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Effects of Fatty Acids on Growth and Poly-3-hydroxybutyrate Production in Bacteria

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Abstract

The effects of saturated and unsaturated fatty acids (lauric acid, palmitic acid, steric acid, oleic acid, linoleic acid, soybean oil) on *Sphaerotilus natans*, *0B17* (*Pseudomonas* sp.), and recombinant *Escherichia coli DH5(/pUC19/CAB* were studied. Oleic acid enhances Poly-3-hydroxybutyrate (PHB) production in these three bacterial strains, suggesting that the single double bond of the acid activates the polyhydroxylkanoate accumulation enzymatic reaction. Under the effect of lauric acid and linoleic acid, the growth of *S. natans* and *0B17* were totally inhibited. However, the enhanced PHB accumulation in recombinant *E. coli* was observed.

Index Entries: Fatty acids; poly-3-hydroxybutyrate; *Sphaerotilus natans;* recombinant *Escherichia coli; Pseudomonas* sp.

Introduction

Polyhydroxyalkanoates (PHAs) are polymers of hydroxyalkanoate that accumulate as carbon/energy storage material in various microorganisms. PHAs are synthesized and intracellularly accumulated as distinct granules inside the microorganism. Many artificial polymers are harmful to the environment owing to their nonbiodegradable properties. One important characteristic of PHA is its biodegradability by bacteria; however, PHA production by microorganisms is expensive owing to the production medium. Hence, the present study explores the effect of fatty acids as nutritional supplement in the poly-3-hydroxybutyrate (PHB) production in different bacterial strains.

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It was reported that oleic acid (C18:1) increases PHA production when used as a nutritional supplement (*1*). The enhancement effect of oleic acid is also found in the following bacterial strains: *Aeromonas hydrophilia* (*2*), recombinant *Escherichia coli XY1-Blue pSYL105* (*3*), and *Ralstonia eutropha* (*1*). In addition, lauric acid enhances PHB accumulation in recombinant *E. coli* and *A. hydrophilia* (*2*).

However, nonanoic acid (C9:0) and octanoic acid (C8:0) were reported to inhibit the production of PHA (*4*). Oleic acid contains unsaturated carbon bonds, so it is doubtful that the unsaturated carbon bond would catalyze the PHA enzymatic process. Hence, in the present study, the effects of six different types of fatty acid—lauricacid (C12:0), palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and soybean oil—as additives were investigated. In addition, the effect of these additives on the PHB content were also compared.

Materials and Methods

Bacterial Strains

Three different types of bacterial strains were adopted: *Sphaerotilus natans;* recombinant *E. coli* (*E. coli DH5(/pUC19/CAB*); and a bacterial strain, *0B17* (*Pseudomonas* sp.), isolated from activated sludge at the Tai Po Sewage Treatment Plant, Hong Kong. The construction method for the recombinant *E. coli* is summarized in ref. *5*. The strains were stored at −20°C in the presence of 10% (v/v) glycerol and were maintained on a nutrient agar slant at 4°C by monthly subculture.

Production Media

Different bacterial strains require different media to grow. The following two types of production media were used for PHB production by *S. natans*, recombinant *E. coli,* and *0B17*. The production medium for *S. natans* consisted of 10 g/L of glucose, 3 g/L of peptone, 0.2 g/L of $MgSO_4 \cdot 7H_2O$, 0.5 g/L of $CaCl_2$, 1 g/L of $NaH_2PO_4 \cdot 2H_2O$, 2.6 g/L of K_2HPO_4, 0.005 g/L of $Fecl_3$, and 0.005 g/L of boric acid. This type of medium maximizes PHB production (*6*). For recombinant *E. coli* and *0B17*, because the specified production media for these two bacterial strains are unknown, common PHB production medium, 10 g/L of glucose. 2 g/L of tryptone, 1 g/L of $MgSO_4 \cdot 7H_2O$, 7 g/L of KH_2PO_4, 1 g/L of citric acid, and 2 g/L of yeast extract the adopted. The pH of those production media was set to 7.0.

Fatty Acids

Six types of fatty acids—lauric acid (12:0), palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:3), and soybean oil (58% polyunsaturated fats, 23% monounsaturated fats, 15% saturated fats),—were used as nutritional supplements in studying PHA production.

Before fermentation, a pulse of 0.3 g/L of fatty acid was introduced into the production medium.

Fermentation

One milliliter of the inoculated culture media was introduced into 100 mL of production media as seeding. Afterward, the production media were fermented in a 250-mL shake flask under the following conditions: *S. natans* at 30°C for 48 h recombinant *E. coli DH5(/pUC19/CAB* at 37°C for 24 h and *0B17* at 30°C for 42 h. The fermentation time is based on the maximum growth rate of the bacterial strain considered. The fermentation was performed inside an automatic temperature-controlled 250-rpm shaker.

