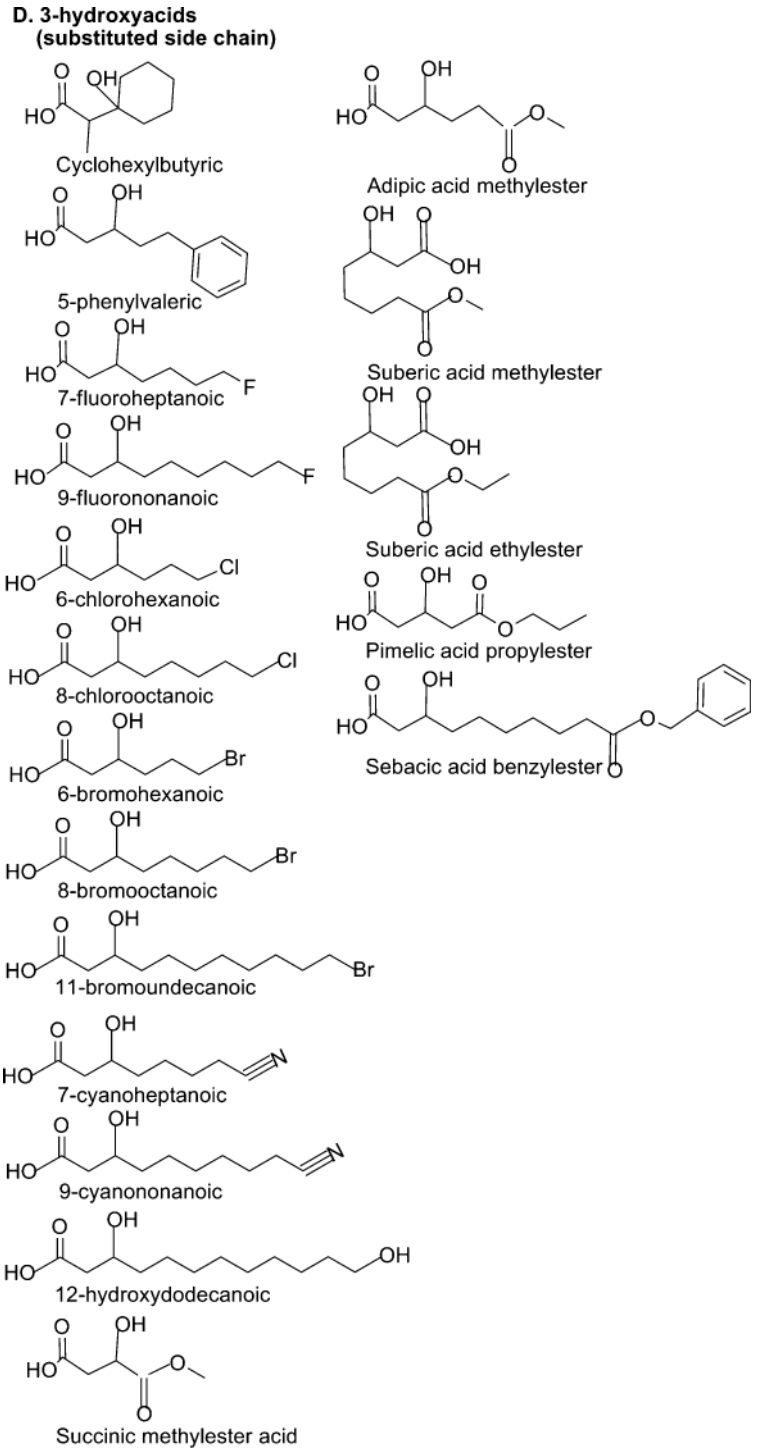

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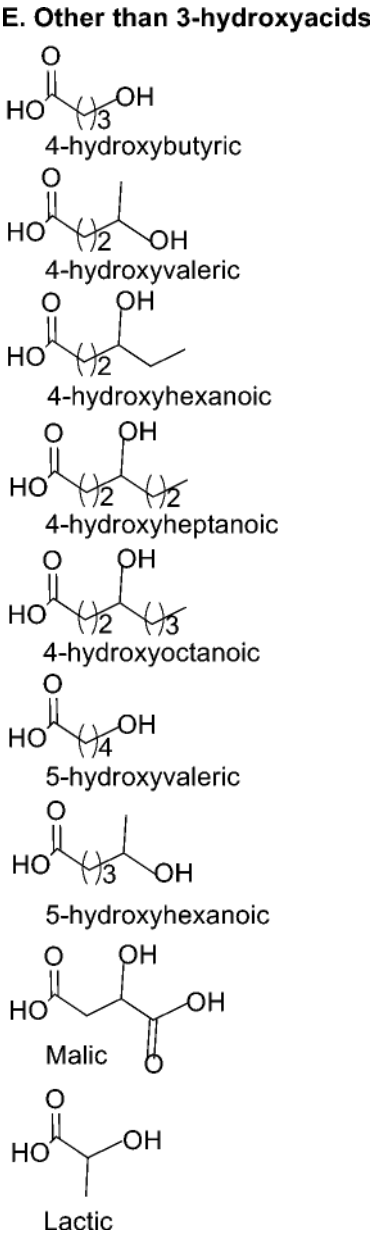

Figure 1. (*Continued*)

monomer-supplying enzymes has the potential to enhance PHA production from the β-oxidation pathway (Fig. 2D).

2.2. Production of SCL PHA from Unrelated Carbon Sources

The most common SCL monomer in SCL-PHA polymers is 3-hydroxybutyrate (3HB) which is the monomer of poly(3HB) or PHB. PHB can be produced from a number of

Table 2
Comparison of the properties of various PHAs with petroleum-based plastics adapted from[1]

Polymer	T_m(°C)	T_g(°C)	Young's modulus (GPa)	Tensile strength (MPa)	Elongation to break (%)	Reference
P(3HB)	180	4	3.5	40	5	[1]
P(3HB-*co*-20 mol% 3HV)	145	−1	0.8	20	50	[1]
P(3HB-*co*-6 mol% 3HA)	133	−8	0.2	17	680	[1]
P(4HB)	53	−48	250	0.97–1.64	150	[136]
Polypropylene	176	−10	1.7	38	400	[1]
Low-density polyethylene (LDPE)	130	−30	0.2	10	620	[1]
High-density polyethylene (HDPE)	130	—	7.5	155	65	[137]

different carbon sources[13,18] and generally produces a stiff, thermoplastic material with relatively poor impact strength (Table 2). However, incorporation of other SCL monomers, such as 3-hydroxyvalerate (3HV)[2,42] or 4-hydroxybutyrate (4HB),[43–45] into PHAs can dramatically improve the physical properties of the polymer. These improvements broaden the number of applications in which SCL PHA polymers can be used.