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Review

Biosynthesis of polyhydroxyalkanoates: Current research and development

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Polyhydroxyalkanoates (PHAs) as an attractive biopolymer with a wide range of applications have been extensively studied. Many new strategies have been employed to effectively and economically produce PHAs. This mini-review mainly focuses on the research and development of new PHAs-producing strains, utilization of renewable materials or industrial wastes, and high cell density culture technologies for PHAs production developed in recent years. The status of PHAs mass production is also introduced here.

Key words: Polyhydroxyalkanoates, biosynthesis, new research progress.

INTRODUCTION

Polyhyroxyalkanotes (PHAs) are a family of polyhydroxyesters synthesized by numerous bacteria as an intracellular carbon and energy storage compound under nutrient-limiting conditions with excess carbon. They can be used in many fields, and new potential applications are still emerging (Philip et al., 2007). Therefore much attention has been paid on the research and development of PHAs production, and much progress has been achieved. Although several review papers (Dias et al., 2006; Khanna and Srivastava, 2005; Rezzonico et al., 2002; Li et al., 2007; Sun et al., 2007; Suriyamongkol et al., 2007) about PHAs production and applications had been published already, this paper introduces the latest research progress on PHAs synthesis reported in the past 2 or 3 years.

NEW PHAs-PRODUCING SPECIES

The best PHAs-producing species should satisfy several demands such as the fast growing population, being able to utilize cheap carbon and having a high production rate. There are two ways of acquiring new bacterial species to meet the above demands: to isolate from the natural environment and design genetic recombination strains. Both of them could gain desired species for producing PHAs (Lopez-Cortes et al., 2008). Recently, about 19 new bacterial species were isolated or re-constructed for producing PHAs (Table 1). It can be seen that most of the new strains were just used in flask or batch cultures, such that the final cell densities and PHAs contents in most cases were not so high and there is still much work to be done. However, for the fed-batch culture of recombinant *Escherichia coli* K24K, the cell density and PHAs content reached 70.1 g/L and 72.9%, respectively, when whey was used as the sole carbon source. In addition, it can be seen that about 63% of the new strains listed on Table 1 are recombinant strains. This again, shows the power of genetic recombination in the production of desired species.

Besides the bacterial species mentioned above, transgenic plants have also been used to produce PHAs. It has been confirmed that potato, tobacco leaf, corn, sugarcane and cotton have the ability to synthesize PHAs, but the PHAs content in transgenic plants was still at a low level (Bohlmann, 2006; Kadouri et al., 2005; Nielsen, 2007).

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Table 1. New PHAs producing bacteria.

Strains	Source/ Gene	Carbon source	Culture time (h)	PHAs monomer	Cell conc. (g/L)	PHAs conc. (g/L)	PHAs content (wt %)	References
Bacillus cereus EGU3	contaminated food	glucose	16-20	PHB	0.750	0.500	66.6	Porwal et al., 2008
Cupriavidus sp.USMAA1020	Lake Kulim	γ-butyrolactone	48	P(3HB-4HB)	3.96±0.04		52.4±1.6	Amirul et al., 2008
Rhodobacter sphaeroides 14F	Bangkok area	fructose	54	PHB	3.5		60	Lorrungruang et al., 2006
P. guezennei biovar. *tikehau*	Atoll of Tikehau	coprah oil	36	P(HO-HD-HDD)			63	Simon-Colin et al., 2008
Zoogloea sp1 GY3	Activated sludge	sucrose	32			9.44		Yang and Huang 2006
P. oleovorans m26	Pctivated sludge	glucose	46	P(HD-HDD)		3.27	31.37	Huang et al., 2006
Corynebacterium glutamicum	*phbCAB*		72	PHB	12.5		22.5	Jo et al., 2006
Bacillus spp.	*Bacillus*	decanoate	24	PHB		0.448	80.14	Valappil et al., 2007
R. eutropha PHB$^-$4	PhaC2$_{Ps}$	gluconate, octanoate	48	PHB	5.05±0.01		40.89±1.89	Chen et al., 2007
Aeromonas hydrophila CQ4	PhaC2$_{Ps}$	dodecanoate, gluconate	48	P(HB-HHx)	4.53±0.63		44.67±0.75	Qin et al., 2007
Aeromonas hydrophila 4AK4	*phaAB*	dodecanoic acid, propionic acid	60	P(HB-HV-HHx)	2.67±0.06		37.2±1.22	Zhao and Chen, 2007
Aeromonas hydrophila 4AK4	*phaPCJ*	lauric acid, 1,4-butanediol	72	P(3HB-4HB - 3HHx)	3.26±0.05		23.6±1.2	Xie and Chen, 2008
P. putida KTOY06	*fadB , fadA* Knockout Mutant	dodecanoate, gluconate	48	P(HD-HDD -HO-HHx)	5.31±0.24		84.3±4.6	Ouyang et al., 2007
R. eutropha PHB$^-$4	*PhbC$_{Re}$* Mutation	fructose	72	PHB	3.65±0.09		68.4±0.6	Zheng et al., 2006
E. coli XL1-Blue (pKSABC) and (pKSSE5.3)		glucose		P(3HB-4HB)			44	Jia et al., 2006
E. coli JM 109	*phaC1*	glucose	36	P(HHx-HO)	1.005	0.54	54.0	Tao et al., 2008
E. coli K24K	*pha*	Whey	24	PHB	70.1	51.1	72.9	Nikel et al., 2006
E. coli CT1061-PHB	Mutation *arcA2*	glycerol	60	PHB	21.17	10.81	51	Nikel et al., 2008
R. eutropha PHB$^-$4	*phaC$_{Re}$* mutants	fructose	72	PHB	5.35 ± 0.13		81.0 ± 2.2	Ye et al., 2008

