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SHORT COMMUNICATION

Magnesium affects poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) content and composition by affecting glucose uptake in *Delftia acidovorans*

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ABSTRACT

Precise control of polyhydroxyalkanoate (PHA) composition is necessary in order to synthesize polymers with specific properties. Among the various types of PHA that have been identified, those that contain 4-hydroxybutyrate (4HB) monomers are especially useful in the medical and pharmaceutical fields as absorbable biomaterial. In this study, we have investigated the effect of magnesium concentration on the biosynthesis of poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] by *Delftia acidovorans* DS-17. Our results show that, magnesium affects the copolymer content and composition by affecting glucose uptake from the culture medium. Higher concentrations of magnesium resulted in lower molar fractions of 3HB in the copolymer and reduced uptake of glucose. The results show for the first time that magnesium may be used to achieve fine control of biologically synthesized PHA copolymer composition.

Keywords: polyhydroxyalkanoates, 4-hydroxybutyrate, *Delftia acidovorans*, 1,4-butanediol, magnesium

INTRODUCTION

Among the various polyhydroxyalkanoate (PHA) constituents, poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)] copolymers has been identified as suitable bioabsorbable materials that have potential applications in the medical field (Martin and Williams, 2003). In this study, we have investigated the ability to control the biosynthesis of this copolymer in order to tailor-make polymers with desired physical and chemical properties. The physical properties of P(3HB-*co*-4HB) is directly dependent on the molar fractions of 3HB and 4HB monomers (Saito and Doi, 1994). The precise control of 4HB monomers in the copolymer composition is very important for implantable medical products. Besides that, the copolymers with precise 4HB monomers will show predictable degradation pattern, which is important for the controlled delivery of therapeutic drugs. Our recent studies have also shown that P(3HB-*co*-4HB) copolymers produced by *D. acidovorans* have excellent biocompatibility comparable to that of commercially available medical products (Siew *et al*., 2006). At present, microbial fermentation is the only successful method for the production of this copolymer that contains 4HB monomers with higher molecular weight (Sudesh *et al*., 1999; Hein *et al*., 1997; Saito *et al*., 1996). Therefore, it is necessary to be able to control the biosynthesis of P(3HB-*co*-4HB) and to modulate the 4HB contents. In this study, it was found that the concentration of Mg^{2+} affected the biosynthesis of P(3HB-*co*-4HB) in *D. acidovorans* by affecting glucose uptake from the culture medium. In addition, we were also able to improve the synthesis and accumulation of P(3HB-*co*-4HB) by *C. acidovorans* (Lee *et al.,* 2004).

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MATERIAL AND METHODS

Bacterial strain and media

D. acidovorans (formerly *Comamonas acidovorans* JCM 10181) that was used in this study was kindly given by Prof. Y. Doi of RIKEN Institute and Tokyo Institute of Technology, Japan. *D. acidovorans* strain was cultivated in 100 mL nitrogen-rich medium (Lee *et al*., 2004) supplemented with 1% (w/v) glucose at 30 °C for 24 h.

Production of PHA

PHA synthesis was carried out by a two-step cultivation of *D. acidovorans*. After, 24 h the cells were harvested and transferred into 100 mL nitrogen-free medium (Lee *et al*., 2004). Magnesium in the form of $MgSO_4 \cdot 7H_2O$ or $MgCl_2$ was added to second stage medium.

Analytical procedures

The *D. acidovorans* cells were harvested, washed twice with distilled water, and lyophilized.

Determination of the cellular PHA content and polymer composition by gas chromatography (GC) were carried out according to standard methods (Braunegg *et al*., 1978). To determine the residual glucose, 1 mL of the cell-free culture broth was mixed with 2 mL of dinitrosalicylic acid (DNS). After mixing, the solution was heated in boiling water for 10 min followed by cooling with tap water. The absorbance of each sample was measured by a UV-VIS Spectrophotometer set at a wavelength of 500 nm.

Table 1: Effect of different concentrations of $MgSO_4 \cdot 7H_2O$ on the production of P(3HB-*co*-4HB) by using1,4-butanediol and glucose[a]

Carbon Source	Concentration of $MgSO_4 \cdot 7H_2O$ (mM)	Dry cell weight (g/L)	PHA content[b] (wt%)	PHA composition[c] (mol%)	
				3HB	4HB
0.6% (w/v) glucose 0.4% (v/v) and 1,4-butanediol	0.05	2.12	28	69	31
	0.10	2.15	37	55	45
	0.15	2.26	35	47	53
	0.20	2.14	30	32	68
	0.25	1.96	30	27	73
	0.30	1.97	28	25	75
0.8% (w/v) glucose 0.2% (v/v) and 1,4-butanediol	0.05	2.01	26	90	10
	0.10	2.17	46	72	28
	0.15	2.30	40	67	33
	0.20	2.27	34	60	40
	0.25	2.07	31	53	47
	0.30	1.90	27	48	52