[2] Figure 3 depicts the demonstrated and potential SCL-PHA monomer-supplying pathways from a non-related carbon source (glucose) in recombinant *Escherichia coli*. Generally, glucose is metabolized via glycolysis to produce pyruvate. For aerobic growth, pyruvate is converted to acetyl-CoA and is used to make reducing equivalents through the tricarboxylic acid cycle (Fig. 3A). One of the most significant developments in the biosynthetic pathways for specialized PHA polymers from nonrelated carbon sources is the production of poly(lactide-*co*-3-hydroxybutyrate) by Taguchi and co-workers.[28] Figure 3b shows the synthetic metabolic pathway to convert pyruvate to lactate via lactate dehydrogenase (LDH) and the subsequent conversion by propionyl-CoA transferase (PCT) of lactate to lactyl-CoA, a substrate for engineered PHA synthases to copolymerize with 3HB monomers produced from the PhaAB pathway depicted in Fig. 3C. The synthetic pathway developed by Taguchi and coworkers represents a significant development for the PHA field because the production of lactyl-CoA was limited and a poor substrate for polymerization in PHA polymers based on previous studies.[46] Because Taguchi et al. used specially engineered PHA synthase enzymes having broad substrate specificity, they were able to overcome this inability to polymerize lactyl-CoA. Figure 3C shows the most well known pathway to produce PHB. The first reaction is catalyzed by beta-ketothiolase (PhaA) to convert two molecules of acetyl-CoA to acetoacetyl-CoA. This reaction is followed by the reduction of acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA by the reductase PhaB. Finally, 3-hydroxybutyryl-CoA is polymerized into PHB by PHA synthase.

