BIOSYNTHESIS AND CHARACTERIZATION OF POLYHYDROXYALKANOATES BY A LOCALLY ISOLATED *Chromobacterium* sp. USM2

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

%	percentage
°C	degree Celsius
3HA	3-hydroxyalkanoate
3HB	3-hydroxybutyrate
3HB-CoA	D(–)-3-hydroxybutyryl-coenzyme-A
3HV	3-hydroxyvalerate
3HV-CoA	D(–)-3-hydroxyvaleryl-coenzyme-A
4HB	4-hydroxybutyrate
$CaCl_2$	calcium chloride
cm	centimetre
CME	caprylic acid methyl ester
CoA	coenzyme-A
$CoSO_4$	cobalt sulphate
$CuCl_2$	copper (II) chloride
$FeSO_4$	ferrum sulphate
g	gram
GC	gas chromatography
h	hour
HCl	hydrochloric acid
H_2O_2	hydrogen peroxide
KH_2PO_4	potassium dihydrogen phosphate
K_2HPO_4	dipotassium hydrogen phosphate
kPa	kilopascal
L	litre
LB	Luria-Bertani
M	molar

MCL	medium-chain-length
mg	milligram
$MgSO_4$	magnesium sulphate
min	minute
mL	millilitre
mm	millimetre
$MnCl_2$	manganese chloride
mol%	mol percentage
µg	microgram
µL	microlitre
µm	micrometre
NA	nutrient agar
NaCl	sodium chloride
NADH	reduced nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NaOH	sodium hydroxide
NB	nutrient broth
nm	nanometre
$(NH_4)_2SO_4$	ammonium sulphate
OD	optical density
OsO_4	osmium tetroxide
P(3HB)	poly(3-hydroxybutyrate)
P(3HB-*co*-3HV)	poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate)
P(3HB-*co*-4HB)	poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate)
P(3HV)	Poly(3-hydroxyvalerate)
PHA	polyhydroxyalkanoate
rpm	revolution per minute
SCL	short-chain-length
sp.	species

TEM	transmission electron microscope
UV	ultraviolet
vol	volume
W	watt
wt	weight
wt%	weight percentage
$ZnSO_4$	zinc sulfate
β	beta

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Biosintesis dan pencirian polihidroksialkanoat oleh pencilan tempatan *Chromobacterium* sp. USM2

ABSTRAK

Terdapat banyak mikroorganisma semula jadi yang mampu menghasilkan polihidroksialkanoat (PHA). Namun, mikrob yang dapat mensintesis PHA yang mengandungi komposisi monomer 3-hidroksivalerat tinggi adalah amat jarang ditemui. Kajian ini melaporkan biosintesis dan pencirian kopolimer poli(3-hidroksibutirat-*co*-3-hidroksivalerat) [P(3HB-*co*-3HV)] dengan komposisi 3HV yang tinggi oleh *Chromobacterium* sp. USM2. Strain ini dipencilkan dari sampel air tawar Air Terjun Telaga Tujuh yang terkenal di Pulau Langkawi. Berdasarkan ciri-ciri morfologi, fisiologi dan biokimia serta analisis 16S rRNA, pencilan tersebut telah dikenalpasti sebagai *Chromobacterium violaceum*. Pencilan tersebut berupaya menghasilkan homopolimer poli(3-hidroksibutirat) dan kopolimer P(3HB-*co*-3HV) bergantung kepada keadaan pengkulturan dan jenis sumber karbon yang dibekalkan. Komposisi 3HV yang tertinggi sebanyak 98 mol% dicatatkan apabila natrium valerat digunakan sebagai sumber karbon tunggal dalam pengkulturan dua peringkat. Tambahan pula, telah dibuktikan bahawa komposisi 3HV dalam PHA yang dihasilkan boleh dikawal dengan menggunakan jenis dan kepekatan sumber karbon yang berpelbagaian.