[a] Incubated for 48h at 30 ºC, pH 7.0, 150 rpm in nitrogen-free mineral medium.
[b,c] Polyester content and composition in freeze-dried cells determined by GC
3HB, 3-hydroxybutyrate; 4HB, 4-hydroxybutyrate

RESULTS

Table 1 lists the results of the effect of different concentrations of $MgSO_4 \cdot 7H_2O$ on the production of P(3HB-*co*-4HB) by using glucose and 1,4-butanediol. It is obvious that the concentration of $MgSO_4 \cdot 7H_2O$ in the culture medium affects the content and the molar fraction of monomers in the copolymer. PHA content was maximum when a final concentration of 0.1 mM $MgSO_4 \cdot 7H_2O$ was added into the culture medium. However, the 4HB molar fraction was relatively low (45 mol%). Interestingly, an increase in the $MgSO_4 \cdot 7H_2O$ concentration resulted in increased 4HB content in the copolymer. Similar trend was observed when the concentrations of the carbon sources were changed. By using a mixture of 0.6% (w/v) glucose and 0.4% (v/v) 1,4-butanediol, 75 mol% 4HB monomers was obtained when the $MgSO_4 \cdot 7H_2O$ concentration was 0.3 mM. The increase in $MgSO_4 \cdot 7H_2O$ concentration above 0.1 mM resulted in a decrease in the P(3HB-*co*-4HB) content. This suggests that the relative increase in the 4HB molar fraction is due to a decrease in the 3HB molar fraction.

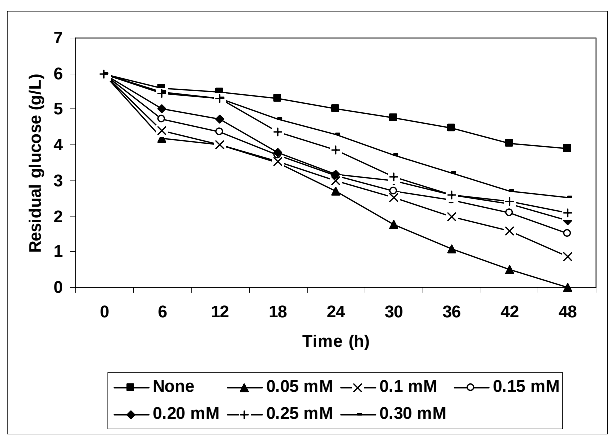

Figure 1: The effect of $MgSO_4.7H_2O$ concentrations on glucose uptake by *D. acidovorans*

Figure 1 shows the influence of $MgSO_4 \cdot 7H_2O$ concentration on glucose uptake. In the absence of $MgSO_4 \cdot 7H_2O$ the glucose uptake was most affected and after 48 h, the remaining glucose in the culture medium was 4.0 g/L. 0.05 mM $MgSO_4 \cdot 7H_2O$ resulted in the highest glucose uptake. When the concentration of $MgSO_4 \cdot 7H_2O$ was further increased the residual glucose in the medium also increased. Similar trends were observed when $MgSO_4 \cdot 7H_2O$ was replaced with $MgCl_2 \cdot 6H_2O$ (results not shown).

Figure 2 shows the results of copolymer biosynthesis without $MgSO_4 \cdot 7H_2O$ supplementation. It can be seen that the remaining PHA content after 48 h was only 1 wt% with the highest content of 3HB monomer in the copolymer. Similar trends were observed when $MgCl_2 \cdot 6H_2O$ was used instead of $MgSO_4 \cdot 7H_2O$. When the concentration of $MgSO_4 \cdot 7H_2O$ was further increased it resulted in an increase in the 4HB content. Both 0.1mM (Figure 3) and 0.3 mM (Figure 4) resulted in increased 4HB contents by decreasing the incorporation of 3HB monomers. 0.3 mM $MgSO_4 \cdot 7H_2O$ favored the accumulation of copolymers containing higher molar fractions of 4HB monomers compared to 0.1 mM.

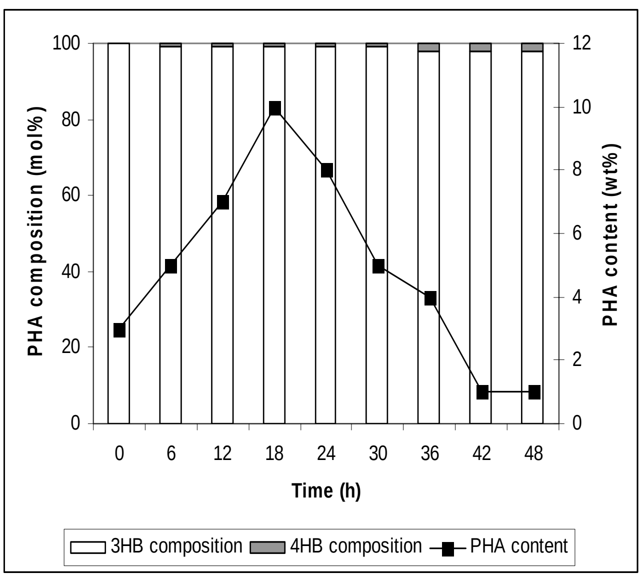

Figure 2: Production of P(3HB-*co*-4HB) by using 0.4% (v/v) 1,4-butanediol and 0.6% (w/v) glucose without $MgSO_4.7H_2O$ supplementation

