



Review paper

Polyhydroxyalkanoates: an overview

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Abstract

Polyhydroxyalkanoates have gained major importance due to their structural diversity and close analogy to plastics. These are gaining more and more importance world over. Different sources (natural isolates, recombinant bacteria, plants) and other methods are being investigated to exert more control over the quality, quantity and economics of poly(3-hydroxybutyrate) (PHB) production. Their biodegradability makes them extremely desirable substitutes for synthetic plastics. The PHB biosynthetic genes *phbA*, *phbB* and *phbC* are clustered and organized in one *phbCAB* operon. The PHB pathway is highly divergent in the bacterial genera with regard to orientation and clustering of genes involved. In spite of this the enzymes display a high degree of sequence conservation. But how similar are the mechanisms of regulation of these divergent operons is as yet unknown. Structural studies will further improve our understanding of the mechanism of action of these enzymes and aid us in improving and selecting better candidates for increased production. Metabolic engineering thereafter promises to bring a feasible solution for the production of “green plastic”. © 2002 Elsevier Science Ltd. All rights reserved.

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1. Introduction

Plastics are utilized in almost every manufacturing industry ranging from automobiles to medicine. Plastics are very much advantageous because as synthetic polymers, their structure can be chemically manipulated to have a wide range of strengths and shapes. They have molecular weights ranging from 50,000 to 1,000,000 Da (Madison and Huisman, 1999). The synthetic polyethylene, polyvinyl chloride and polystyrene are largely used in the manufacture of plastics. Plastics can be easily molded into almost any desired shape including fibers and thin films. They have high chemical resistance and are more or less elastic, hence popular in many durable, disposal goods and as packaging materials.

What makes plastics undesirable is the difficulty in their disposal. Plastics being xenobiotic are recalcitrant to microbial degradation (Flechter, 1993). Excessive molecular size seems to be mainly responsible for the resistance of these chemicals to biodegradation and their persistence in soil for a long time (Atlas, 1993). In the recent years, there has been increasing public concern

over the harmful effects of petrochemical-derived plastic materials in the environment. Nature's built-in mechanisms and self-regulation ability cannot tackle novel pollutants since these are unfamiliar to it. This has prompted many countries to start developing biodegradable plastic. According to an estimate, more than 100 million tonnes of plastics are produced every year. The per capita consumption of plastics in the United States of America is 80, 60 Kg in the European countries and 2 Kg in India (Kalia et al., 2000a). Forty percent of the 75 billion pounds of plastics produced every year is discarded into landfills. Several hundred thousand tonnes of plastics are discarded into marine environments every year and accumulate in oceanic regions. Incinerating plastics has been one option in dealing with non-degradable plastics, but other than being expensive it is also dangerous. Harmful chemicals like hydrogen chloride and hydrogen cyanide are released during incineration (Johnstone, 1990; Atlas, 1993). Recycling also presents some major disadvantages, as it is difficult sorting the wide variety of plastics and there are also changes in the plastic's material such that its further application range is limited (Johnstone, 1990; Flechter, 1993). Replacement of non-biodegradable by degradable plastic is of major interest both to decision-makers and the plastic industry (Song et al., 1999). Making eco-friendly products such as bioplastics is one such reality that can help us overcome

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the problem of pollution caused by non-degradable plastics. Thus, it becomes inevitable for us to improve upon the method of production, selection of raw materials, recycling, conversion to suitable forms of certain wastes, so that we do not add any material waste into the environment which nature cannot take care of.

2. The solution for plastics

The three types of biodegradable plastics introduced are photodegradable, semi-biodegradable, and completely biodegradable. Photodegradable plastics have light sensitive groups incorporated directly into the backbone of the polymer as additives. Extensive ultraviolet radiation (several weeks to months) can disintegrate their polymeric structure rendering them open to further bacterial degradation (Kalia et al., 2000a). However, landfills lack sunlight and thus they remain non-degraded. Semi-biodegradable plastics are the starch-linked plastics where starch is incorporated to hold together short fragments of polyethylene. The idea behind starch-linked plastics is that once discarded into landfills, bacteria in the soil will attack the starch and release polymer fragments that can be degraded by other bacteria. Bacteria indeed attack the starch but are turned off by the polyethylene fragments, which thereby remain non-degradable (Johnstone, 1990). The third type of biodegradable plastic is rather new and promising because of its actual utilization by bacteria to form a biopolymer. Included are polyhydroxyalkanoates (PHA), polylactides (PLA), aliphatic polyesters, polysaccharides, copolymers and/or blends of the above.