Poly-3-hydroxybutyrate-*co*-3-hydroxyvalerate [P(3HB-*co*-3HV)] copolymers have a variety of uses as single use, bulk-commodity plastics in the marine environment, and in biomedical applications.[47] Normally, P(3HB-*co*-3HV) is synthesized in bacteria grown on a mixture of glucose and propionate.[48] Figure 3d shows a pathway for the conversion

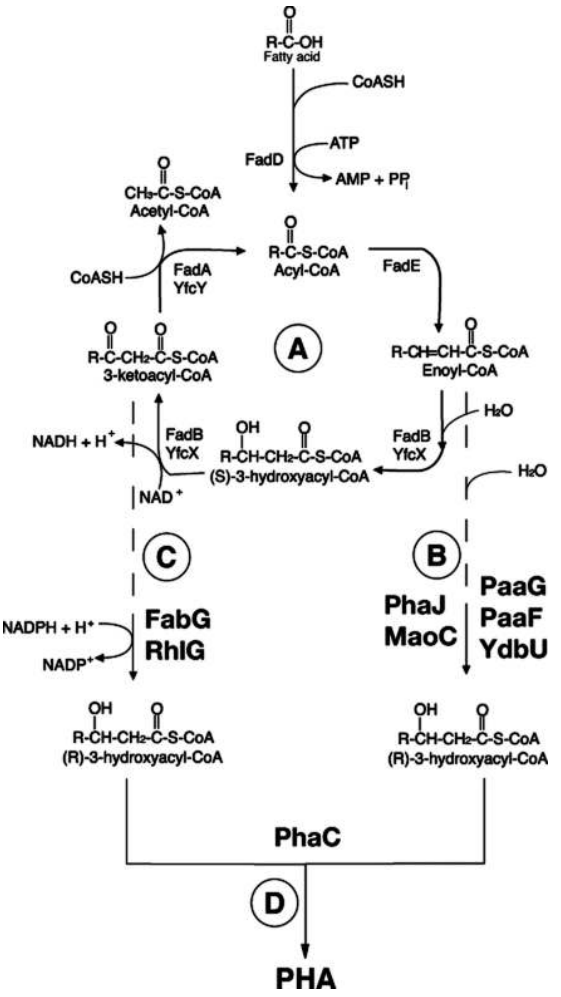

Figure 2. β-oxidation and PHA production. A. Bacterial β-oxidation pathway. Fatty acids are converted to fatty acyl-CoA substrates by fatty acyl-CoA synthetase (FadD) in an ATP-dependent manner. Fatty acyl-CoA is oxidized by acyl-CoA dehydrogenase (FadE, YafH). 2-enoyl-CoA is hydrated by enoyl-CoA hydratase (FadB) to produce *S*−3-hydroxyacyl-CoA, which is subsequently oxidized to 3-ketoacyl-CoA. FadA acts as a 3-ketoacyl-CoA thiolase and releases acetyl-CoA resulting in a fatty acyl-CoA that is 2 C shorter. B. In strains deficient in FadB, YfcX, PaaG, PaaF, and YdbH can produce monomers for PHA production. In addition, *R*-specific enoyl hydratases such as PhaJ and MaoC can intercept enoyl-CoA intermediates of fatty acid oxidation to produce PHA monomers. C. 3-ketoacyl reductases such as FabG and RhlG can intercept 3-ketoacyl-CoA intermediates to produce *R*-3-hydroxyacyl-CoA monomers for PHA production.

of threonine (derived from the TCA cycle) to 3-hydroxyvalerate by threonine deaminase (IlvA), to 2-ketobutyrate, followed by reduction to propionyl-CoA by pyruvate dehydrogenase. BktB then catalyzes the formation of the 3-(*R*)-hydroxyvaleryl-CoA substrate which can be polymerized into a P(3HB-*co*-3HV) copolymer.[49] Although this pathway has been demonstrated in plants, potentially it could be used in bacteria.