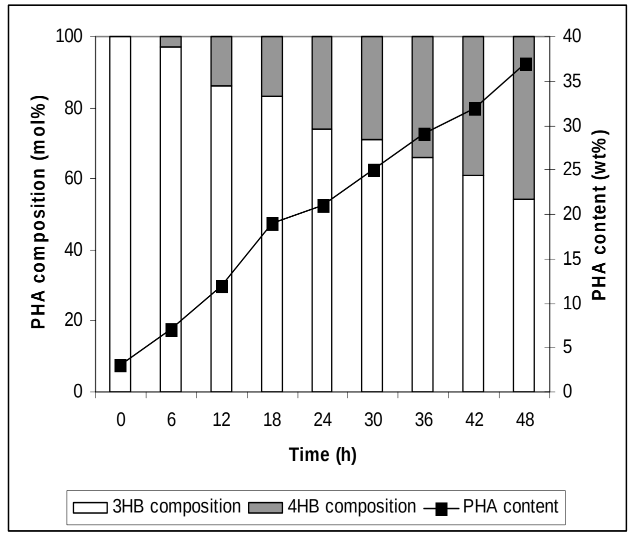

Figure 3: Production of P(3HB-*co*-4HB) by using 0.4% (v/v) 1,4-butanediol and 0.6% (w/v) glucose with (0.1 mM) $MgSO_4.7H_2O$ supplementation

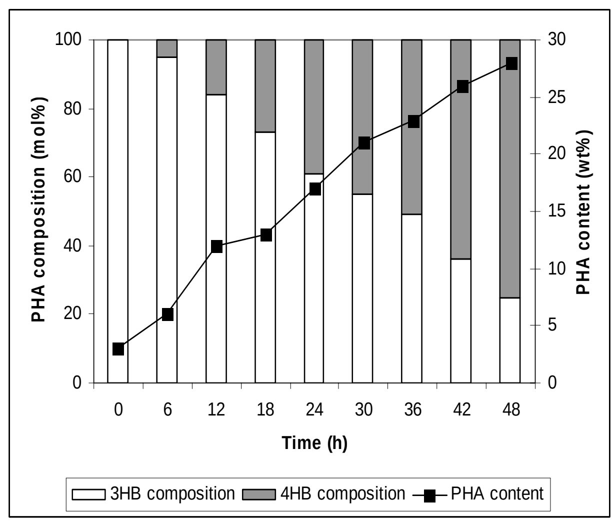

Figure 4: Production of P(3HB-*co*-4HB) by using 0.4% (v/v) 1,4-butanediol and 0.6% (w/v) glucose with (0.3 mM) $MgSO_4.7H_2O$ supplementation

DISCUSSION

Precise control of biologically synthesized copolymer composition is crucial as it affects the physical properties, which is important for specific applications. In a recent study we've shown that various culture parameters can be used to regulate the monomer compositions. In cases where block copolymers need to be synthesized biologically, more than one culture parameters may have to be adjusted. In addition, it may be necessary to completely stop the uptake of certain carbon sources so that the monomer composition can be modulated. In this study we show that Mg^{2+} affects the uptake of glucose. Increased concentrations of Mg^{2+} decrease the uptake of glucose, which results in decreased 3HB molar fraction in the copolymer.

Divalent cations such as Ca^{2+} and Mg^{2+} also affect the stability of membranes because of ionic interactions with phosphonyl groups that result in decreased membrane mobility. (Chen *et al.*, 1995) This may influence the transport of glucose across the membrane and eventually affect the concentration of acetyl-CoA that is needed to generate 3HB monomers. Mg^{2+} is involved in regulating cellular membrane stability (Wee and Wilkinson 1999). Mg^{2+} forms stable complexes with membrane phospholipid and it is important for maintenance of normal membrane fluidity. It is suggested that Mg^{2+} plays an important role in the uptake of glucose and therefore affects the 3HB content P(3HB-*co*-4HB) in copolymer. The resulted obtained in this study clearly demonstrate that Mg^{2+} concentration affects glucose uptake and therefore the P(3HB-*co*-4HB) composition is affected.

Most of the enzymes required the cofactor for catalytic activity and Mg^{2+} plays role as cofactor. Mg^{2+} will bind together to substrate to orient them properly for the reaction. And this maybe why in the absence of Mg^{2+}, the copolymer content is very low. This suggested that those enzymes involved in the conversion of glucose and 1,4-butanediol to 3HB and 4HB monomers need Mg^{2+} as a cofactor.

This study has shown the effect of Mg^{2+} concentration on the compositions of P(3HB-*co*-4HB) produced by *C. acidovorans*. Mg^{2+} affects the copolymer composition and content by affecting glucose uptake, which will affect the generation of 3HB monomers from acetyl-CoA.

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