3. Polyhydroxyalkanoates

PHA are polyesters of various hydroxyalkanoates that are synthesized by many gram-positive and gram-negative bacteria from at least 75 different genera. These polymers are accumulated intracellularly to levels as high as 90% of the cell dry weight under conditions of nutrient stress and act as a carbon and energy reserve (Madison and Huisman, 1999). Non-storage PHA that are of low molecular weight, poly(3HB), have been detected in the cytoplasmic membrane and cytoplasm of *Escherichia coli*. It is also a membrane constituent in yeasts, plants and animals. Putative functions include a role in voltage-gated calcium channels or DNA transport, protection of the macromolecules, to which it is bound, from degradative enzymes (Dawes and Senior, 1973). More than 100 different monomer units have been identified as constituents of the storage PHA (Fig. 1). This creates a possibility for producing different types of biodegradable polymers with an extensive range of properties. The molecular mass of PHA is in the range of 50,000–1,000,000 Da and varies with the PHA

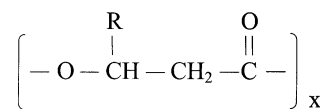


Fig. 1. Chemical structure of the major PHA produced in bacteria. PHA are commonly composed of (*R*)- β -hydroxy fatty acids, where the 'R' group varies from methyl (C1) to tridecyl (C13) (Madison and Huisman, 1999).

producer. The monomer units are all in D(-) configuration owing to the stereospecificity of biosynthetic enzymes (Senior et al., 1972; Dawes and Senior, 1973; Oeding and Schlegel, 1973; Wang and Bakken, 1998).

Bacterially produced polyhydroxybutyrate and other PHA have sufficiently high molecular mass to have polymer characteristics that are similar to conventional plastics such as polypropylene (Madison and Huisman, 1999). poly(3-hydroxybutyrate) (PHB) is the best characterized PHA. Copolymers of PHB can be formed by co-feeding of substrates and may result in the formation of polymers containing 3-hydroxyvalerate (3HV) or 4-hydroxybutyrate (4HB) monomers. The incorporation of 3HV into PHB results in a poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-3HV)] that is less stiff and more brittle than P(3HB) (Marchessault, 1996). Together, polymers containing such monomers form a class of PHA referred to as short-side-chain PHA (ssc-PHA). In contrast, polymers composed of C₆–C₁₆ 3-hydroxy fatty acids or aliphatic carbon sources are referred to as medium-side-chain PHA (msc-PHA). The composition of the resulting PHA depends on the growth substrate used (Brandl et al., 1988; Lageveen et al., 1988; Huisman et al., 1989). The msc-PHA are also synthesized from carbohydrates, but the composition is not related to the carbon source (Lageveen et al., 1988; Huisman et al., 1989; Haywood et al., 1990). The msc-PHA have a much lower level of crystallinity than PHB or P(3HB-3HV) and are more elastic. They have a potentially different range of applications compared to ssc-PHA (Gross et al., 1989; Preusting et al., 1990). The vast majority of microbes synthesize either ssc-PHA containing primarily 3HB units or msc-PHA containing 3-hydroxyoctanoate (3HO) and 3-hydroxydecanoate (3HD) as the major monomers (Anderson and Dawes, 1990; Steinbüchel, 1991; Steinbüchel and Schlegel, 1991; Lee, 1996a). PHA are produced from a wide variety of substrates such as renewable resources (sucrose, starch, cellulose, triacylglycerols), fossil resources (methane, mineral oil, lignite, hard coal), byproducts (molasses, whey, glycerol), chemicals (propionic acid, 4-hydroxybutyric acid) and carbon dioxide.

4. PHA biosynthesis in natural isolates

The extensive information on the P(3HB) metabolism, biochemistry, and physiology since 1987, has been