ABSTRACT

Although various naturally occurring microorganisms are capable of producing polyhydroxyalkanoates (PHAs), microbes with the ability to synthesize PHAs that contain predominantly 3-hydroxyvalerate monomers are rare. This study reports on the biosynthesis and characterization of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] copolymers with high 3HV composition by *Chromobacterium* sp. USM2. This interesting strain was isolated from the freshwater samples of the famous Telaga Tujuh Waterfalls at Langkawi Island. On the basis of morphological, physiological, biochemical characteristics and 16S rRNA sequence analysis, the isolate was provisionally identified as *Chromobacterium violaceum*. The isolate was able to produce homopolymer of poly(3-hydroxybutyrate) and copolymers of P(3HB-*co*-3HV) depending on the cultivation conditions and carbon sources provided. The highest 3HV composition recorded was 98 mol% when sodium valerate was used as the sole carbon source in a two stage cultivation. In addition, it was shown that the 3HV composition of the PHA produced can be controlled by varying the type and concentration of the carbon sources.

1.0 Introduction

The introduction of synthetic polymers (plastics) has significantly changed our daily life due to their widespread use in industrial, domestic and environmental applications. Plastics structure can be manipulated to have a wide range of strength and shape. Due to the high molecular weight which ranges from several to 150,000 Da, plastics are resistant to biodegradation. They are able to persist in soil environment for very long time (Atlas, 1993). Even though plastics are recalcitrant, they are regarded to be more aesthetic nuisance rather than hazardous because they are biologically inert.

Great demand for plastics over the past few decades has caused serious environmental problem. Existing methods to deal with disposed plastics are inefficient. Incineration of plastics releases hazardous chemicals such as hydrochloric acid and hydrogen cyanide and the process is costly (Fiechter, 1990). Recycling also has some drawbacks. The recycled plastics have limited usage since their structure has been changed (Fiechter, 1990). Wide variety of plastics makes the process of sorting different plastics to be recycled even more difficult. Meanwhile, landfills usually reach their maximum capacity very rapidly as the amount of plastics produced and thrown away is rising. Discarding plastics into marine environment posts serious threat to marine life as millions of them

were killed either by choking on plastics or entangled in non-degradable plastics debris (Fiechter, 1990).

In response to these problems, the idea of substituting non-degradable synthetic plastics with biodegradable plastics has drawn interest from both academic and commercial world.

Currently, biodegradable plastics can be categorized into three main types; photodegradable, starch-linked and bacterial plastics. There has been increasing effort to study and develop the production and application of bacterial plastics. In 1925, Lemoigne had discovered and firstly described a polymer, polyhydroxyalkanoate (PHA) produced by *Bacillus megaterium* (Lemoigne, 1926). Later, many bacteria were found capable of producing various PHAs.

This natural polymer exists as intracellular inclusions that are synthesized and accumulated by certain bacteria under unfavorable growth condition caused by limiting nutrients and in the presence of excess carbon sources. PHA granules serve as carbon and energy source during starvation and are crucial for the survival in harsh conditions caused by fluctuating osmotic pressure and radiation. PHA degrades naturally and completely into carbon dioxide and water under aerobic conditions and into methane under anaerobic conditions (Doi *et al*., 1992).

The physical and chemical properties of PHA can be controlled and manipulated by altering the medium on which the bacteria grow (Pool, 1989). Thus, polymers with desirable properties can be produced.

1.1 The objectives of this study

Due to relatively poor physical properties of P(3HB) homopolymer, extensive efforts are being directed towards the synthesis of copolymers that possess better qualities. P(3HB-*co*-3HV) have improved physical properties in terms of its melting point, crystallinity, plasticity and biodegradability (Doi, 1990). Researches from a British chemical company, Imperial Chemical Industry developed and marketed this copolymer under the trade name Biopol® (Lee, 1996). P(3HB-*co*-3HV) with high 3HV molar fraction has improved processibility and wider applications (Pool,1989). Therefore, the objectives of this study are:

i. to isolate and identify a PHA producing bacteria from natural environment.

ii. to produce P(3HB-*co*-3HV) copolymer with high 3HV composition.

LITERATURE REVIEW

2.1 *Chromobacterium violaceum*

C. violaceum is a Gram negative bacterium that was firstly described at the end of the 19th century (Hungria *et al*., 2004), after which several reports confirmed its presence in a variety of ecosystems in tropical and subtropical regions. *C.violaceum* is found abundantly in the water and the borders of the Negro River located in the Amazon region, Brazil (Durán *et al*., 1989). *C. violaceum* is able to live under aerobic and anaerobic conditions. It can survive under a series of diverse environmental conditions which require great adaptability and strong protective system (Vasconcelos *et al*., 2003).