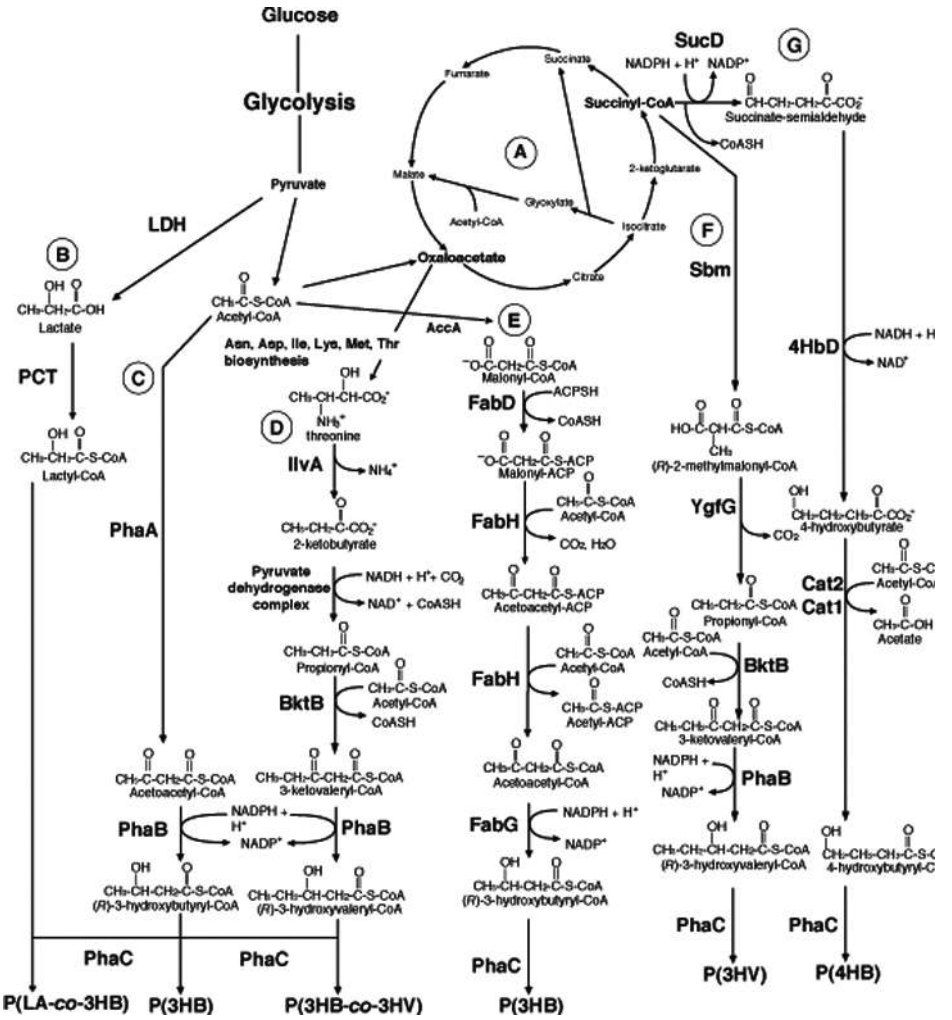

Figure 3. SCL-PHA production pathways from glucose as a carbon source. PhaC represents PHA synthase in all pathways and catalyzes the polymerization of monomers into PHA polymers. A. Tricarboxylic acid cycle. B. Synthetic pathway for the production of lactyl-CoA monomers. LDH, lactate dehydrogenase; PCT, propionyl-CoA transferase. C. *Ralstonia eutropha* derived pathway(s) for the production of 3-hydroxybutyrl-CoA (3HB-CoA). PhaA, ketothiolase; PhaB, ketoreductase. D. Pathway for the production of 3-hydroxyvaleryl-CoA (3HV-CoA). IlvA; threonine deaminase, BktB; ketothiolase. E. Pathway for the production of 3HB-CoA from fatty acid biosynthesis. AccA, acetyl-CoA carboxylase; FabD, malonyl-CoA:ACP transacylase; FabH, 3-ketoacyl-ACP synthase III; FabG, 3-ketoacyl-ACP reductase. F. Alternative 3HV synthetic pathway. Sbm, Sleeping beauty mutase; YgfG, methylmalonyl-CoA decarboxylase; BktB, ketothiolase. G. Pathway for the production of 4-hydroxybutyryl-CoA. SucD, succinate dehydrogenase; 4HbD, 4-hydroxybutyrate dehydrogenase; Cat2, Cat1, 4-hydroxybutyrate transferase.