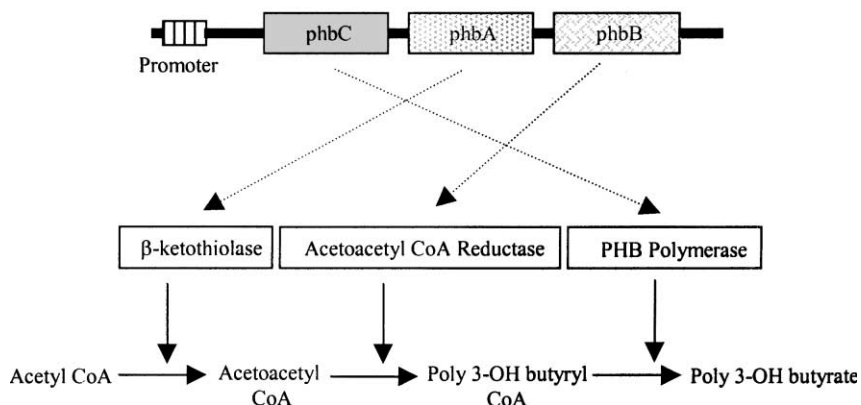


Fig. 2. Biosynthetic pathway of poly(3-hydroxybutyrate). P(3HB) is synthesized by the successive action of β -ketoacyl-CoA thiolase (*phbA*), acetoacetyl-CoA reductase (*phbB*) and PHB polymerase (*phbC*) in a three-step pathway. The genes of the *phbCAB* operon encode the three enzymes. The promoter (P) upstream of *phbC* transcribes the complete operon (*phbCAB*) (Madison and Huisman, 1999).

enriched with molecular genetic studies. Numerous genes encoding enzymes involved in PHA formation and degradation have been cloned and characterized from a variety of microorganisms. The picture is now clear that nature has evolved several different pathways for PHA formation, each suited to the ecological niche of the PHA-producing microorganism. Genetic studies have conferred insights into the regulation of PHA formation with respect to the growth conditions. The prime role of central metabolism and the cellular physiology have become evident by studying PHA mutants with genetic manipulation other than the *phb* genes (Madison and Huisman, 1999). Such studies have made clear the microbial physiology and do provide a powerful tool for designing and engineering recombinant organisms for PHA production.

The biosynthetic pathway of P(3HB) consists of three enzymatic reactions catalyzed by three different enzymes (Fig. 2). The first reaction consists of the condensation of two acetyl coenzyme A (acetyl-CoA) molecules into acetoacetyl-CoA by β -ketoacylCoA 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* (Huisman et al., 1989).

5. PHA production by recombinant bacteria

Natural PHA-producing bacteria have a long generation time and relatively low optimal growth temperature. These are often hard to lyse and contain pathways for PHA degradation. Bacteria such as *E. coli* are incapable of synthesizing or degrading PHA; however *E. coli* grows fast, even at high temperature and is easy to lyse. Fast growth will enable it to accumulate a large amount of polymer. The easy lysis of the cells saves the

cost of the purification of the PHA granules (Steinbüchel and Schlegel, 1991; Wang and Bakken, 1998; Madison and Huisman, 1999).

Metabolic engineering is being intensely explored to introduce new metabolic pathways to broaden the utilizable substrate range, to enhance PHA synthesis and to produce novel PHA. Recombinant *E. coli* strains harboring the *Alcaligenes eutrophus* PHA biosynthesis genes in a stable high-copy-number plasmid have been developed and used for high PHA productivity (Zhang et al., 1994; Lee et al., 1994). Since *E. coli* can utilize various carbon sources, including glucose, sucrose, lactose and xylose, a further cost reduction in PHA is possible by using cheap substrates such as molasses, whey and hemicellulose hydrolysate (Lee et al., 1995). This strategy can be extended to virtually any bacterium if it possesses metabolic advantages over those currently in use. Heterologous expression of PHA biosynthetic genes of *A. eutrophus* in *Pseudomonas oleovorans* (which synthesizes only medium chain length PHA), has allowed the production of a blend of P(3HB) and msc-PHA (Preusting et al., 1993). The production of various PHA using natural isolates and recombinant bacteria with different substrates is presented in Table 1. Two approaches can be taken in the development of bacterial strains that produce PHA from inexpensive carbon substrates. First, the substrate utilization genes can be introduced into the PHA producers. Second, PHA biosynthetic genes can be introduced into the non-PHA producers, which can utilize cheap substrates. At present, the second approach seems to be more promising (Lee, 1996b).