Indeed, the most notable feature of this bacterium is its ability to produce a purple pigment called violacein. Both pigmented and non-pigmented strains are known to exist, though the non-pigmented strain is very rare (Sneath, 1984).

Upon the completion of the entire genome sequence of wild type *C.violaceum*, a wide range of genes related to its adaptability has been revealed (Vasconcelos *et al*., 2003).

2.2 Pathogenicity

Wooley first described the pathogenicity potential of *C.violaceum* in 1905 when he isolated it from water buffaloes in the Phillipines (Wooley, 1905). Since the first report of human infection by Lessor in 1927, several other cases had also been reported. Most of these infections occurred in Brazil, South East Asia and the South Eastern of the United States (Chattopadhyay *et al*., 2002).

Infection in human usually occurs when broken skin is exposed to contaminated soil and water (McGowan *et al*., 1995). Cellulitis is the early symptom of infection which resembled to Staphylococcal infection (Victorcia *et al*., 1974). This is occasionally followed by lumphadenitis and then bacteremia with multiple abscesses in the liver, lungs and brain which is usually fatal (Shao *et. al*., 2002).

Diagnosis of *C.violaceum* infections are made by the culture of blood, lumph nodes, cutaneous lesions, exudates, or abscess fluid (Auerbach, 1987). Treatment usually consists of surgical drainage of purulent collections and appropriate antibiotic administration. Recommendation for the duration of antibiotic treatment includes 3 to 4 weeks of intravenous therapy followed by a month or more of oral antibiotics (Fisher *et al*., 1998).

Despite the ubiquitous distribution, human infection with this organism is very rare and the awareness of the disease is limited so far.

2.3 Biotechnological potentials of *C.violaceum*

Even though *C.violaceum* is pathogenic, it has been known to produced many useful genes, enzymes and other secondary metabolites (Vasconcelos *et al*., 2003). The purple pigment, violacein which is a notable characteristic of this bacteria exhibits antimicrobial (Fisher *et al*., 1998, Vasconcelos *et al*., 2003), antibiotic, antiviral (Durán *et al*., 2001), antitumor (Furumai *et al*., 2002) and anticoagulant properties. *C.violaceum* also secretes antibiotics such as aerocavin (Singh *et al*., 1988) and aerocyanidine (Parker *et al*., 1988).

Campbell *et al*. (2001) have shown that *C.violaceum* cultures can produce cyanide which is very useful in gold production enhancement. Cyanide forms a complex anion when react with the gold on the ore surface. This solubilised gold are then extracted easily without the usage of mercury, thereby avoiding environmental contamination (Campbell *et al*., 2001).

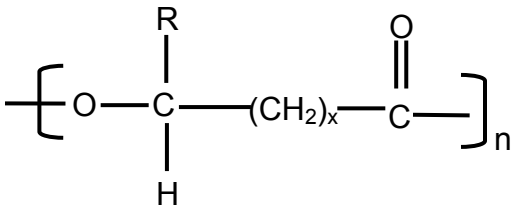
Besides that, acid dehalogenase produced by *C.violaceum* could be used to detoxify contaminated soils at industrial sites from chlorinated compounds (Lee *et al*., 1998). *C.violaceum* is also known to synthesis polyhydroxyalkanoate (PHA) polymers. This has made it a possible

candidate to produce renewable source of biodegradable plastics. PHA is a viable alternative to synthetic plastic derived from petrochemical since it exhibits similar physical properties to most commonly used plastic, polypropylene (Steinbüchel *et al*., 1993). Furthermore, PHAs can be completely degraded in natural environment.

2.4 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are natural polymers biosynthesized and accumulated as intracellular granules in some bacteria. PHAs are produced as an energy and carbon reserve, under unfavourable conditions of limiting nutrients in the presence of excess carbon source (Madison and Huisman, 1999). These intracellular inclusions are mobilized and metabolized as energy and carbon source once the limiting nutrient is restored. The general structure of PHAs is shown in Figure 2.1.