Figure 3E shows the production of (*R*)-3-hydroxybutyryl-CoA via the fatty acid biosynthesis pathway. Native FabH proteins have a low transacylase activity,[50] so when overexpressed, they are able to catalyze the conversion of 3-ketoacyl-ACP and acetoacetyl-ACP to 3-ketoacyl-CoA and acetoacetyl-CoA, respectively.[51] Although

overexpression of FabH and PHA synthase leads to PHA production, the additional coexpression of recombinant FabG with FabH and a PHA synthase can further enhance PHA production.[52]

Figure 3F shows an alternative pathway to produce 3HV from succinyl-CoA in the tricarboxylic acid cycle. Via a coenzyme B12-dependent methylmalonyl CoA mutase, Sbm, succinyl-CoA is made into 2-(*R*)-methylmalonyl-CoA, which is converted by a methylmalonyl-CoA decarboxylase, YfgG, to propionyl-CoA. Propionyl-CoA is converted to 3-ketovaleryl-CoA by BktB and then to the (R)-3-hydroxyvaleryl-CoA, which is a substrate for P(3HV) synthesis by PhaB.[53]

P(3HB-*co*-3HV) has been studied for potential biomedical applications, but recently poly-4-hydroxybutyrate [P(4HB)] and poly-3-hydroxybutyrate-*co*-4-hydroxybutyrate [P(3HB-*co*-4HB)] have also been studied for their potential use as biomedical polymers.[4] These potential uses stress the importance of developing metabolic pathways for the economic production of 4HB monomers. Figure 3 shows a monomer-supplying pathway for 4-hydroxybutyryl-CoA production via succinyl-CoA from the tricarboxylic acid cycle. Succinate dehydrogenase (SucD) catalyzes the change of succinyl-CoA to succinate-semialdehyde, which is further reduced to 4-hydroxybutyrate by 4-hyroxybutyrate dehydrogenase (4HbD). This 4-hydroxybutryate is then converted to 4-hydroxybutyryl-CoA by a 4-hydroxybutyric acid-CoA transferase (either Cat1 or Cat2).[44,54]

3. Biosynthetic Pathways for the Production of MCL PHA from Unrelated Carbon Sources

Several pathways for the production of MCL monomers from non-related carbon sources are available (Fig. 4). All of these pathways are derived from the dissociated fatty acid biosynthesis pathway. Unlike the β-oxidation pathway, which reduces fatty acyl substrates by two carbons by releasing a molecule of acetyl-CoA per turn of the cycle and where intermediates are linked to coenzyme A, fatty acid biosynthesis builds up fatty acids by the addition of two carbons per cycle via acyl carrier protein (ACP) linked intermediates (Fig. 4A). Fatty acid biosynthetic pathways are present in all organisms, and many carbon sources can be used to generate the intermediates for PHA production. Previous studies demonstrated that co-expression of 3-ketoacyl acyl carrier protein synthase III genes (*fabH*), carrying site-specific mutations which changed their substrate specificity with various PHA synthase genes led to the production of SCL-MCL PHA copolymer (pathway outlined in Fig. 4B and 4E) in recombinant *E. coli* grown in the presence of excess glucose.[52,55,56]

Previous studies showed that genetically modified thioesterases were capable of producing MCL PHA monomers via the βoxidation pathway, even in microorganisms grown on unrelated carbon sources.[57,58] This pathway is outlined in Fig. 4c and requires the deletion of genes in the host strain encoding enzymes involved in the β-oxidation pathway (*fadR* and *fadB*) in order to be effective.[57]

PhaG was originally identified as an acyl-ACP:CoA transacylase.[59] In the original studies to identify the activity of PhaG monomer-supply pathway, recombinant *E. coli* strains required the presence of the fatty acid biosynthesis inhibitor triclosan in order for the strain to be effective as an MCL-PHA monomer supplier.[60] However, recent studies have shown that PhaG actually acts as a hydroxyacyl-ACP specific thioesterase and that the additional expression of acyl-CoA synthetase (AlkK) will activate 3-hydroxyacid intermediates generated by PhaG for PHA biosynthesis,[61,62] as shown in Fig. 4D. The identification of this "missing link" has opened the door for future studies to improve the