6. Genetic basis of PHA formation

The PHB biosynthetic genes *phbA* (for 3-ketothiolase), *phbB* (NADPH-dependent acetoacetyl-CoA

Table 1
Production of PHA by various bacteria

Microorganism	Carbon source	PHA	PHA content (%w/v)	References
<i>Alcaligenes eutrophus</i>	Gluconate	PHB	46–85	Liebergesell et al. (1994)
	Propionate	PHB	26–36	
	Octanoate	PHB	38–45	
<i>Bacillus megaterium</i> QMB1551	Glucose	PHB	20	Mirtha et al. (1995)
<i>Klebsiella aerogenes</i> recombinants	Molasses	PHB	65	Zhang et al. (1994)
<i>Methylobacterium rhodesianum</i> MB 1267	Fructose/methanol	PHB	30	Ackermann and Babel (1997)
<i>M. extorquens</i> (ATCC55366)	Methanol	PHB	40–46	Borque et al. (1995)
<i>Pseudomonas aeruginosa</i>	Euphorbia and castor oil	PHA	20–30	Eggink et al. (1995)
<i>P. denitrificans</i>	Methanol	P(3HV)	0.02	Yamane et al. (1996)
	Pentanol	P(3HV)	55	
<i>P. oleovorans</i>	Glucanoate	PHB	1.1–5.0	Liebergesell et al. (1994)
	Octanoate	PHB	50–68	
<i>P. putida</i> GPP104	Octanoate	PHB	14–22	Liebergesell et al. (1994)
<i>P. putida</i>	Palm kernel oil	PHA	37	Tan et al. (1997)
	Lauric acid	PHA	25	
	Myristic acid	PHA	28	
	Oleic acid	PHA	19	
<i>P. putida</i> BM01	11-Phenoxyun-decanoic acid	5POHV	15–35	Song and Yoon (1996)
<i>Sphaerotilus natans</i>	Glucose	PHB	40	Takeda et al. (1995)

PHB—polyhydroxybutyric acid), P(3HV)—polyhydroxvaleric acid, 5POHV—poly(3 hydroxy-5-phenylvalerate).

reductase), and *phbC* (PHB synthase) from acetyl-CoA are clustered and are presumably organized in one operon *phbCAB*. The loci encoding the genes for PHA formation have been characterized from 18 different species (Madison and Huisman, 1999). The diversity of the P(3HB) biosynthetic pathways implies how far the *pha* loci have diverged. The *phb* (genes encoding enzymes for ssc-PHA) and *pha* (genes encoding enzymes for msc-PHA) are not necessarily clustered and the gene organization varies from species to species. In *Acinetobacter* sp., *Alcaligenes latus*, *Pseudomonas acidophila*, and *Ralstonia eutropha*, the *phbCAB* genes are in tandem on the chromosome although not necessarily in the same order. In *Paracoccus denitrificans*, *Rhizobium meliloti*, and *Zoogloea ramigera*, the *phbAB* and *phbC* loci are unlinked (Umeda et al., 1998). The PHA polymerase has two sub-units in *Chromatium vinosum*, *Thiocystis violacea*, and *Synechocystis* encoded by *phbE* and *phbC* genes. The *phbAB* and *phbEC* genes are in one locus but divergently oriented (Hein et al., 1998). The *phb* loci in *C. vinosum*, *P. acidophila*, *R. eutropha*, *R. meliloti* and *T. violacea* have an additional gene, *phbF*, that has an unknown function in PHA metabolism (Povolo et al., 1996), while part of the gene encoding a protein homologous to the hypothetical *E. coli* protein yfiH is located upstream of the *P. acidophila*, *R. eutropha*, and *Z. ramigera* P(3HB) polymerase genes. In msc-PHA-producing *P. oleovorans* and *Pseudomonas aeruginosa*,

the *pha* loci contain two *phaC* genes separated by *phaZ*, which encodes an intracellular PHA depolymerase (Hein et al., 1998).

It is possible that functional constraints against co-expression of genes may be so weak that the organization of gene clusters in operon structures can be readily changed during the course of time. In the CAB operon too the gene order is not conserved when different species are compared. This leads us to ask questions as to how exactly is the operon regulated in the different states. It may also be possible that the selective pressures at the time resulted in the clustering of genes in an operon in some microbes such as *P. acidophila*, *R. eutropha*, *Acinetobacter*, *A. latus* and *Aeromonas caviae* or as separate transcriptional units in others (*Z. ramigera*, *P. denitrificans*, *R. meliloti*, *C. vinosum*, *T. violacea*, *P. oleovorans* and *Pseudomonas putida*). A second evolutionary force may have worked on the *pha* genes since only some of these diversely structured loci contain *phbF* and *phbP* genes or homologs of yfiH (Hein et al., 1998; Umeda et al., 1998; Madison and Huisman, 1999). The above discussion allows some conjecture on the evolution of PHA formation. In the first PHA producers, the PHA formation was most probably a minor metabolic pathway, and the purpose of the pathway was probably different from synthesis of storage material (Haywood et al., 1990). As the PHA formation became beneficial for the microbe, evolution selected PHA-

accumulating strains. Over the course of time, the *phaC* gene was sometimes combined with genes that supply monomer, such as *phbAB* or *phaJ* or *phaZ*.