Among all, polyhydroxybutyrate (PHB) that consists of only 3HB monomers is the most commonly found PHAs in the nature (Horowitz and Sanders, 1994). PHB has physical properties that resembled synthetic petrochemical-based plastics. PHAs are thermoplastics, elastomeric, insoluble in water, enantiomeric pure, non-toxic, biocompatible, piezoelectric and exhibit high degree of polymerization and molecular weights (Hocking *et al*., 1994).



		Type of monomer
x = 1	R = methyl	3-hydroxybuytrate; 3HB
	R = ethyl	3-hydroxyvalerate; 3HV
	R = propyl	3-hydroxyhexanoate; 3HHx
x = 2	R = hydrogen	4-hydroxybutyrate; 4HB
x = 3	R = hydrogen	5-hydroxyvalerate; 5HV

Figure 2.1. General structure of PHAs (Sudesh *et al*., 2000)

In natural environment, PHAs degrade naturally and completely into CO_2 and H_2O (water) by the enzymatic activities of microbes. This is the most important feature of PHAs (Jendrossek *et al*., 1996).

Simple, renewable resources such as sucrose, starch and cellulose can be used to produce PHAs. In contrary, production of synthetic plastics consumes non-renewable fossil resources such as petroleum. PHAs' desirable physical properties such as biodegradability and readily produced from renewable resources have make it a viable substitute to petrochemical-based plastics (Byrom, 1987).

PHAs can be observed under phase contrast light microscope, as discrete granules sized between 0.2 to 0.5 μm in diameter which is localized in the cell cytoplasm. Under transmission electron microscope (TEM), it appears as electron-densed bodies. In terms of molecular weight, PHA can weigh between 2 X 10^5 to 3 X 10^6 Daltons (Hocking *et al*., 1994). Native PHA granules can be stained with Sudan Black B (Burdon, 1946), Nile Blue A (Ostle and Holt, 1982) and Nile Red (Garenflo *et al*., 1999). PHAs are more specifically stained by Nile Blue A, where its presence is indicated by strong orange fluorescence. The structure, physio-chemical properties, monomer composition and the number and size of the PHA granules vary depending on the organism (Anderson and Dawes, 1990).

2.5 Classification of PHAs

A wide variety of PHAs has been discovered in various bacteria. So far, approximately 150 different constituents of PHAs have been identified (Steinbüchel and Valentin, 1995).

PHAs can be divided into two broad groups, depending on the number of carbon atoms in the polymer chain. They are short chain length and medium chain length PHAs. The short chain length polyhydroxyalkanoates (scl-PHAs) consists of 3-5 carbons atoms. As for polyhydroxyalkanoates with medium chain length (mcl-PHAs), it usually consists of 6-14 carbon atoms.

The composition of the PHAs produced varies depending on the growth substrates used. This is due to the substrate specificity of the PHA synthases that only accept 3-hydroxyalkanoates (3HAs) of a certain range of carbon length (Anderson and Dawes, 1990).

2.6 Poly(3-hydroxybutyrate) P(3HB)

P(3HB) is the most common type and best characterized PHA. In fact, it was the first PHA to be discovered that contains only 3HB monomers. It is comparable with conventional plastic like polypropylene in terms of melting point, crystallinity, molecular weight and tensile strength (Table 2.1).

Most of the available biodegradable plastics have certain disadvantages since they are water soluble and moisture sensitive (Lee, 1996). P(3HB) has overcome these problems as it exhibits resistance to moisture, insoluble in water and has better optical purity (Lindsay, 1992). It also shows good oxygen permeability (Lindsay, 1992). Besides heat resistance (up to 130 °C), P(3HB) also has better resistance to Ultra Violet (UV) lights than polypropylene (Lee, 1996).

However, the practical applications of P(3HB) is limited due to its poor impact strength. P(3HB) is a highly stiff, crystalline and a relatively brittle thermoplastic. Its melting point at 175 °C is only slightly lower than the degrading temperature, making it difficult to be heat processed. Hence, extensive works are being directed towards the synthesis of copolymers that have better properties than the homopolymer of P(3HB). For example, incorporating substrates such as 3-hydroxyvalerate may result in greater strength and flexibility.