Extraction

After fermentation, the culture broth was concentrated by centrifuging at 4000 rpm for 20 min. The residues were filtered and freeze-dried. PHAs were extracted from the dried cell through esterification, which consists of the following reagents: 0.29 g of benzoic acid, 3 mL of concentrated 98% H_2SO_4, 97 mL of methanol, (*7*). During extraction, 1 mL of the esterification solution and 1 mL of chloroform were added to 10 to 14-mg samples. The mixed samples were heated to 100°C for 4 h. Afterward, 1 mL of distilled water was added to the cooled mixture and the mixture was vortexed for 1 min. The mixture was allowed to stand overnight to separate into two layers. The bottom layer, which contained dissolved PHA, was used for subsequent analysis.

Gas Chromatography

Samples for gas chromatography were prepared according to Braunegg et al. (*8*). Analysis was performed on a Varian Model 3700 gas chromatograph, using a 1/8-in-diameter Chromosorb-WAW column with 80/100 mesh size and 6-ft length (from Supelco, Bellefonte, PA). The recorder was a Shimadzu C-R5a Chromatopac. N_2 was the carrier gas at a flow rate of 10 mL/min. Analysis started at 100°C for 3 min, whereupon the temperature was increased to 220°C at a rate of 8°C/min. After reaching 220°C, the temperature was maintained for 5 min before the analysis was terminated.

Results and Discussion

Effect of Fatty Acids on S. natans

Figure 1 shows the effect of fatty acids on *S. natans*. Lauric acid (C12:0) and linoleic acid (C18:2) inhibited bacterial growth, suggesting that these two types of fatty acid are toxic to bacterial metabolism. For oleic acid, a decrease in the cell biomass was also observed, which suggests that oleic acid suppresses cell growth. By contrast, palmitic acid (16:0), steric acid (18:0), and soybean oil promoted cell growth. Among the five fatty acids con-

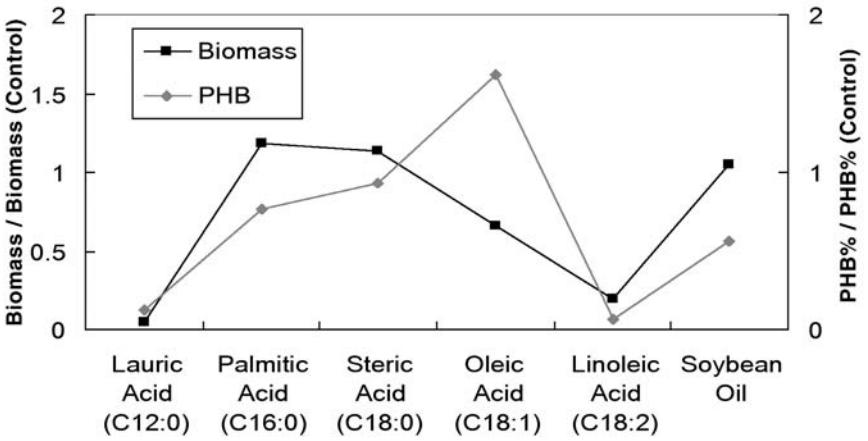

Fig. 1. Effect of fatty acids on biomass and percentage of PHB accumulation of *S. natans.*

sidered, only oleic acid promoted PHB accumulation inside the cell. In our study, there was a 50% increase in the percentage of PHB accumulation. An enhanced PHA formation by oleic acid has also been observed in *Ralstonia eutropha* and recombinant *E. coli* (*1*,*3*). In those studies, oleic acid increased PHA by 50–100%, which is similar to the findings of the present study.

According to Lee et al. (*3*), oleic acid saves acetyl-co-A by providing precursors for the synthesis of C_{2n} fatty acids in the metabolic pathway of recombinant *E. coli* XL-1 Blue pSYL105. Hence, more acetyl-co-A and/or NADPH are available for PHB synthesis. Consequently, the cell mass and the amount of PHB are enhanced. When we compared the cell mass production and structures among steric acid, oleic acid, and linoleic acid in the present study, we found that the double bond seemed to suppress cell growth but enhanced PHB production. For two double bonds, cell growth was totally suppressed, resulting in no formation of PHB.

Effect of Fatty Acids on Recombinant E. coli DH5α/pUC19/CAB

Figure 2 shows the effect of fatty acids on the growth of recombinant *E. coli DH5(/pUC19/CAB.* Palmitic acid (C16:0) and oleic acid (C18:1) greatly promoted cell growth but had a slightly negative effect on PHB production. Unlike *S. natans*, lauric acid and linoleic acid did not significantly suppress cell biomass. Instead, an increase in PHB accumulation was observed, suggesting different metabolic systems for *S. natans* and recombinant *E. coli DH5(/pUC19/CAB*. For recombinant *E. coli*, its metabolic system can utilize different carbon sources for growth. Hence, lauric acid and linoleic acid are degraded and have little effect on the TCA cycle of the recombinant *E. coli*. For oleic acid, unlike that of *S. natans*, its effect on recombinant *E. coli* is to promote cell growth rather than accumulate PHA. No significant increase in PHB accumulation was observed in the soybean oil. This may be owing to the combined effect of the saturated and unsaturated fatty acids.