7. Multiple sequence alignment and domain structure of beta-ketothiolase, acetoacetyl-CoA reductase and PHB polymerase

Eight commercially important bacterial genera were chosen, for which the sequences of all the three enzymes were available from the National Center for Biotechnology Information (NCBI). The multiple sequence alignments were done using CLUSTALW (Thompson et al., 1994) and edited with GeneDoc, Version 2.6.002 (Nicholas and Nicholas, 1997). Domains were detected by RPS-BLAST (Altschul et al., 1997) against the Conserved Domain Database (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) of PFAM HMMs (Sonhammer et al., 1998).

The PHB pathway is highly divergent in these bacterial genera with regard to orientation and clustering of the genes involved (Madison and Huisman, 1999). However, the principal enzymes remain similar in sequence, as in all the alignments done the sequences show a high degree of conservation across all genera. Especially in acetoacetyl-CoA reductase the conservation is evident all along the length. Beta-ketothiolase sequence alignment also reveals highly conserved stretches. In the case of PHB polymerase, the abhydrolase fold and the regions surrounding it are also significantly similar. The domain structure in these enzymes is also nearly identical. Two domains (Thiolase, N terminal domain, pfam00108 and Thiolase, C terminal domain, pfam02803) are present in all sequences of beta-ketothiolase examined. The thiolase domain is reported to be structurally related to beta-ketoacyl synthase (pfam00109), and also chalcone synthase. The abhydrolase, alpha/beta hydrolyase fold (pfam00561) occurs in the PHB polymerase of all the organisms chosen. It is a catalytic domain and is found in a wide range of enzymes. The *adh_short*, short chain dehydrogenase domain (pfam00106) is present in all the eight sequences of acetoacetyl-CoA reductase. This family contains a wide variety of dehydrogenases.

A threading model of PHA synthase based on the homology to the *Burkholderia glumae* lipase (whose structure has been resolved by X-ray crystallography) has been proposed (Rehm et al., 2002). This model is built on residues that are conserved in structure. However, another study, an in vitro enzyme evolution system comprising PCR-mediated random mutagenesis targeted to a limited region of the *phaC* gene and screening mutant enzymes with higher activities based on polyester accumulation. It has shown that the same enzyme with higher activities were those that had mutation in the not so highly conserved region of the enzyme (Ki-

chise et al., 2002). This shows that there is a lot to be understood about the nature of conservation and the enzyme sequences.

8. Production of PHA by genetically engineered plants

Crop plants are capable of producing large amounts of a number of useful chemicals at a low cost compared to that of bacteria or yeast. Commercialization of plant derived PHA will require the creation of transgenic crop plants that in addition to high product yields have normal plant phenotypes and transgenes that are stable over several generations (Snell and Peoples, 2002). Production of PHA on an agronomic scale could allow synthesis of biodegradable plastics in the million tonne scale compared to fermentation, which produces material in the thousand tonne scale. PHA could potentially be produced at a cost of US\$0.20–0.50/kg if they could be synthesized in plants to a level of 20–40% dry weight and thus be competitive with the petroleum based plastics.

In contrast to bacteria, plant cells are highly compartmentalized hence *phb* genes must be targeted to the compartment of the plant cells where the concentration of acetyl-CoA is high. *Arabidopsis thaliana* was the first choice for transgenic studies since it is the model for heterologous expression studies in plants (Madison and Huisman, 1999). As 3-ketoacyl-CoA thiolase is already present in *A. thaliana* only the *phbB* and *phbC* genes were transfected from *R. eutropha*, resulting in accumulation of PHB granules in the cytoplasm, vacuole and nucleus, but it also resulted in growth defects in the plant. Later on expressing *phbCAB* genes in the plastid of *A. thaliana* subsequently developed an improved production system. The maximum amount of PHB in the leaves obtained now was 14% dry weight (Nawrath et al., 1994). Plastid is the site of a high flux of carbon, through acetyl-CoA. It was therefore hypothesized that since there is a larger flux of acetyl-CoA in the plastid, expression of the PHB biosynthetic pathway in this compartment may lead to a significant increase in the PHB production without any deleterious effects on the plant.

