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STUDY ON INTERACTION BETWEEN ANTIBIOTICS AND *ESCHERICHIA COLI* DH5α BY MICROCALORIMETRIC METHOD

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A microcalorimetric technique based on the bacterial heat output was applied to evaluate the influence of antibiotics PIP (*Piperacillin Sodium*) and composite preparation of PIP and SBT (*Sulbactam Sodium*) on the growth of *E. coli* DH5α. The power–time curves of the growth metabolism of *E. coli* DH5α were studied using a TAM Air Isothermal Microcalorimeter at 37°C. By analyzing the power–time curves, the parameters such as growth rate constants (k), inhibitory ratio (I), the maximum heat power (P_m) and the time of the maximum heat power (t_m) were obtained. The results show that different concentrations of antibiotics affect the growth metabolism of *E. coli* DH5α. The PIP in the concentration range of 0–0.05 μg mL^{-1} has a stimulatory effect on the *E. coli* DH5α growth, while the PIP of higher concentrations (0.05 –0.25 μg mL^{-1}) can inhibit its growth. It seems that the composite preparation composed of PIP and SBT cannot improve the inhibitory effect on *E. coli* DH5α as compared with the PIP.

Keywords: *E. coli DH5α, inhibition, microcalorimetry, Piperacillin Sodium, Sulbactam Sodium*

Introduction

Piperacillin Sodium (PIP) is a semisynthetic broad-spectrum penicillin antibiotic. It is used to treat many gram-positive and some gram-negative bacteria that cause infections of the lungs, urinary tract, and skin [1, 2].

The composite preparation of the *Piperacillin sodium* and *Sulbactam sodium* (PIP–SBT) is normally in a fixed combination with a ratio of 4:1 (mass/mass). And the SBT is a competitive, irreversible β-lactamase inhibitor [3]. It has been reported that the SBT has inhibitory effect to Neisseriaceal [4].

Microcalorimetry is a versatile technique for thermochemistry and thermodynamic study in life sciences [5–11]. It is a non-invasive, non-destructive manner and allowed in situ biochemical analysis of the samples [12, 13]. Moreover, because the microcalorimetry has a high sensitivity and can provide much information of both qualitative and quantitative aspects of samples, it has been widely used to study the metabolism in cells and whole organism [14–20].

In this paper, the microcalorimetric method has been used to investigate the effects of the PIP and the PIP–SBT on the growth of *E. coli* DH5α and the relationship between the rate of heat production and microbial growth. The results are very important for clinic applications of the antibiotics.

Experimental

Materials

The *E. coli* DH5α was provided by Biomass Conversion Technology Group, Dalian Institute of Chemical Physics, CAS, Dalian 116023, P.R. China. The strain of *E. coli* DH5α was stored in 10% glycerol solution at –20°C.

The LB culture medium per 200 mL contained tryptone 2 g, yeast extract powder 1 g and NaCl 2 g (pH 7.0–7.2). It was sterilized in high pressure steam at 120°C for 20 min before experiment.

The *E. coli* DH5α used in the present paper was prepared as follows: first, a single colony of *E. coli* DH5α from LB plates was inoculated into a 10 mL LB culture medium and cultivated at 37°C in a rotary shaker (220 rpm) for 12 h in aerobic condition; second, 200 μL of the above suspension was inoculated into 10 mL LB culture medium at 37°C in a rotary shaker (220 rpm) for 2.5 h once again; finally, the value of OD (optical density) of the *E. coli* DH5α suspension obtained was measured to be about 0.6 by spectrophotometry at λ=600 nm.

The PIP and the PIP–SBT drugs were kindly supplied by the Hunan Institute of Drug Detection, P.R. China.

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Instrument

A TAM Air Isothermal Microcalorimeter with an eight-channel twin, manufactured by Thermometric AB Company of Sweden, was used to measure the power–time curves of the metabolism of *E. coli* DH5α at 37°C. A computer was employed to record the voltage–time signals continuously which were converted to power–time signals through calibration. The performance of the instrument and the details of its construction have been described elsewhere by Wadsö [10].

