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A MICROCALORIMETRIC STUDY OF THE BIOLOGIC EFFECT OF Mn(II) ON *Bacillus thuringiensis* GROWTH

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Abstract

A microcalorimetric technique based on the bacterial heat-output was explored to evaluate the effect of Mn(II) on *Bacillus thuringiensis*. The power-time curves of the growth metabolism of *B. thuringiensis* and the effect of Mn(II) on it were studied using an LKB-2277 BioActivity Monitor, ampoules method, at 28°C. For evaluation of the results, the maximum peak-heat output power (P_{max}) in the growth phase, the growth rate constants (k), the log phase heat effects ($Q_{\log}$), and the total heat effect in 23 h (Q_T) for *B. thuringiensis* were determined. Manganese has been regarded as the essential biological trace element. Mn(II) of different concentration have different effects on *B. thuringiensis* growth metabolism. High concentration (800–1600 μg mL^{-1}) of Mn(II) can promote the growth of *B. thuringiensis*; low concentration (500–800 μg mL^{-1}) can inhabit its growth.

Keywords: *Bacillus thuringiensis*, growth metabolism, Mn(II), microcalorimetry

Introduction

Bacillus thuringiensis is one of the major microbial insecticides. It has a major role in the shift from chemical to microbial insecticides. It is a Gram positive soil bacterium, and it belongs to Bacillus according to taxology of bacteria. *B. thuringiensis* is widely applied to control field crop, forestry, horticulture and medical with remarkable ecological benefit. Therefore, the study of *B. thuringiensis* metabolic process is important for the research on the mode of action of this valuable species.

Mn is one of the necessary biological trace elements, because it plays an important role in the living body. Many studies indicate that the lack of Mn may cause sev-

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eral diseases. Mn is involved in bone formation, glucose metabolism, reproduction and cancer development. Consequently, the study of the effect of Mn on microorganism is significant for understanding life phenomenon.

Microcalorimetry is an important method that can be applied to study microbial growth. Many calorimetric studies have been made on microbial systems in order to obtain quantitative information about their biochemical and physiological activities [1–5]. Microcalorimetry has the advantage of being specific only to the initial and final energy states of a system. It is independent of the organisms or the reaction pathway. The heat output is derived largely from the catabolic breakdown of substrate, anabolic reaction contributing little to the overall balance [6].

In the present work, microcalorimetry was used to study the influence of Mn(II) on the growth of *B. thuringiensis* and its relation with the rate of heat production.

Experimental

Materials

Bacillus thuringiensis was provided by Key Laboratory of Agricultural Microbiology of Chinese Agriculture Ministry, Huazhong Agricultural University, Wuhan 430070, P. R. China.

$MnCl_2$ (analytical grade) was supplied by the Shanghai Yuelong Chemical Factory, Shanghai, P. R. China.

The culture medium was LB medium, containing the tryptone 10 g, beef extract 5 g and NaCl 5 g per 1000 mL (pH=7.0–7.2). It was sterilized in high pressure steam at 120°C for 30 min.

Instrument

A microcalorimeter, LKB-2277 BioActivity Monitor by LKB corporation of Sweden, was used to determine the growth metabolic power-time curves of *B. thuringiensis* cells. 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 W/24 h. The performance of this instrument and the details of its construction have been described previously [7–9].

Preparation of the sample

Initially, *B. thuringiensis* was inoculated in the prepared LB culture medium, containing $1 \cdot 10^6$ cells mL^{-1}. The cells were suspended in the LB culture medium, then Mn(II) of different concentration 50, 100, 200, 400, 800, 1200, 1400, and 1600 μg mL^{-1} were added into the culture medium.

Microcalorimetric determination

Solutions of *B. thuringiensis* were put into stainless steel ampoule, containing 5 mL LB medium. Hook the lifer on the ampoules, then lower them slowly to the thermal equilibration position. The ampoule were kept at this position for 30 min for pre-heating. Then ampoules were lowered to the measurement position. Run the chart recorder and its monitor, then record the power-time curves of *B. thuringiensis* growth.