Oilseed crops are considered as good targets for seed-specific polyhydroxyalkanoate production. As PHB and oil are derived from acetyl-CoA, metabolic engineering of plants for the diversion of acetyl-CoA towards PHB accumulation can be more directly achieved in the seeds of crops having a naturally high flux of carbon through acetyl-CoA. Also a substantial increase in the PHB synthesis may potentially be achieved through a decrease in fatty acid biosynthesis in the seed by antisense mediated down-regulation of acetyl-CoA carboxylase (catalyzing formation of malonyl CoA from acetyl-CoA). Thus, many oil crops such as rapeseed,

Table 2
Manufacturers and the microorganisms, raw materials used for the production of biodegradable plastics

Microorganism/raw material	Manufacturer
<i>Alcaligenes eutrophus</i> (HI6)	ZENECA Bio-products, UK (formerly ICI Ltd.)
<i>A. latus</i>	Biotechnologische Forschungs gesellschaft mbH (Austria) Petrochemie Danubia
Transgenic plants	Metabolix Inc. (USA) Monsanto (USA) ZENECA Seeds (UK)
Recombinant <i>Escherichia coli</i>	Bio Ventures Alberta Inc. (Canada)
Starch	Warner's Lambert (USA) Fertec, Italy (Ferruzzi e Tecnologia) Biotec (Melitta) Emmerich (Germany) BASF Ludwigshafen (Germany) Bayer/Wolf Walsrode Leverkusen (Germany) Novamont Novara (Italy)
Cheap substrates	Polyferm Inc. (Canada)
Bacteria	Biocorp (USA) Asahi Chemicals and Institute of Physical and Chemical Research (Japan)

sunflower and soybean could be potentially engineered for the production of PHA. The other plants currently in use for PHA production are *Gossypium hirsutum* and *Zea mays*. The advantage looks more with the starch-producing crops than oil crops in terms of yield (kg/hectare) but the diversion of acetyl-CoA towards PHB synthesis is likely to be more complex in starch crops since the flux of carbon is primarily directed towards sucrose instead of acetyl-CoA. In the UK, ZENECA Seeds is focussing its efforts on rapeseed while in the USA, Monsanto is working on both rapeseed and soybean. Many other companies are also intensely involved in the manufacture and marketing of biodegradable plastics (Table 2).

9. Production of PHA by anaerobic digestion of biological wastes

Anaerobic digestion is a multi-step process (Hobson et al., 1981; Kalia et al., 2000a; Mata-Alvarez et al., 2000). In the first step, complex biomolecules are hydrolysed into soluble, biodegradable organics. Then acidogenic and acetogenic bacteria metabolise the hydrolytic products to lower volatile fatty acids (VFAs) (Sans and Mata-Alvarez, 1995). These lower VFAs are the precursors to formation of PHA. Sources of VFAs include Municipal wastes (Kalia and Joshi, 1995), banana (Kalia et al., 2000b), damaged food grains, pea shells, apple pomace and palm oil mill effluent (Kalia et al., 1992). Various microbes like *A. eutrophus*, *Bacillus megaterium*, *P. oleovorans*, *Azotobacter beijerincki*, *Rhizobium*, *Nocardia* utilize lower VFAs as substrate for PHA production (Kalia et al., 2000a).

10. Properties and practical applications of PHA

The PHA are non-toxic, biocompatible, biodegradable thermoplastics that can be produced from renewable resources. They have a high degree of polymerization, are highly crystalline, optically active and isotactic (stereochemical regularity in repeating units), piezoelectric and insoluble in water. These features make them highly competitive with polypropylene, the petrochemical-derived plastic.

PHA have a wide range of applications owing to their novel features. Initially, PHA were used in packaging films mainly in bags, containers and paper coatings. Similar applications as conventional commodity plastics include the disposable items, such as razors, utensils, diapers, feminine hygiene products, cosmetic containers—shampoo bottles and cups. In addition to potential as a plastic material, PHA are also useful as stereoregular compounds which can serve as chiral precursors for the chemical synthesis of optically active compounds (Oeding and Schlegel, 1973; Senior and Dawes, 1973). Such compounds are particularly used as biodegradable carriers for long term dosage of drugs, medicines, hormones, insecticides and herbicides. They are also used as osteosynthetic materials in the stimulation of bone growth owing to their piezoelectric properties, in bone plates, surgical sutures and blood vessel replacements. However, the medical and pharmaceutical applications are limited due to the slow biodegradation and high hydraulic stability in sterile tissues (Wang and Bakken, 1998). The PHA are considered as a source for the synthesis of chiral compounds (enantiometrically pure chemicals) and are raw materials for the production of paints. PHA can be easily depolymerised to a rich source

