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MICROCALORIMETRIC RESEARCH ON RECOMBINANT *ESCHERICHIA COLI* WITH HIGH PRODUCTION OF POLYHYDROXYALKANOATES (PHAs)

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The thermogenic curves of metabolism of the four strains of *Escherichia coli* pUC19cab/JM109, pUC19cab/XL-IBlue, JM109 and XL-IBlue were determined using the LKB-2277 BioActivity Monitor and the ampoule method at 37°C. The pUC19cab/JM109 and pUC19cab/XL-IBlue are recombinant *E. coli* strains bearing the same foreign plasmid pUC19cab, which confers the ability to produce polyhydroxyalkanoates (PHAs). The yield of PHAs of pUC19cab/XL-IBlue was higher than that of pUC19cab/JM109. XL-IBlue and JM109 were the host bacteria. The heat flow of these strains was XL-Blue≈JM109>pUC19cab/JM109>pUC19cab/XL-IBlue. These results indicate an obvious interrelation between the PHAs production and the heat flow rate of *E. coli* strains.

Keywords: *microcalorimetry, PHAs, recombinant Escherichia coli, thermokinetics*

Introduction

Polyhydroxyalkanoates (PHAs) are polymers of hydroxyalkanoates which are synthesized and accumulated as an intracellular carbon and energy storage material by most genera of bacteria, usually under nutrient-limited conditions such as those lacking nitrogen, potassium, sulphur, oxygen or magnesium [1]. PHAs have drawn considerable attention because of their potential as an alternative to the petrochemical-based plastics. PHAs have many advantages over the conventional plastics. In particular, their biodegradability means they can be used as thermoplastics that are compatible with the environment. However, the broad application of PHAs has been hampered by their high cost compared to the petrochemical-based plastics [2]. Much effort has been devoted to reducing the manufacturing costs, including constructing efficient recombinant bacteria with the desired products [3], as well as developing efficient fermentation and economical recovery processes [4].

The microcalorimetric technique is one of the important methods for thermodynamic study used in the biological field. Microcalorimetry can directly determine the biological activity of a living system and provide a continuous measurement of heat production, thereby giving much information in both qualitative and quantitative ways. For example, a highly-sensitive differential scanning microcalorimeter has been used to study the structure of DNA [5]. In recent years, microcalorimetry has been used to determine the metabolic out-put of *B. thuringiensis* [6].

In this study, we used the microcalorimetric technique to evaluate the influence of PHAs production on the thermogenetic curve of *E. coli* strains. The result revealed that there is an obvious interrelation between the PHA production and the heat flow rate of *E. coli* strains. The more PHAs were produced, the less heat was released. The purpose of this research was to provide a new approach for screening recombinant *Escherichia coli* with a high polyhydroxyalkanoates (PHAs) production.

Experimental

Bacteria

Escherichia coli pUC19cab/XL-IBlue, XL-IBlue, pUC19cab/JM109 and JM109 are preserved in our Lab.

Growth medium

LB medium consisted of 0.5% NaCl, 1% tryptone, 0.5% yeast-extract, pH=7.2. The medium was sterilized by autoclaving for 20 min at 0.1 MPa.

The PHA production medium comprised 1% glucose, 0.2% tryptone, 1.3% KH_2PO_4, 0.1% citric acid, 0.1% $MgSO_4 \cdot 7H_2O$, 0.1% yeast-extract, pH 7.2. The medium was sterilized by autoclaving for 30 min at 0.07 MPa.

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Methods

Heat conduction microcalorimetry

The LKB 2277 Bioactivity Monitor, a type of heat conduction microcalorimeter, was used to determine the metabolism of cells. The microcalorimeter was maintained at 37°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000-mv range). The baseline stability was 0.2 W/24 h. For details of the performance and structure of the instrument [7–9].

The metabolic thermogenic curves of *E. coli* were recorded using the ampoule method. A 20 mL stainless steel ampoule was cleaned and sterilized. Once the system was cleaned and sterilized and the baseline stabilized, 5 mL bacterial suspension which had been cultured in LB medium for 12 h was placed in the ampoule. The temperatures of the calorimeter system and the isothermal box were controlled at 37°C. Meanwhile, the LKB-2210 recorder recorded the thermogenic curves of *E. coli* growth continuously.

Measurement of PHAs

1 mL esterification solution (3 mL 95–98% H_2SO_4, 0.29 g benzoate and 97 mL methanol), freeze-dried cells and 1 mL chloroform were heated at 100°C for 4 h. 1 mL H_2O was added to the mixture after the mixture cooled to room temperature, then the mixture was vortexed for phase separation. 1 μL portion of the lower organic phase was subjected to Gas Chromatograph (Hewlett Packard 5890 Series II) analysis.