Results and discussion

Figure 1 is the power-time curve for growth of *B. thuringiensis* at 28°C, which is a typical growth curve for *B. thuringiensis*. The metabolic process can be divided into two phases:1) grow and 2) sporulation [10–12]. Figure 2 shows the power-time

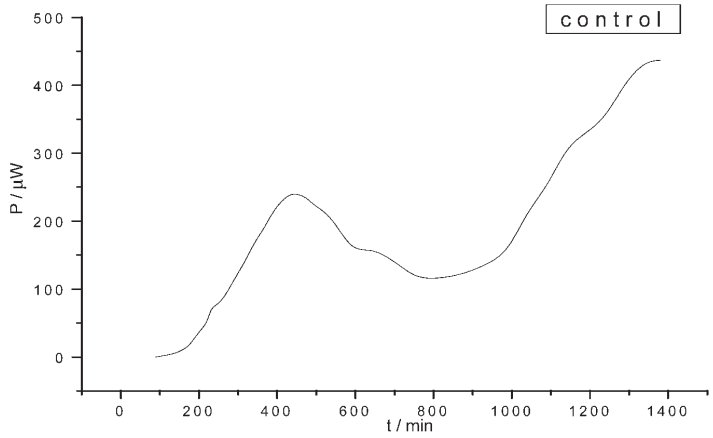

Fig. 1 Growth thermogenic curve of *Bacillus thuringiensis* at 28°C

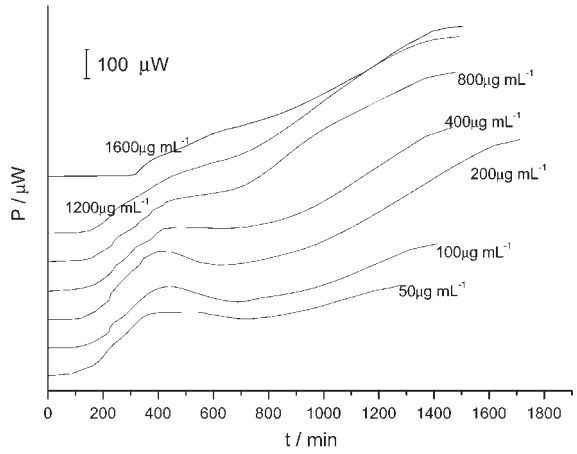

Fig. 2 The power-time curves of *Bacillus turingiensis* at different concentrations of Mn(II)

curves for the growth of *B. thuringiensis* in the presence of Mn(II) ion of different concentrations. Some similarities and differences can be observed from a qualitative point of view. The power-time curves in the presence of Mn(II) can still be divided into two phases, in which the second phase corresponds to the sporulation process.

From analysis of the power–time curves for *B. thuringiensis* in Figs 1 and 2, we can see that the heat power during the log phase of the curves were exponential. We can calculate the growth rate constants (k) of *B. thuringiensis*, using the exponential equation:

$$P_t=P_0\exp(kt)$$

or

$$\ln P_t=\ln P_0+kt$$

From this, we have calculated the values of k and the correlation coefficient R. These are listed in Tables 1 and 2.

Table 1 Rate constant (k) for the growth of *Bacillus thuringiensis* at 28°C

Exp.	1	2	3	4	5	6	mean
k/min^{-1}	0.02621	0.02616	0.02587	0.02633	0.02576	0.02583	0.02603±0.00022
R	0.99502	0.99953	0.99687	0.99731	0.99589	0.99789	0.99709

Table 2 Experimental result of effects of Mn^{2+} ions on *Bacillus thuringiensis* growth

C/g mL^{-1}	k/min^{-1}	t_G/min	t_F/min	t_S/min	Q_F/J	Q_T/J
0	0.02603	26.9	209	429	0.15	14.78
50	0.02533	27.4	203	403	0.25	17.16
100	0.01889	36.7	221	414	0.22	15.20
200	0.02193	31.6	227	447	0.19	18.41
400	0.02409	28.8	229	470	0.25	20.03
800	0.02550	27.2	235	516	0.21	25.09
1200	0.03584	19.3	238	548	0.20	25.66
1600	0.04626	14.9	352	543	0.11	15.71

As shown in Table 1, all the correlation coefficients are larger than 0.9950, indicating a good reproducibility and correlativity. Rate constants (k) increased with increasing the concentration of Mn(II) in the range of 0–1200 μg mL^{-1}. Figure 3 shows the relation of the rate constants and concentrations of Mn(II). When the concentration of Mn(II) reaches 1600 μg mL^{-1}, Mn(II) starts inhibiting growth of *B. thuringiensis*.