of optically active, pure, bi-functional hydroxy acids. PHB for instance is readily hydrolyzed to *R*-3-hydroxybutyric acid and used in the synthesis of Merck's anti-glaucoma drug 'Truspot'. In tandem with *R*-1,3-butanediol, it is also used in the synthesis of β -lactams. Plant derived PHA can be depolymerized and used, directly or following esterification, in the manufacture of bulk chemicals (Brandl et al., 1988). Besides helping to replace the existing solvents, β -hydroxy acid esters and derivatives are likely to find growing use as 'green solvents' similar to lactic acid esters. The conversion of hydroxy acids into crotonic acids such as 1,3-butanediol, lactones, etc. would help improve the market value as they have an existing market demand in thousands of tonnes.

11. Biodegradability of PHA

The property that distinguishes PHA from petroleum based plastics is their biodegradability. PHA are degraded upon exposure to soil, compost, or marine sediment. Biodegradation is dependent on a number of factors such as microbial activity of the environment, and the exposed surface area, moisture, temperature, pH, molecular weight (Boopathy, 2000). For PHA, polymer composition and crystallinity also assume importance (Lee, 1996b). The nature of the monomer units also has been found to affect degradation. Copolymers containing PHB monomer units have been found to be degraded more rapidly than either PHB or 3HB-co-3HV copolymers. Microorganisms secrete enzymes that break down the polymer into its molecular building blocks, called hydroxyacids, which are utilized as a carbon source for growth. The principal enzyme for the degradation of PHB and oligomers derived from the polymer is PHB depolymerase. Studies on the extracellular PHB depolymerase of *Alcaligenes faecalis* have indicated it to be an endo-type hydrolase. Other prominent organisms in which PHB depolymerase has been identified and worked upon are *Rhodospirillum rubrum*, *B. megaterium*, *A. beijerinckii*, and *Pseudomonas lemoignei*. Biodegradation of PHA under aerobic conditions results in carbon dioxide and water, whereas in anaerobic conditions

the degradation products are carbon dioxide and methane. PHA are compostable over a wide range of temperatures, even at a maximum of around 60 °C with moisture levels at 55%. Studies have shown that 85% of PHA were degraded in seven weeks (Johnstone, 1990; Flechter, 1993). PHA have been reported to degrade in aquatic environments (Lake Lugano, Switzerland) within 254 days even at temperatures not exceeding 6 °C (Johnstone, 1990).

12. Economics of PHA production

It is a prerequisite to standardize all the fermentation conditions for the successful implementation of commercial PHA production systems. The price of the product ultimately depends on the substrate cost, PHA yield on the substrate, and the efficiency of product formulation in the downstream processing (Lee, 1996b). This implies high levels of PHA as a percentage of cell dry weight and high productivity in terms of gram of product per unit volume and time (de Koning and Witholt, 1997; de Koning et al., 1997). Commercial applications and wide use of PHA is hampered due to its price. The cost of PHA using the natural producer *A. eutrophus* is US\$16 per Kg which is 18 times more expensive than polypropylene. With recombinant *E. coli* as producer of PHA, price can be reduced to US\$4 per Kg, which is close to other biodegradable plastic materials such as PLA and aliphatic polyesters. The commercially viable price should come to US\$3–5 per Kg (Lee, 1996b). The effect of various substrate costs and the yield on the P(3HB) production cost are described in Table 3. The development of PHB was begun by Imperial Chemical Industries (ICI) in 1975–76 as a response to the increase in oil prices. ICI started making 'Biopol' as early as in 1982 from *A. eutrophus* (H16). Cargill Dow Polymers is marketing its 'EcoPla' resins in Japan, Novamont's starch-based material, 'Mater-Bi', is marketed in Japan by Nippon Gohsei. Mater-Bi has been used in transport packaging for electrical goods, agricultural mulch film and in composting trials. Mitsubishi and Nippon Shokubai under the trade names 'LUNARE ZT' and 'Lunare SE' market 'EnviroPlastic'.