UTILIZATION OF INEXPENSIVE OR/AND RENEWABLE CARBON SOURCES

Although there are considerable industrial interests in PHAs, their cost of production is a major issue. To make PHAs production more economical, some researchers have tried to produce PHAs from inexpensive carbon sources such as plant oils (Shang et al., 2008; Bhubalan et al., 2008; Lee et al., 2008; Kek et al., 2008), molasses (Albuquerque et al., 2007; Solaiman et al., 2006), starch (Chen et al., 2006; Haas et al., 2008), whey (Koller et al., 2008), and industrial wastes (Nielsen, 2007, Goff et al., 2007; Yu and Heiko, 2008; Bengtsson and Werker, 2008). The detailed results are showed on Table 2. The highest cell density reached 179 g/L with a PHAs content of 55% when waste tomato starch was used. A high cell density could also be achieved when crude glycerol or hydrolyzed corn oil was used as the carbon source. In addition, it can be seen that, in most cases, the cell concentrations and PHAs contents obtained from the low cost carbon substrates were at a low level, making it more difficult for PHAs to be separated from the dilute culture broth. Meanwhile, the fermentation time also became longer. All these may become

Table 2. PHAs biosynthesis from inexpensive carbon sources.

Carbon resource	Stains	PHAs monomer	Cell conc. (g/L)	PHAs conc. (g/L)	PHAs content (wt.%)	References
Hydrolyzed corn oil	*P. putida*	mcl-PHAs	103	28	27.2	Shang et al., 2008
Whey	*P. hydrogenovora*	PHB	5	1.27		Koller et al., 2008
Sugar cane molasses	Mixed bacteria	PHBV			30	Albuquerque et al., 2007
Soy molasses	*P. corrugata*	P(HDD-HO-HTDE)	3.6		5-17	Solaiman et al., 2006
Enzymatic extruded starch	*Haloferax mediterranei*	PHBV	39.4	20	50.8	Chen et al., 2006
Petrochemical plastic waste	*P. putida* CA-3		1.14	0.84	43	Goff et al., 2007
Paper mill wastewater	Activated sludge	PHBV			48.2	Bengtsson & Werker, 2008
Bagasse hydrolysates	*R. eutropha*	PHB	11.1±0.4		56.5±0.5	Yu et al., 2008
Waste tomato starch	*R. eutropha* NCIMB 11599	PHB	179	94	55	Haas et al., 2008
Crude glycerol	*Cupriavidus necator* JMP 134	PHB	50		48	Mothes et al., 2007
Palm kernel oil	*Cupriavidus necator* PHB–4	P(HB-HV-HHx)	7.9		79	Bhubalan et al., 2008
Plant oils	*Cupriavidus necator* H16	PHBV	4.4-5.6	6.8	80	Lee et al., 2008

key barriers for the mass production of PHAs in the future.

Compared to other processes, the use of mixed cultures as a cheaper method to produce PHAs has gained much attention in recent years, because it can use some industrial wastes as carbon sources, resulting in a decrease in the cost of production of PHAs. Considering both the environmental and resource factors, mixed cultures could become the most effective and potential means of producing PHAs in the future (Lu, 2007). Up to now, much research on mixed culture has been done by using industrial wastes with much success (Dias et al., 2006). A metabolic model was established to describe the production of PHAs in mixed cultures, using mixtures of acetic acid and propionate as carbon sources. Metabolic flux analysis and flux balance analysis indicated that acetate was a more conducive PHAs storage than propionate, and the PHAs production process by mixed microbial cultures had the potential to be comparable or even more favorable than pure cultures (Dias et al., 2008).