Figure 4 shows the maximum time ($t_{max\text{-}log}$) of the log phase appeared progressively later with increasing concentrations of Mn(II), the second phases ($t_{max\text{-}grow}$) are similar to the first phases.

Figure 5 shows the maximum power heights (P_{log}, P_{total}) on the growth curves of the log phase and the growth phase for *B. thuringiensis* without and with different concentration of Mn(II), respectively. It is seen that in the log phase and the growth phase, the max-

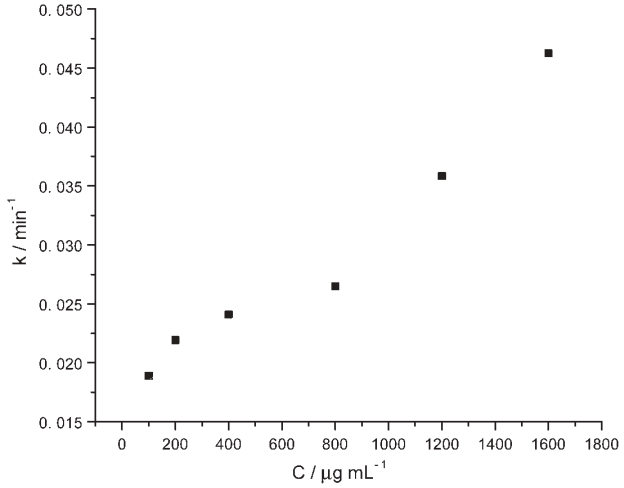

Fig. 3 Relationship between the growth rate constant (k) and C (100–1600 μg mL^{-1})

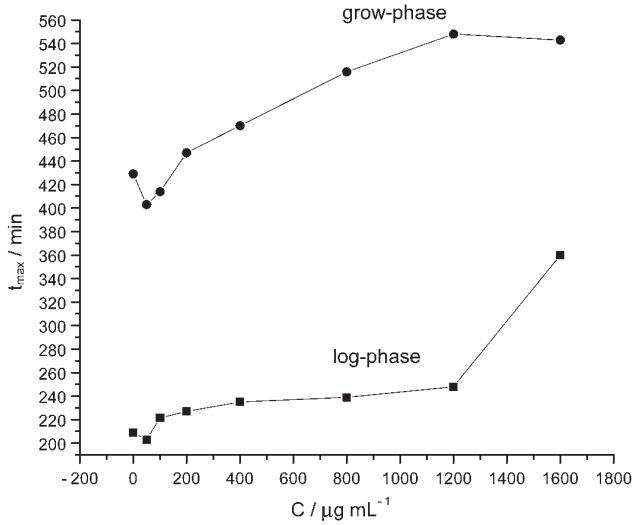

Fig. 4 Relationship between the time of maximum power (t_m) and C (Mn)

imal peak-heights decrease substantially in the range of 0–1200 μg mL^{-1}. When the concentrations of Mn(II) are in the range of 1200–1600 μg mL^{-1}, the peak-heights of both decrease drastically. By comparing the power-time curves for different concentration of Mn(II), we have observed that the peak-height for the grow phase corresponding to 265 μW is the greatest. From these measurements, we concluded that in the range of 0–800 μg mL^{-1}, Mn(II) can inhibit the growth of *B. thuringiensis*, whereas in the range 800–1200 μg mL^{-1}, Mn(II) can stimulate the growth of *B. thuringiensis*. Beyond concentrations of 1600 μg mL^{-1}, Mn(II) inhibits bacterial growth. At the same time, we observed

the temporal details of the growth for *B. thuringiensis* under the presence of Mn(II) during the different phases.