Results and discussion

There are four phases in the growth period of bacteria: lag phase, log phase, stationary phase and dead stage. In the log phase, the cell growth is exponential. If the cell number is n_0 at time 0, and n_t at time t, then

$$n_t=n_0\exp(k_t) \quad (1)$$

k is the growth rate constant. If the heat power flow rate of each cell is w, then

$$n_tw=n_0w\ \exp(k_t) \quad (2)$$

$P_0=n_0w$ and $P_t=n_tw$, giving

$$P_t=P_0\exp(kt) \text{ or } \ln P_t=\ln P_0+k_t \quad (3)$$

The calorimetric curves of the exponential phase correspond to Eq. (3). So, making use of the data $\ln P_t$ and t taken from the curves to fit a linear equation, we can obtain the growth rate constants (k).

The recombinant *E. coli* pUC19cab/XL-IBlue and pUC19cab/JM109 were obtained by transforming the plasmid pUC19cab into *E. coli* host XL-IBlue and JM109, respectively. The plasmid pUC19cab confered the new phenotype's ability to produce PHAs to *E. coli* XL-IBlue and JM109. The four *E. coli* strains were detected by microcalorimetric measurements. The power-time curves of these strains are shown in Fig. 1. The thermokinetic parameters of these strains are shown in Table 1. All of the experimental results had a very good reproducibility and consistency. From the data in Table 1, we observed that the total heat flow rate (Q_T) of strain XL-IBlue and JM109 were approximately the same. The total heat flow rate (Q_T) of strain pUC19cab/XL-IBlue and pUC19cab/JM109 were lower than that of strain XL-IBlue and JM109, respectively. Furthermore, the total heat flow rate (Q_T) of strain pUC19/XL-IBlue was lower than that of pUC19/JM109. We can deduce from the results that there were PHAs production in the two recombinant *E. coli* strains and the yield of PHAs from pUC19cab/XL-IBlue was higher than that from pUC19cab/JM109. Our conclusion was affirmed by further determining the percentage of PHAs in these two recombinant strains. The yield-time curves of these two strains are shown in Fig. 2. From Fig. 2, we can observe that the yield of PHAs from pUC19cab/XL-IBlue was higher than that from pUC19cab/JM109. The maximum yield in pUC19cab/Xl-IBlue was 54.77% (mass of PHA/mass of freeze-drier cells), while that in pUC19cab/JM109 was 39.45%. The results obtained by examining the percentage of PHAs corresponded with the results assayed from the thermogenic curves. As we have known, PHAs are a kind of energy storage material. Energy was saved as PHAs in the recombinant *E. coli* pUC19cab/XL-IBlue and pUC19cab/JM109 while energy was released as heat in XL-IBlue and JM109. Therefore, the heat output of the recombinant strains which produced PHAs was lower than that of the host strains which did not produce PHAs. The more PHAs were produced, the less heat was released. So microcalorimetry can serve as a new method screening for the recombinant *E. coli* with a high polyhydroxyalkanoates (PHAs) production.

A major problem in the commercialization of PHAs is their high cost. The most effective method to reduce the cost is to improve the yield of PHAs. *E. coli* is widely regarded as the optimal host for foreign gene expression. We often construct different recombinant *E. coli* to improve the yield of PHAs [10].

Table 1 The thermokinetic parameters for the four *E. coli* strains

Strain	P_m/mW	t_m/min	Q_t/J
XL-IBlue	0.62	438	44.1
JM109	0.58	480	46.6
pUC19cab/XL-IBlue	0.46	545	29.9
pUC19cab/JM109	0.54	467	37.3