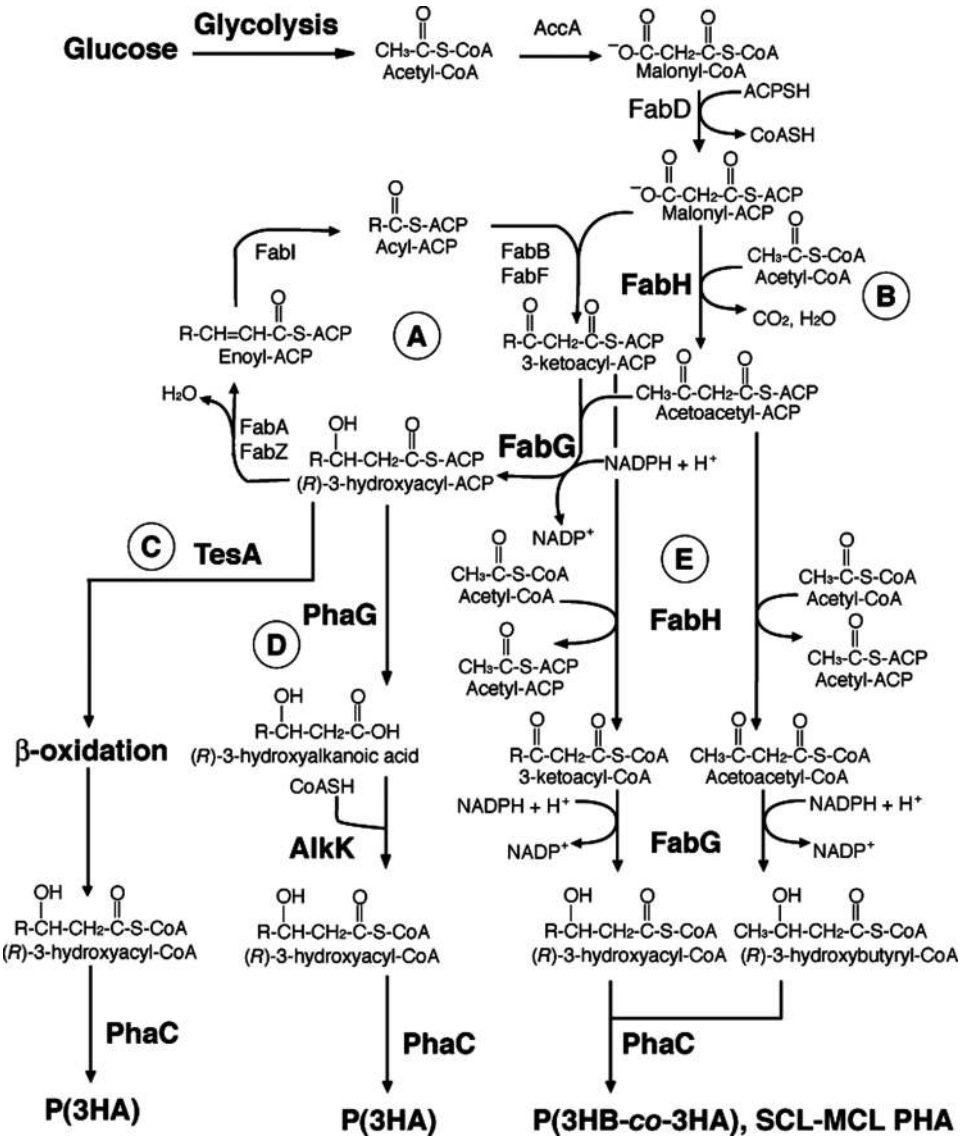

Figure 4. MCL-PHA production pathways from glucose as a carbon source. A. Dissociated fatty acid biosynthesis. B. Condensation reaction of wild type FabH. C. Thioesterasae (Tes) dependent pathway for 3-hydroxyacyl-CoA production. D. 3-hydroxyacyl-ACP thioesterase (PhaG) and acyl-CoA synthetase specific MCL-PHA monomer supplying pathway. E. Engineered FabH, FabG-mediated SCL-MCL PHA monomer supplying pathway.

production of MCL-PHA monomer supply from the fatty acid biosynthetic pathway through enzyme evolution techniques that have been successfully applied to PHA synthase enzymes (see below). The ubiquity of fatty acid biosynthesis pathways in all organisms makes the fatty acid biosynthesis derived production of SCL and MCL monomers attractive, since this system may be transferred to photosynthetic organisms to further reduce production costs by utilizing CO_2 instead of processed plant oils or sugars as carbon sources.