Table 2.1. Physical properties of poly(3-hydroxybutyrate) P(3HB) compared to polypropylene (PP) (Evans and Sikdar, 1990)

Properties	P(3HB)	PP
Crystalline melting point, °C	175	176
Crystallinity, %	80	70
Molecular weight, Daltons	5×10^5	2×10^5
Glass transition temperature, °C	15	− 10
Density, g/cm^3	1.25	0.905
Young's modulus, GPa	3.5	1.7
Tensile strength, MPa	40	38
Extension to break, %	6	400
UV resistance	Good	Poor

2.7 P(3HB) biosynthesis pathway

The synthesis of P(3HB) involves three successive enzymatic reactions which is catalyzed by three distinctive enzymes and their respective encoding genes as shown in Figure 2.2. *phb*A gene encodes β-ketothiolase which catalyzes the first reaction; condensation of two acetyl-CoA molecules into acetoacetyl-CoA. A reduction process is followed in which acetoacetyl-CoA is reduced into (*R*)-3-hydroxybutyryl CoA by an NADPH-dependent acetoacetyl-CoA reductase. This enzyme is encoded by *phb*B. The last reaction is the polymerization of (*R*)-3-hydroxybutyryl CoA monomers into P(3HB) by PHA synthase, which is encoded by *phb*C (Huisman *et al*., 1989).