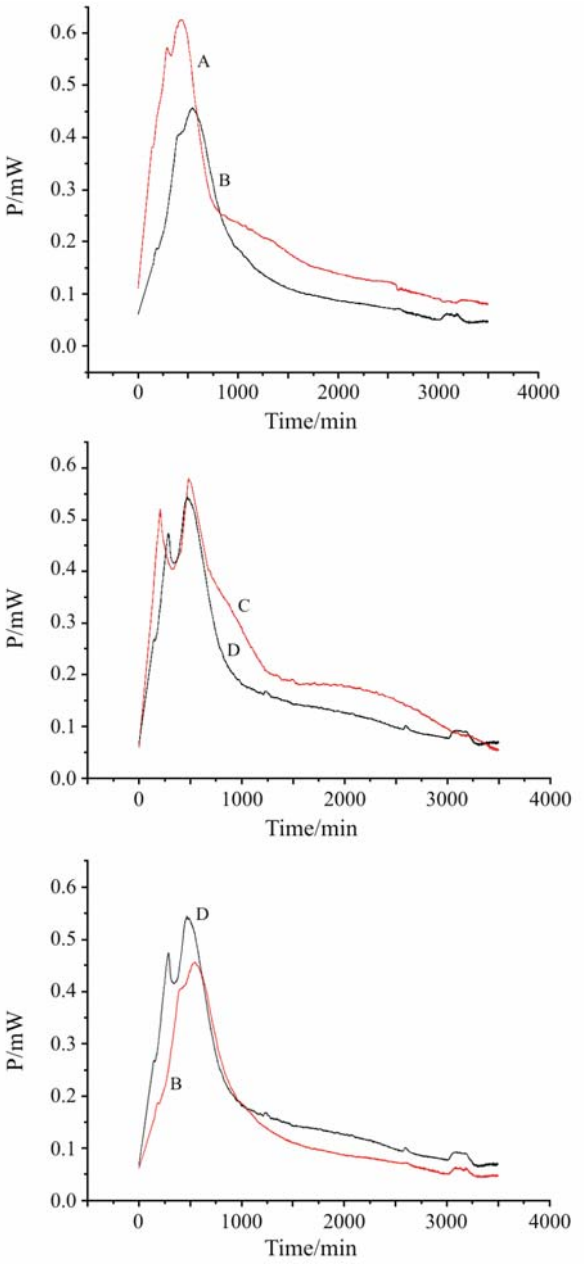

Fig. 1 Power–time curves of the four *E. coli* strains A – XL-IBlue, B – pUC19cab/XL-IBlue and C – JM109, D – pUC19cab/JM109

The PHAs synthesis occurs in a three-step reaction, starting with two acetyl-CoA that are derived from the tricarboxylic acid cycle. The enzyme β-thiolase (PhaA) condensed the acetyl-CoA to acetoacetyl-CoA, which is reduced at position 3 by the NADH-dependent enzyme acetoacetyl-CoA reductase (PhaB). In the final step, the monomers are esterified to the PHA polymer by the polymerase (PhaC). The formation of PHAs is regulated by the intracellular concentration of acetyl-CoA [11]. There are many available *E. coli* hosts with different genetic types [12]. Different *E. coli* strains carrying the same gene produced

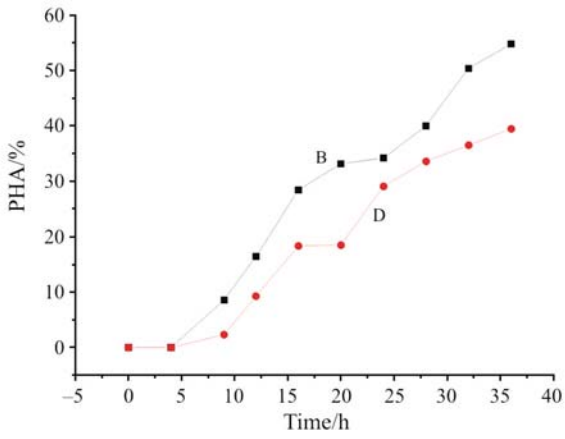

Fig. 2 Yield–time curves of pUC19cab/XL-IBlue and pUC19cab/JM109, B – pUC19cab/XL-IBlue and D – pUC19cab/JM109

different yield of PHAs. Perhaps this results from the intracellular concentration of acetyl-CoA in different *E. coli* host strains. In order to screen for the recombinant *E. coli* strain with the highest yield of PHAs, we normally measure the percentage of PHAs in different recombinant *E. coli* strain after different incubation period by GC ('Experimental'). In this study, we introduced a more efficient method, the microcalorimetric method, to screen for the recombinant *E. coli* strain with a high PHAs production.

Conclusions

In this study, we described a new strategy to screen for the recombinant *E. coli* strains with a high PHAs production. We reported the relationship between PHA production and the heat flow rate of the recombinant *E. coli*. The results directly confirmed that the thermogenetic curves reflect the PHAs production and even the yield of PHAs in different recombinant *E. coli*. The microcalorimetric techniques were more efficient and convenient than the biological methods. The yield of PHAs in different recombinant *E. coli* was also measured using traditional methods. The conclusion drawn from the results from the traditional methods corresponded to those from the thermal analysis.

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References

1 L. L. Madison and G. W. Huisman, Microbiol. Mol. Rev., 63 (1999) 21.
2 J. Choi and S. Y. Lee, Bioprocess. Eng., 17 (1997) 335.
3 T. Kichise, S. Taguchi and Y. Doi, Appl. Environ. Microbiol., 68 (2002) 2411.
4 R. Chen, Y. H. Yang and P. H. F. Yu, Agro-Environment Protection, 20 (2001) 424.
5 Z. Heng, Z. Congyi and W. Cunxin, J. Therm. Anal. Cal., 79 (2005) 45.
6 L. Ruan, Y. Liu, Z. Gao, P. Shen and S. S. Qu, J. Therm. Anal. Cal., 70 (2002) 521.
7 L. Li, X. Wang and J. Shen, J. Therm. Anal. Cal., 82 (2005) 103.
8 L. Núńez-Regueira, J. A. Rodríguez-Ańón and J. Proupín-Castińeiras, J. Therm. Anal. Cal., 80 (2005) 35.
9 Y.-M. Kim, L. K. Kostanski and J. F. MacGregor, J. Therm. Anal. Cal., 78 (2004) 153.
10 K. Takase, S. Taguchi and Y. Doi, J. Biochem. (Tokyo), 133 (2003) 139.
11 M. D. Williams, J. A. Rahn and D. H Sherman, Appl. Environ. Microbiol., 62 (1996) 2540.
12 J. Sambrook, E. F. Fritsch and T. Maniatis, Molecular cloning: A laboratory Manual, 2nd Cold Spring Harbor Laboratory Press, NY 1996.

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