Journal of Thermal Analysis and Calorimetry, Vol. 70 (2002) 521–525

MICROCALORIMETRIC STUDY ON EXPRESSION OF FOREIGN GENES IN *Bacillus thuringiensis*

L. Ruan[1], *Y. Liu*[1,2], *Z. Gao*[2], *P. Shen*[1*] *and Q. S. Sheng*[2]

[1]College of Life Sciences, Wuhan University, Wuhan 430072
[2]Department of Chemistry, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

(Received October 23, 2001; in revised form January 27, 2002)

Abstract

The thermogenic curves of the aerobic metabolism of the three strains of *Bacillus thuringiensis B.t.* A, *B.t.* B and *B.t.* C have been determined by using an LKB-2277 BioActivity Monitor. *B.t.* A was the host bacterium without foreign gene. *B.t.* B and *B.t.* C were constructed by transforming different foreign genes into the host *B.t.* A, respectively. *B.t.* B expressed erythromycin resistant gene, while *B.t.* C expressed both erythromycin resistant gene and tyrosinase gene. The heat flow rate of these strains is *B.t.* A> *B.t.* B >*B.t.* C. These results indicated that there is obvious interrelation between expression of foreign genes and heat flow rate of *B.t.* strains.

Keywords: *Bacillus thuringiensis*, expression, foreign gene, microcalorimetry

Introduction

Bacillus thuringiensis (*B.t.*) is a spore-forming bacterium that produces highly specific insecticidal proteins, the δ-endo-toxins [1]. The most widely used microbial pesticides are those based on preparations of the bacterium *B.t.*. In contrast to chemical insecticides, this pesticide has many advantages such as safety to human, animal and low-pollution to the environment [2,3]. To date, products containing *B.t.*. for pest control have some shortages. In general, its effective employment is improved by changing its genetic characteristics which will involve in the expression of foreign genes [4]. The expression of foreign gene is often identified by complex genetics and molecular biology methods [5]. We report here the study on dynamic relationship between expression of foreign gene and heat flow rate in *B.t.* strains and attempt to find a simple and speedy supplementary method.

The microcalorimetric technique is one of the important methods for thermodynamic study. 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. The microcalorimetric

* Author for correspondence: E-mail : whubmg@whu.edu.cn

1418–2874/2002/ $ 5.00
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Kluwer Academic Publishers, Dordrecht

technique has been widely used in biological field. A high-sensitive differential scanning microcalorimeter have been used to study the structure of DNA [6]. In recent years the microcalorimetory have been used to determine the metabolic out-put of the competence development of *Escherichia coli* [7].

The purpose of this study was to establish the interrelation between expression of foreign genes and heat flow rate in *B.t.* with different genes, and therefore, to provide a new approach for detecting the expression of foreign genes.

Experimental

Bacteria and plasmid

B.t. A strain and Plasmid pHT3101 containing erythromycin resistant gene were kindly provided by the Key Laboratory of Agro-Microbiology of the Ministry of Agriculture, Huazhong Agriculture University, Wuhan 430070, P. R. China. Plasmid pHTAM containing both erythromycin resistant gene and tyrosinase gene was preserved in our laboratory.

B.t. B and *B.t.* C were constructed by transforming the plasmids pHT3101 and pHTAM into *B.t.* A, respectively.

Growth medium

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

CL medium consists of 0.25% glycerol, 0.5% polypeptone, 0.5% casein, 0.3% NaCl. The medium was sterilized by autoclaving for 20 min at 0.07 MPa.

Instrument

An LKB-2277 BioActivity Monitor, manufactured by LKB corporation of Sweden, was used to measure heat output of the metabolism of *Bacillus thuringiensis.* The microcalorimeter was thermostated at 28°C. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). The baseline stability was 0.2 μW/24 h. For details and structure of the instruments, [8, 9].

Microcalorimetric measurements

The metabolic thermogenic curves of *Bacillus thuringiensis* 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 had been stabilized, 5 mL bacterial suspension was put into the ampoule. The temperatures of the calorimeter system and the isothermal box were controlled at 28°C. Meanwhile, the LKB-2210 recorder recorded the thermogenic curves of *Bacillus thuringiensis* growth continuously.

Results and discussion

The heat flow rate change of recombinants B.t. A, B and C strains in LB medium

There are two stages in the growth process of *B.t.*: the vegetative period and the spore-forming period. The vegetative period is divided into three phases: lag phase, exponential phase, stationary phase. During the exponential phase, the bacterium grows exponentially. If the cell number is n_0 at time 0, and n_t at time t, then

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

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

$$n_t w = n_0 w \exp(kt) \quad (2)$$

$$P_0 = n_0 w \text{ and } P_t = n_t w \text{, giving}$$

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

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).