Table 3
Effect of substrate cost and P(3HB) yield on the production cost (Madison and Huisman, 1999)

Substrate	Substrate price (US\$ kg ⁻¹)	P(3HB) yield (g P(3HB) (g substrate) ⁻¹)	Product cost (US\$ (kg P(3HB)) ⁻¹)
Glucose	0.493	0.38	1.30
Sucrose	0.290	0.40	0.72
Methanol	0.180	0.43	0.42
Acetic acid	0.595	0.38	1.56
Ethanol	0.502	0.50	1.00
Cane molasses	0.220	0.42	0.52
Cheese whey	0.071	0.33	0.22
Hemicellulose hydrolysate	0.069	0.20	0.34

'Bionolle', a thermoplastic aliphatic polyester, is manufactured by Showa Highpolymer and Denko of Japan. It is produced by the polycondensation of glycol with dicarboxylic acids. 'Lacea' is another type of bioplastic manufactured by Mitsui Chemicals, Japan, from fermented starch, derived from a variety of renewable resources, such as corn, beet, cane, and tapioca. Lacea is comparable to polyethylene in terms of transparency and similar to polystyrene or polyethylene in terms of processability. It also claims good mould resistance, low heat of combustion which is similar to that of paper, superior stability in processing use and biodegradability superior to that of earlier polylactic acid-based materials. Daicel Chemical Industries, Japan has developed biodegradable blends of two different kinds of material, polycaprolactone and acetyl cellulose resin with the brand name 'Celgreen'. Shimadzu developed a fermentation process for lactic acid and collaborated with Mitsubishi Plastics Industries to develop poly-L-lactic acid. The resins are marketed under the trade name 'Lacty'. The other bioplastics manufactured by Japan based firms are 'Eco-Ware' and 'Eco-Foam' (which are starch-based) and Cardoran and Pulluran (which are based on polysaccharides).

13. Poly lactides and polyglycolides

Other biodegradable plastics that have gained attention are the PLA and polyglycolides (PGA). PLA and PGA are thermoplastics and biodegradable polyesters. Low molecular PLA and PGA are made by direct polymerization of lactide and glycolide whereas the high molecular PLA and PGA are made by ring-open polymerization of lactide and glycolide, which are cyclic diesters of the respective acids. Polyglycolic acid and polylactic acid have degradation times from a few days to a few weeks, respectively. The degradation times of PLA and PGA range from a few months to years. Dexon, a polyglycolic acid homopolymer, was the first absorbable suture material developed by the American Cyanamid Corporation. Vicryl was developed by Dupont which has 92:8 glycolic acid:lactic acid as copolymer. The Ecological Chemical Products Co. (ECOCHEM) a joint venture of DUPONT and Con. Agra. Inc. has opened a US\$20 million commercial plant in Adel, Wisconsin to produce lactic acid and polylactide polymers for food and pharmaceutical applications. The production of lactic acid-based plastics from starchy food wastes and byproducts such as whey is on the increase (Hocking and Marchessault, 1997).

PLA and PGA find their main importance in medical applications, as sutures, as ligament replacements for resorbable plates and screws in orthopedic repairs, for controlled drug release and for arterial grafts. Production is in two key steps, one converts glucose to lactic

acid and the other converts lactic acid to polylactic acid. The properties of polylactic acid are similar to polystyrene and can also be modified to make it similar to polyethylene or polypropylene. Polyglutamic acid (PGU) is a water-soluble polymer produced by *Bacillus* sp. with maximum yields reported up to 40 g/l of PGU in 5 days. Biodegradable enviroplastic is developed by Planet Technologies, USA using sucrose, adipic acid, and the action of lipases and proteases is sought to link sugar and di-acid into a copolymer chain (Preusting et al., 1990; Lee, 1996b). The only plant-based plastic that is currently being commercialized is Cargill Dow's PLA.

Aliphatic polyesters are now produced from natural resources like starch and sugars through large-scale fermentation processes and used to manufacture water-resistant bottles, eating utensils and a few other products.

Triglycerides have become the basis for a new family of sturdy composites. Infusion of glass fiber, jute fiber, hemp, flax, wood and straw or hay with triglycerides is useful in making long lasting durable materials with applications in the manufacture of agricultural equipment, the automobile industry, building construction, etc.

14. Conclusions

The current advances in metabolic engineering supported by the genome information and bioinformatics have opened a cascade of opportunities to introduce new metabolic pathways. This would help us not only to broaden the utilizable substrate range and produce tailor-made PHA but also enhance the current PHA yields. The studies should also be focussed towards understanding the host-plasmid interactions so as to develop stable plasmids such that the recombinant bacteria surpass the wild-type bacteria currently in use. Transgenic plants harboring the microbial PHA biosynthesis genes have been recently developed with the aim of ultimately reducing the price of the polymer. However, much more effort is required in this area to increase the production of bioplastics to successfully replace the non-degradable plastics. Thus the future of bioplastics depends on the efforts towards fulfilling requirements of price and performance.

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