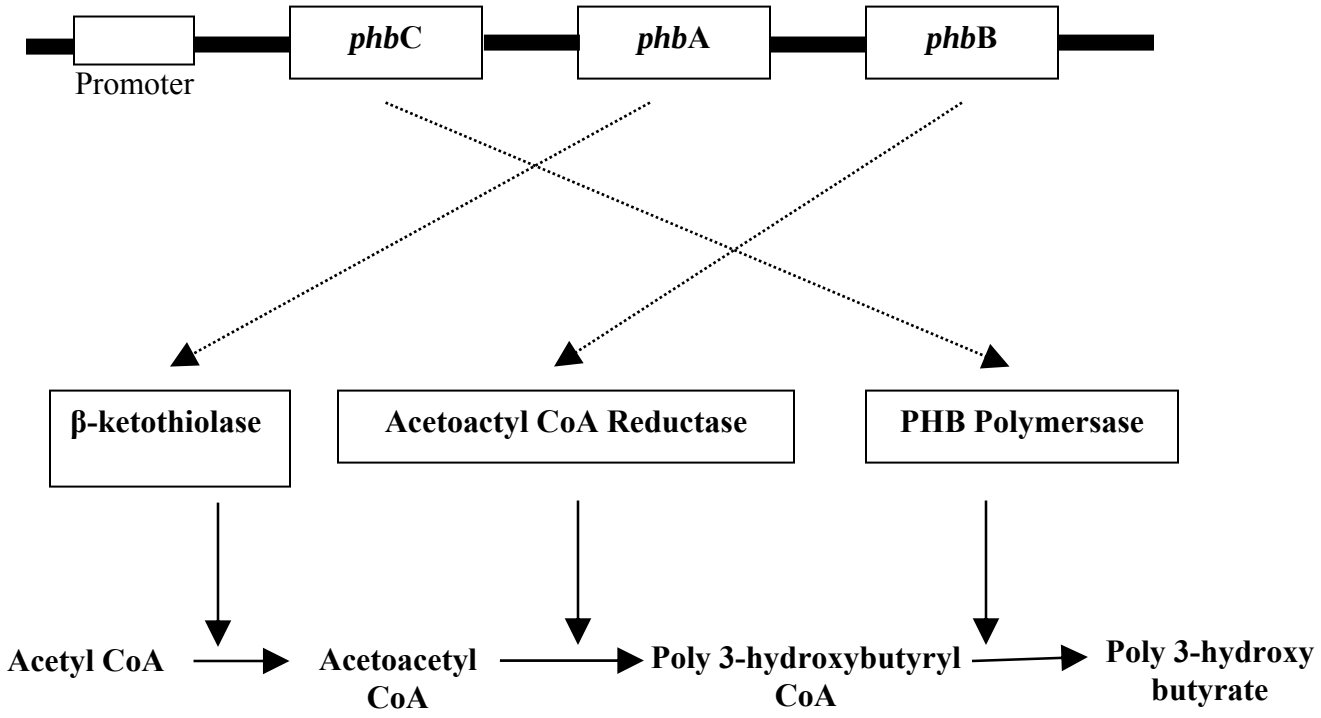


Figure 2.2. Biosynthetic pathway of poly(3-hydroxybutyrate). P(3HB) is synthesized by three successive reactions by three different enzymes. These enzymes are encoded by the genes of the *phb*CAB Operon. A promoter upstream of *phb*C transcribes the complete operon *phb*CAB (Madison and Huisman, 1999).

2.8 Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), P(3HB-*co*-3HV)

P(3HB) homopolymer form stiff crystalline materials which are brittle and unextendable without breakage, thus limiting its range of applications. It is also hard to process because it degrades at 10 ºC above its 177 °C melting point (Luzier, 1992). Therefore, extensive efforts are being directed towards the improvement of its properties, including the formation of a copolymer.

P(3HB-*co*-3HV) is the most extensively studied PHA copolymer. Its' mechanical properties depends on the molar fraction of 3HV. Incorporation of 3 to 5 carbon monomers into a polymer consisting mainly of 3HB, leads to a decrease in crystallinity and melting point, but improved flexibility, strength and easier processing without greatly affecting degradation temperature (Lee, 1996b). P(3HB-*co*-3HV) copolymer is much more flexible than P(3HB) homopolymer since it exhibits a five fold decrease in Young's modulus to 0.7 GPa as the fraction of 3HV unit increases (Williams and Peoples, 1996). The elongation to break is also reported to increase as the co-monomer fraction increases (Lee, 1996b). The chemical structure of P(3HB-*co*-3HV) is shown in Figure 2.3 and its typical properties are shown in Table 2.2.

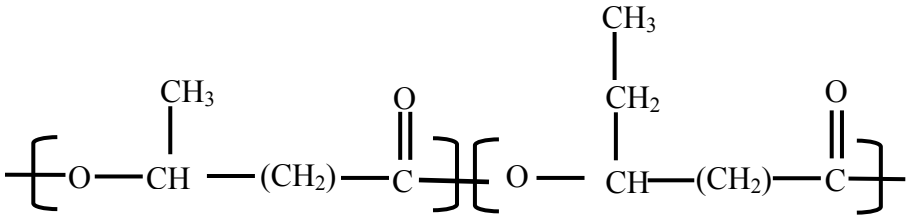


Figure 2.3. Chemical structure of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), P(3HB-*co*-3HV) (Holmes, 1988).

Table 2.2. Physical properties of poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate), P(3HB-*co*-3HV) (Luzier,1992).

	3HV content, mol %		
Properties	0	10	20
Melting point, °C	177	140	130
Crystallinity, %	80	60	35
Tensile strength, MPa	40	25	20
Flexural modulus, GPa	3.5	1.2	0.8

P(3HB-*co*-3HV) was developed by Imperial Chemical Industries (ICI), now known as ZENECA Bio Products. It is known under the trade name Biopol® (Lee, 1996a) and used in Europe to bottle products such as shampoo (Coghlan, 1992).

2.9 P(3HB-*co*-3HV) biosynthesis pathway

Biosynthesis of poly(3HB-*co*-3HV) requires both 3HB-CoA and also 3-hydroxyvaleryl-CoA, 3HV-CoA. 3HV-CoA is obtained from condensation of acetyl-CoA and propionyl-CoA to 3-ketovaleryl-CoA. This reversible process is promoted by a distinct 3-ketothiolase. As shown in Figure 2.4, reduction of 3-ketovaleryl-CoA to (R)-3-hydroxyvaleryl-CoA and subsequent polymerization to form 3HV unit is catalyzed by the same enzymes involved in PHB synthesis, namely acetoacetyl-CoA reductase and PHA synthase (Poirier, 2002). Hence, the biosynthesis of poly(3HB-*co*-3HV) requires formation and occurance of propionyl-CoA in the cells.