There are several factors that may influence mixed culture processes, such as carbon resources, substrate concentration, temperature, pH, and retention time (Bengtsson et al., 2007; Serafim et al., 2008; Kasemsap and Chalermraj, 2007). The use of different carbon sources led to a change in polymer composition: homopolymers of PHB were obtained with acetate and butyrate, whereas a mixture of acetate and propionate as well as propionate and valerate, gave terpolymers of 3-hydroxybutyrate, 3-hydroxyvalerate and 2-methyl- 3-hydroxyvalerate. All these PHAs had molecular weights from 2.0×10^6 to 3.0×10^6. Thermal characterization of the produced PHAs showed values of glass transition temperature, melting temperature, melting enthalpy, and crystallinity slightly lower than those obtained from pure cultures (Serafim et al., 2008). In continuous chemostat experiments with two effluents, increasing the retention time (RT) resulted in increased degree of acidification up to a maximum of 0.93 g COD of VFA/g influent soluble chemical oxygen demand at RT 95 h for whey and 0.75 g COD/g COD at RT 24 h for the paper mill effluent. Increasing pH from 5 to 6 resulted in increased amount of propionate. PHAs monomer composition and associated polymer properties could be regulated by controlling RT and pH during acidogenic pretreatment (Bengtsson et al., 2007). PHAs content of 14–41% could also be achieved by using glycogen accumulating organisms culture under both aerobic and anaerobic conditions (Yu et al., 2007). In the case of PHAs produced by excess activated sludge, it was found that PHAs content reached 51% with a productivity rate of 2.19 g /L·h the optimal pH of 8 (Kasemsap and Chalermraj, 2007; Gurieff and Lant, 2007). All these indicate that PHA production by using activated sludge is a promising alternative to a typical pure culture approach.

It was also reported that static magnetic exposure has definitely influenced the biosynthesis of PHAs from different short-chain fatty acids, by the activated sludge process under the aerobic dynamic feeding technique, and the effect was dependent on field strength: the maximum PHB production occurring at 7 mT, and the minimum one at 42 mT; the maximum PHV production

occurring at 21 mT, and the minimum one at 0 mT (Chen and Li, 2008). With the increase of oxidation-reduction potential, PHAs yield, production rate, cell growth rate and glucose consumption rate increase correspondingly (Cai et al., 2008AB).

In most cases of mixed cultures, PHAs were produced from the organic acids contained in wastewater or transformed from other industrial wastes. Therefore, carrying out mixed culture does not only help to solve the problem of environmental pollution caused by organic wastes, but can also convert them into useful materials, which is important for sustainable development and environmental protection.

The above research works show that mixed culture has the potential to produce large amounts of PHAs at relatively low costs due to lower sterility, equipment and control requirements and the ability to use a wide range of cheap substrates including industrial and agricultural wastes. However, there are also some drawbacks that cannot be neglected, such as the low PHAs content rate and cell density, insufficient substrate utilization ratio, long domestication time for activated sludge and so on. Nevertheless, mixed culture would be a better method for producing PHAs.

PROCESS CONTROL AND OPTIMIZATION

To synthesize PHAs effectively, process control and optimization were carried out to have a high cell density culture, guarantee a high product level and a high productivity, and have significant effects on the product separation processes and the mass-production cost. In an effort to further optimize PHAs production processes, a number of studies on the effects of growth medium, culture conditions and nutrient feeding strategies on PHAs production were carried out. In a 34 h fed-batch culture of *Halomonas boliviensis*, PHB content and DCW reached 81% and 23 g/L respectively after the optimization of culture medium (Quillaguamán et al., 2008). By using two different decaying exponential feeding strategies, a linear decaying strategy and a quadratic one, to control the nonanoic acid feeding rate for delaying oxygen limitation, a higher cell density (109 g/L) with PHA content of 63% was achieved in a fed-batch culture of *Pseudomonas putida* KT2440 (Maclean et al., 2008). When a continuous feeding strategy according to the cumulative CO_2 production analysis and the real-time glucose consumption was used to control the glucose concentration in the broth, a final cell density of 54.2 g/L was gained in 12.5 h, which represents an overall biomass productivity of 4.3 g/L h (Sun et al., 2006). Similarly, in order to eliminate the inhibition of high substrate concentration in the process of PHAs synthesis, an unstructured model was proposed for predicting the result in high cell density cultures of *R. eutropha*. It was found that the optimal glucose concentration was around 9 g/L and that the phosphate concentration played a key role in the accumulation of PHB (Shang et al., 2007). In a fed-batch culture of recombinant *E. coli*, a stress-induced system based on the response of cells to pH change was constructed into *E. coli* DH5α to induce the PHB biosynthesis pathways. Fermentation results showed that the PHB content could reach 85.8% in minimal glucose medium without adding any inducer. This process was very similar to what happened in wild-type PHB producers in which the PHB accumulation was spontaneously induced under nitrogen or phosphate stress (Kang et al., 2008).

MASS PRODUCTION OF PHAs

Even though the price of PHAs is still very high, there are several companies producing PHAs to meet the demands of the market. PHB and PHBV are still the main members of PHAs that are produced on a commercial scale. Presently, there are some PHAs products in the markets, such as Biopol, Mirel, and Nodax made in USA, Biomer in Germany, Biocycle in Brazil, DegraPol in Italy, Tianan PHBV and PHB in China. In fact, most companies produce PHAs just amounting to several hundred tons per year. However, some companies have made schedules or have started to increase their production capacity of PHAs to several thousand tons or 10 thousand tons per year. For example, Tianjin Green Biosciences Limited Company has been building a new factory to produce PHAs at the capacity of 10 thousand tons per year. All these indicate that a new era of PHAs industry will be coming to us soon.

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