In order to show the results in a more quantitative way, we calculated the heat (Q_{log}) produced in the log phages and the total thermal effects (Q_T) in 23 h from power-time curves of *B. thuringiensis* growth at different concentrations of M (II). These results are shown in Fig. 6.

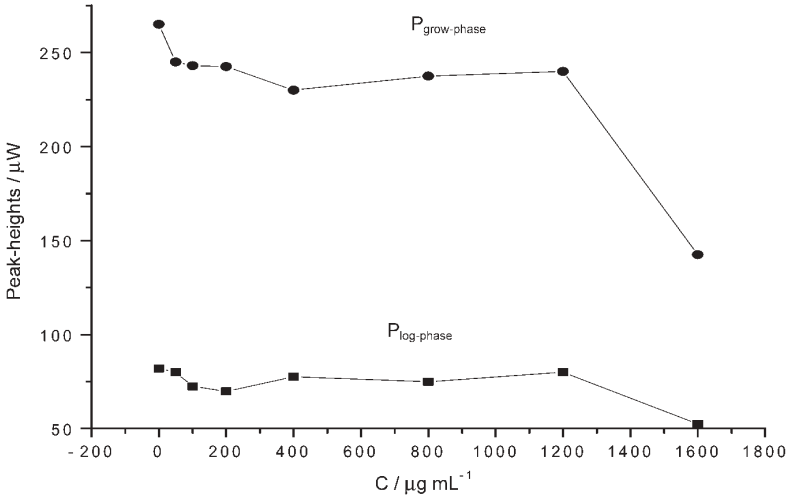

Fig. 5 Relationship betwee peak-height and C (Mn)

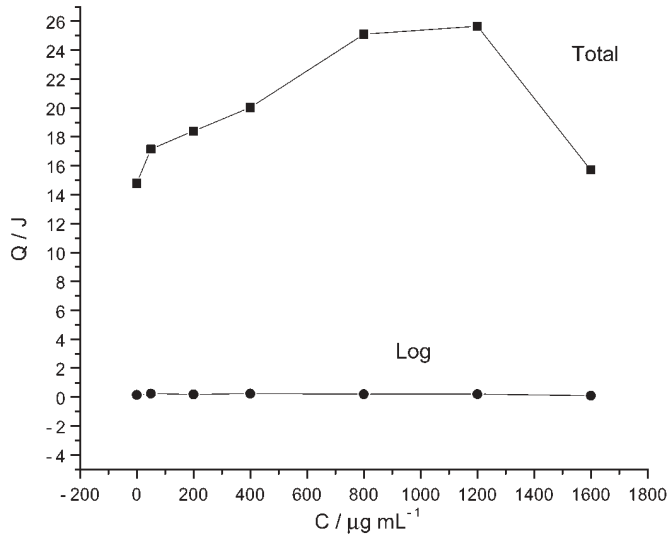

Fig. 6 Relationship between heat-output (Q_T) and *C* (Mn)

Obviously, the heat (Q_{log}) generated in the log phases vary little while the heat produced in 23 h (Q_T) increases as the concentration of Mn(II) increases within the range of 0–800 μg mL^{-1}. In the range of 800–1200 μg mL^{-1}, the total heat-output changed little. Beyond 1200 μg mL^{-1}, the total heat-output decreased with Mn(II) concentration increasing.

This microcalorimetric method was shown to be an approach to quantify the effect of Mn(II) on the growth of *B. thuringiensis*. The experimental results indicated that Mn(II) has stimulating effects in very high concentration, even when the concentration is 800 μg mL^{-1}. The apparent advantage of the automated microcalorimetric method over the former methods is that it allows precise multiple comparisons of different Mn(II)–bacterium combinations, at the same time with high reproducibility and accuracy.

Conclusions

In conclusion, microcalorimetric studies on the effects of the biological trace elements on microorganisms are possible and significant. We believe that microcalorimetry is a useful and accurate system for studying the detailed mechanism of microorganism in the presence of the biological trace elements and can provide important information for microbiology research.

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