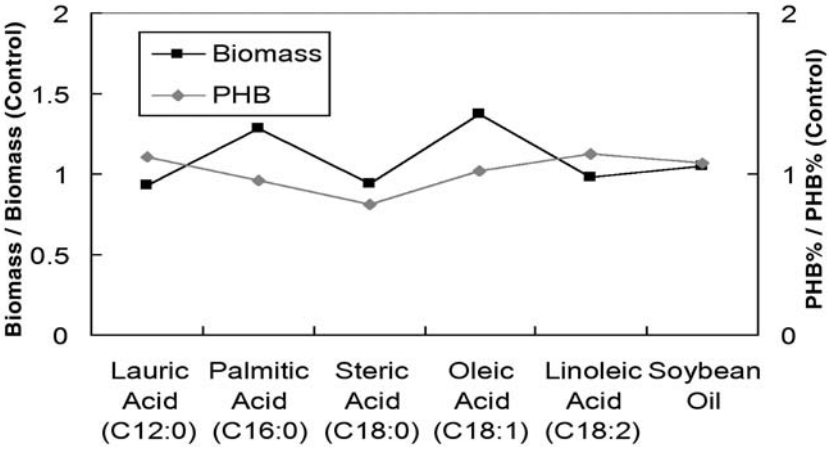

Fig. 2. Effect of fatty acids on biomass and percentage of PHB accumulation of recombinant *E. coli DH5α/pUC19/CAB*.

Although oleic acid saves acetyl-co-A by acting as its precursor (*3*), its effect seems to target the TCA cycle, which increases cell biomass. This may be related to the presence of the high copies of plasmid pUC19, which is more effective in PHB accumulation.

Comparison of PHB accumulation and the biomass of recombinant *E. coli* under the effect of oleic acid, linoleic acid, and lauric acid, revealed that PHB accumulation under the effect of linoleic acid and lauric acid was greater than that of oleic acid. However, there was no significant change in the biomass of recombinant *E. coli* under linoleic acid and lauric acid. These findings suggest that linoleic and lauric acid affect the PHB accumulation enzymatic system instead of acting as precursors of acetyl-co-A, similar to that of oleic acid in recombinant *E. coli*.

Effect of Fatty Acids on 0B17

Figure 3 shows the effect of fatty acids on the growth of *0B17.* Lauric and linoleic acid totally inhibited cell growth. Similar to *S. natans*, this suggests that these two fatty acids are toxic to cell metabolism. In addition, both steric and oleic acid decreased cell mass, suggesting that these two fatty acids suppress cell growth. Palmitic acid and soybean oil, however, did not significantly change cell growth.

Comparison of the PHB accumulation of the six fatty acids, revealed that steric and oleic acid promoted PHB accumulation inside the cell. Steric acid slightly increased PHB accumulation, whereas oleic acid increased PHB accumulation by twofold.

The effect of fatty acids on *0B17* was similar to that of *S. natans*, which suggests that their enzymatic systems may be similar. Hence, instead of acting as a precursor of acetyl-co-A, oleic acid seems to enhance the PHA synthesis enzymatic system.

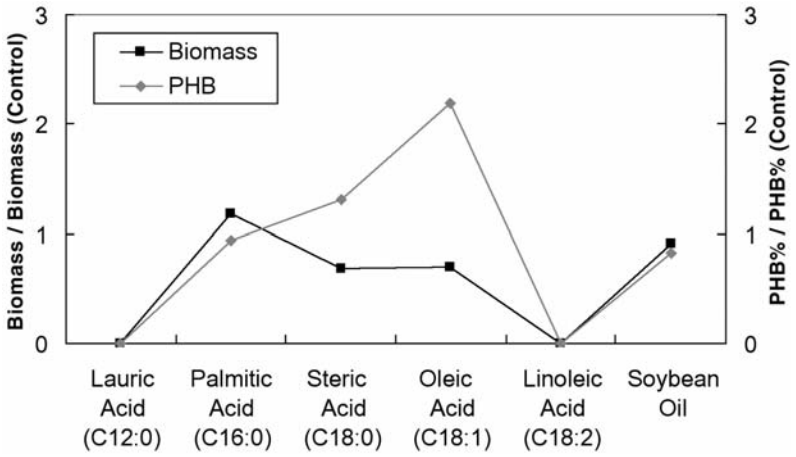

Fig. 3. Effect of fatty acids on biomass and percentage of PHB accumulation of 0B17.

Conclusion

Different saturated and unsaturated medium-chain fatty acids with carbon numbers ranging from 12 to 18 were studied. Six types of fatty acids—lauric acid (C12:0), palmitic acid (C16:0), steric acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and soybean oil—were used as nutrient supplement. For *S. natans,* recombinant *E. coli* and *0B17*, oleic acid enhanced PHB production with glucose as the carbon source. Both lauric and linoleic acid inhibited the growth of *S. natans* and *0B17*. However, enhanced in PHB accumulation was observed in recombinant *E. coli DH5α/pUC19/CAB*.

Acknowledgments

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