Table 3
Classes of PHA synthases and types of PHAs produced

Substrate specificity[a]	Class[b]	Subunit(s)[c]	Microorganism[d]	Polymers produced[e]
SCL-HA-CoA	I	PhaC	*Ralstonia eutropha*	SCL-PHA
(C3-C5)	III	PhaC, PhaE	*Allochromatium vinosum*	
	IV	PhaC, PhaR	*Bacillus megaterium*	
MCL-HA-CoA	II	PhaC	*Pseudomonas oleovorans*	MCL-PHA
(C6-C14)			*Pseudomonas putida*	
			Pseudomonas aeruginosa	
SCL-MCL-HA-CoA	I	PhaC	*Aeromonas caviae FA440*	SCL-MCL-PHA
(C3-C14)	II	PhaC	*Pseudomonas sp. 61-3*	

[a]Substrates preferred by the PHA synthase. SCL-HA-CoA (C3-C5), short-chain-length-hydroxyacyl-coenzyme A (3–5 carbons in length); MCL-HA-CoA (C6-C14), medium-chain-length-hydroxyacyl-coenzyme A (6–14 carbons in length); SCL-MCL-HA-CoA (C3-C14), short-chain-length-medium-chain-length-hydroxyacyl-coenzyme A (3–14 carbons in length). [b]Class of PHA synthase. [c] Name of PHA synthase subunit or subunits if the enzyme consists of more than PhaC. [d] Native microorganism where the PHA synthase and polymer are found. [e] Polymers produced. SCL-PHA, short-chain-length polyhydroxyalkanoate, MCL-PHA, medium-chain-length polyhydroxyalkanoate; SCL-MCL-PHA, short-chain-length-medium-chain-length polyhydroxyalkanoate.

All of the aforementioned pathways may be targeted for enhanced metabolic flux via carbon source supply, protein engineering, and other forms of regulation in order to enhance SCL-PHA production.

3.1. PHA Synthases: Key Catalysts to PHA Biopolyester Production

The key enzymes for PHA polymer production are the PHA synthases or PhaC enzymes. For an in depth review, refer to reference 63. These enzymes catalyze the polymerization of hydroxyacyl monomers to produce PHA polymers. There are several classes of PhaC enzymes that have been isolated from various microorganisms, and these enzymes display a wide range of substrate specificity (Table 3). PhaC from *Ralstonia eutrpha* ($PhaC_{Re}$) has substrate specificity towards SCL PHA monomers. PHA synthases from *Pseudomonas* sp. have substrate specificities toward MCL PHA monomers.[63] PhaC1 from *Pseudomonas* sp. 61-3 ($PhaC_{Ps}$) can recognize SCL PHA monomers but displays substrate specificity predominantly towards MCL PHA monomers.[63] *In vitro* evolutionary techniques have been successfully used to generate PHA synthases with enhanced activity and substrate specificity.[64,65] A key for the successful production of P(LA-*co*-3HA) was the use of an engineered PHA synthase in combination with the engineered monomer-supplying pathway.[28]

4. Conclusions

This review illustrates how knowledge of biosynthetic pathways for the production of PHA monomers can lead to the engineering of enzymes and the introduction of synthetic pathways into organisms for the biosynthetic production of various PHA polymers. Research towards a thorough understanding of native pathways for PHA biosynthesis is still in progress. The genome sequence of *R. eutropha* has revealed a number of potential

ketothiolases and reductases that may be involved in PHA synthesis.[66] It is clear that enzyme engineering combined with metabolic pathway manipulation can lead to the production of tailor-made PHA biopolymers.

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