Propionic acid is the most commonly used precursor substrate for poly(3HB-*co*-3HV) production. However, it has a few disadvantages. Firstly, propionic acid is toxic and it could inhibit cell growth when high concentration is used. Therefore, propionic acid must be fed at relatively low concentration. Secondly, it does not exclusively convert into 3HV units and may condense to acetyl-CoA which is an intermediate substrate for 3HB units (Doi *et al*., 1987). Lastly, it costs higher than other simple carbon sources such as glucose.

Aliphatic fatty acids with a higher carbon chain length and an odd number of carbon atoms such as valeric acid, heptanoic acid and nonanoic acid are also viable 3HV precursor candidates. This is because propionyl-CoA

instead of acetyl-CoA is the final product of the β-oxidation cycle (Steinbüchel and Lütke-Eversloh, 2003). Therefore, the probability of getting polymer with higher 3HV fraction could be increased when cultivated with fatty acids.

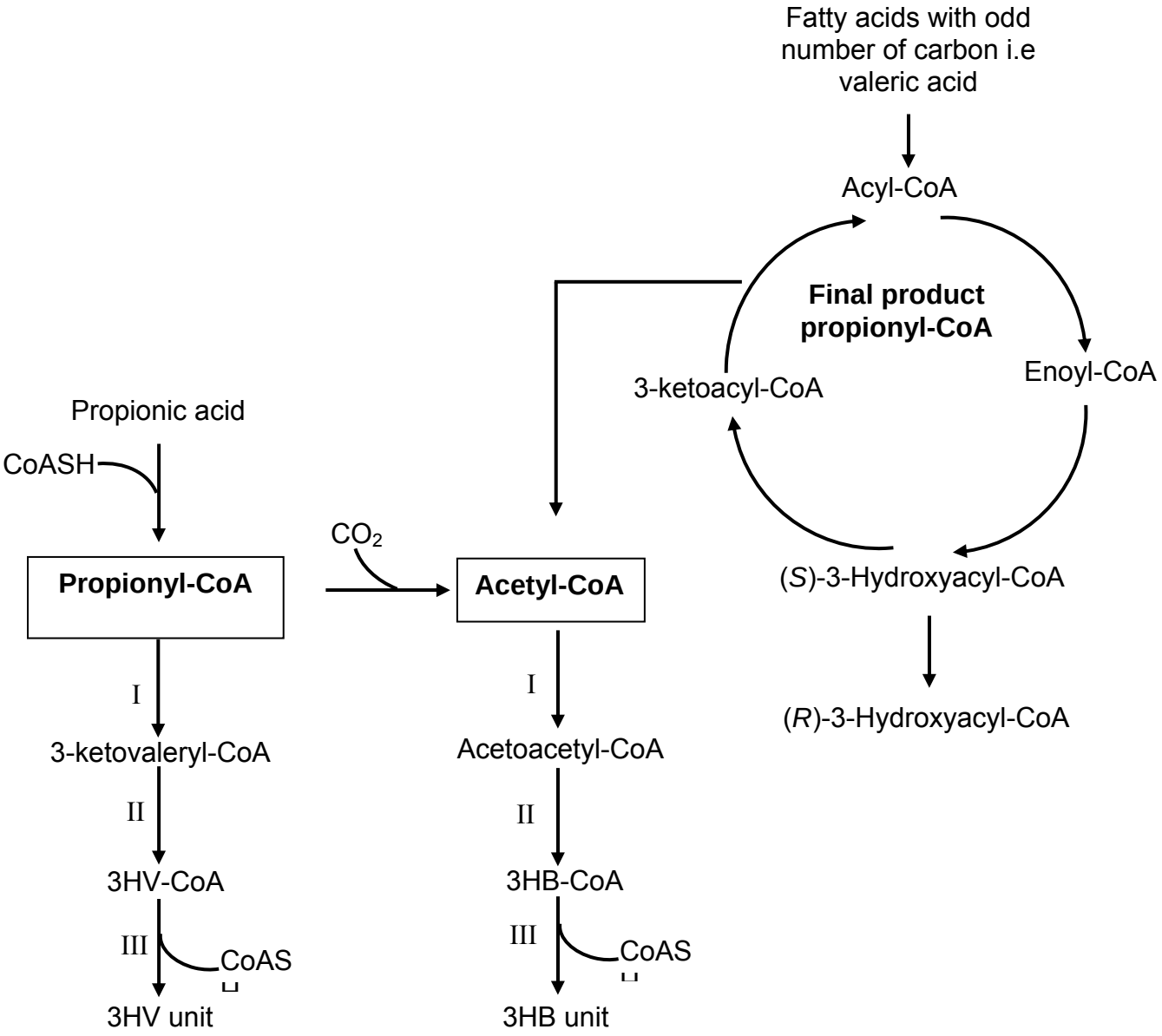


Figure 2.4. Biosynthesis pathways of P(3HB-*co*-3HV) that is involved in the uptake and conversion of propionic acid and other fatty acids with odd numbers of carbon atoms. Enzymes: I - 3-ketothiolase; II -NADPH-dependent acetoacetyl-CoA reductase; III - PHA synthase (Steinbüchel and Lütke-Eversloh, 2003)

2.10 Carbon sources for PHAs production

PHAs are produced and accumulated in most bacteria under unfavorable condition of limited essential nutrients in the presence of excess carbon sources (Madison and Huisman, 1999). Several factors need to be considered in the selection of the bacteria for PHAs production at industrial scale. The ability of the bacteria to utilize cheap carbon sources is very important because the cost of substrates will significantly affect the overall production cost.

Preliminary calculation of PHAs yield on several carbon sources has been derived by calculation (Yu and Wang, 2001). Recently, studies have been conducted to explore cultivation strategies involving inexpensive, renewable carbon substrate in order to reduce the production costs and improve productivity (Doi *et al*., 1988)

PHA producing bacteria are capable of utilizing various carbon sources such as plant oils (Loo *et al.*, 2005), fatty acids (Tan *et al.*, 1997), agricultural waste products such as beef molasses, whey and starch (Omar *et al*., 2001) as well as sugars (Borah *et al*., 2002). Sugars, oils and agricultural wastes are derived from plant as the products of photosynthesis, thus they are natural and renewable. Utilization of these plant derivatives are actually an indirect way of using atmospheric carbon dioxide as the carbon source for PHAs production.