Table 1 The thermokinetic parameters for A, B and C in LB medium

Strains	k/min^{-1}	P_m/μW	t_p/min	Q_{LOG} /J	Q_T/J
A	0.01621	285.7	475	3.2026	35.3193
B	0.01396	280.3	400	2.6568	29.2976
C	0.01929	298.8	645	3.9927	23.7960

Different foreign genes were transformed into *B.t.* A and acquired *B.t.* B and *B.t.* C (Experimental). We do not know if the foreign gene can be expressed in *B.t.*. The three *B.t.* strains were detected by microcalorimetric measurements. The power-time curves of the strains *B.t.* A, B and C are shown in Fig. 1. The thermokinetic parameters of these strains are shown in Table 1. All of the experimental results have a very good reproducibility and consistency. From data in Table 1, we observed that the total heat flow rate (Q_T) of strain *B.t.* A is the highest, since the strain *B.t.* A does not carry any foreign gene. The total heat flow rate (Q_T) of strain *B.t.* B is lower than that of strain A and higher than that of strain C. We can deduce from the result that the erythromycin resistant gene is expressed in *B.t.* C, so it consumed more energy than *B.t.* A.The heat flow rate of *B.t.* C is the lowest because it expresses two foreign genes, the tyrosinase gene and erythromycin resistant gene. These results suggest that there is an interrelation between expression of foreign gene in *B.t.* and its thermogenic curve. It is possible that microcalorimetry can be used directly to determine the expression of foreign gene in *B.t.*. From Fig. 1, we can see that every curve has a peak which represents the maximum heat flow rate (P_m) of the bacterium during the exponential phase, and the corresponding time is t_p. There is a slope which repre-

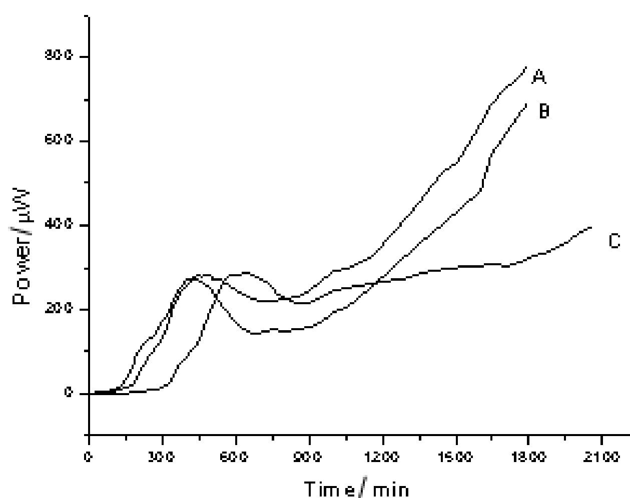

Fig 1 Power-time curves of strains A, B and C LB medium

sents the spore-forming stage after the peaks in the curves [10]. As is shown in Fig. 1, the slope of *B.t.* A and *B.t.* B are steeper than that of strain *B.t.* C. We can deduce that the tyrosinase is synthesized at the onset of sporulation. The high expression of tyrosinase gene at the onset of sporulation consumes much energy. The result revealed that the microcalorimetry could be used to detect the expression and expressing period of foreign gene.

The heat flow rate of recombinant strain A, B and C in different media

We cultivated these three strains in LB medium and CL medium, respectively. The LB medium is high-nutrient medium, while the CL medium is low-nutrient medium. The growth thermokinetic parameters are shown in Table 2. As is shown in Table 2, comparing to that in LB medium, the heat flow rate of these three strains all decrease in CL medium. The maximum heat flow rates (P_m) of the thermogenic curves of these strains in CL medium are all lower than that in LB medium. The heat flow rate of

Table 2 The thermokinetic parameters for strain A, B, C in different medium

	Media	k/min^{-1}	P_m/μW	t_p/min	Q/J	Q_T/J
A	LB	0.01621	285.7	475	3.2026	35.3193
	CL	0.02086	85.0	215	0.3702	20.0533
B	LB	0.01396	280.3	400	2.6568	29.2976
	LC	0.01763	88.4	210	0.3537	17.4382
C	LB	0.01929	298.8	645	3.9927	23.7960
	CL	0.01600	77.5	350	0.4666	12.2139

strain C is the lowest among the three strains in CL medium. This is because it needs express two foreign genes, tyrosinase and erythromycin resistant gene.

Conclusion

In this research, we report the relationship between the expression of foreign gene in *B.t.* and heat flow rate for the first time. The results directly confirm that the expression of foreign gene will consume energy. Furthermore, the more genes are expressed, the more energy is consumed. The thermogenic curves can directly display the energy consumption process. Therefore, the microcalorimetric technique can examine the expression and expression period of foreign gene in *B.t.*. The microcalorimetric techniques are more direct and convenient than biological methods. The expression and expression period of tyrosinase have been detected by biology methods. The conclusion drawn from the results of biology corresponds to that from the results of thermal analysis. The expression period of foreign genes deduced from thermal analysis are more precise than the one from biological methods.

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We gratefully acknowledge the support of the National Natural Science Foundation of China (29973030, 39770009), the Natural Science Foundation of Hubei Province (98052), and Yong Mainstay Teacher Program of Chinese Education Ministry.

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