2.11 Fermentation strategies

In general, bacteria used in PHAs production can be categorized into two groups. The main difference is based on the culture conditions required to induce the production of PHAs. Under unfavorable conditions of limiting nutrients in the presence of excess carbon source, the first group of bacteria such as *Cupriavidus necator* (formerly known as *R. eutropha*) and *Protomonas oleovorans* can produce and accumulate PHAs. On the other hand, the second group of bacteria does not require essential nutrient limitation to produce PHAs such as *Alcaligenes latus* and recombinant *E. coli* (Hahn *et al*., 1995).

Production of PHAs by the first group of bacteria can be done by either fed-batch or continuous fermentation. The fed-batch process employed often is done in two-stage cultivation. In the first stage, culture is fed with simple carbon sources such as glucose or fructose and other essential nutrients. This is to obtain desirable high cell biomass. In the second stage of the culture, supply of essential nutrient such as nitrogen is limited to initiate the synthesis of PHAs. During this stage, cell growth is impaired but the cell biomass still increases due to the accumulation of PHAs inside the bacteria cells. *C. necator* can accumulate PHAs up to 80% of its dry cell weight when nitrogen or phosphorus is completely depleted (Kim *et al*., 1994). PHA yield can be enhanced if the availability of carbon source and the nutrient to be limited are kept at the optimal ratio.

For the second group of bacteria, nutrient limitation is not required and complex nitrogen sources such as yeast extracts or corn steep liquor can be used to increase biomass of cell and also improve PHAs accumulation. For these bacteria, active cell growth and PHAs accumulation occurs simultaneously. However, these two processes need to be kept at a balance state to avoid incomplete accumulation of PHAs. The advantage of using such bacteria for larger-scale production is a shorter fermentation time. It also avoids extra operations associated with the two-stage cultivation strategy.

Ability of bacterial cell to utilize cheap carbon source to achieve high biomass and PHA yield is a crucial factor to be considered in producing PHAs at a larger scale. The efficiency of PHAs recovery is also very important because it significantly affects the overall cost of the production.