Microcalorimetric measurements

The ampoule method was used for the microcalorimetric measurement in this work. Reaction vessels of 20 mL made from glass were cleaned and sterilized. The samples were prepared in the below manners: 10 mL LB culture medium was first put into eight sample ampoules containing antibiotics of different concentrations, respectively; then, the *E. coli* DH5α suspension were inoculated into above eight ampoules. The temperatures of the calorimeter were controlled at 37°C. The power–time signals were recorded at intervals of 1 min.

Results and discussion

Thermokinetics

In the log phase of growth, the growth of *E. coli* DH5α cells is exponential [21]. It can be expressed as follows:

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

where t is the incubation time, n_t is the cell number at time t, n_0 is the initial cell number and k is the constant of cell growth rate. If the power output of each cell is denoted as P_w, then:

$$n_tP_w=n_0P_w\exp(kt) \quad (2)$$

to define P_0 is the initiative power output, P_t is the power output at time t, then Eq. (2) can be rewritten as follows:

$$P_t=P_0\exp(kt) \quad (3)$$

or

$$\ln P_t=\ln P_0+kt \quad (4)$$

According to the Eq. (4), using the experimental data of P_t and t obtained from the bacterial growth curves, the rate constant of cell growth k was calculated and its values are shown in Tables 1 and 2.

Another important thermokinetic parameters – antibiotic inhibitory ratio (I) is defined as:

$$I=[(k_0-k_c)/k_0]\cdot 100\%$$

where k_0 is the rate constant of the control, k_c is the rate constant of *E. coli* DH5α growth inhibited by the antibiotic with a concentration of C. The analysis results are also listed in Tables 1 and 2.

When the inhibitory ratio (I) is 50%, the corresponding half-inhibitory concentration of the antibiotics can be represented as IC_{50}. It can be regarded as the inhibiting concentration causing a 50% decrease of the *E. coli* DH5α growth rate constant.

The effect of the PIP on E. coli DH5α growth

Figure 1 is the power–time curves of *E. coli* DH5α growth in LB medium containing various concentrations of the PIP at 37°C. Figure 1 shows that the metabolic power–curve of *E. coli* DH5α is a typical growth curve, which can be divided into four phases, that is, lag phase, log phase, stationary phase and decline phase.

From Table 1, one can obtain the relationship between C and k. When the concentration of the PIP is 0.05 μg mL^{-1}, the value of k is larger than the control group. It shows the PIP can speed up the growth of *E. coli* DH5α. In the concentration range of 0.05–0.2 μg mL^{-1}, the rate constants decreased with increasing of the concentration of the PIP. The k *vs.* C relationship can be expressed as the following equation by using least square method:

$$K=-11.61C^4+5.221C^3-0.7606C^2+$$
$$+0.03117C+0.001850 \quad R^2=0.97546$$

The growth of *E. coli* DH5α was completely inhibited when the concentration reached 0.25 μg mL^{-1}. The IC_{50} of the PIP was 0.18 μg mL^{-1}.

Table 1 Thermokinetic parameters of growth of *E. coli* DH5α at different concentrations of the PIP at 37°C

C/μg mL^{-1}	k/10^{-3} min^{-1}	R	P_m/μW	t_m/min	I/%
0.00	1.85	0.9997	542.7	2271	0.0
0.05	2.04	0.9993	611.0	2263	–10.3
0.08	1.79	0.9992	521.0	2497	3.20
0.10	1.32	0.9997	549.7	2410	28.6
0.15	1.17	0.9994	461.2	2631	36.8
0.20	0.85	0.9994	407.7	3333	54.3
0.22	1.39	0.9890	372.4	3596	24.9
0.25	0.00	–	–	–	100.0

k – growth rate constants, R – correlated coefficients for k, P_m – maximum power output, t_m – the time of P_m, I – inhibitory ratio

Table 2 Thermokinetic parameters of growth of *E. coli* DH5α at different concentrations of the PIP–SBT at 37°C

C/μg mL^{-1}	k/10^{-3} min^{-1}	R	P_m/μW	t_m/min	I/%
0.00	1.85	0.9997	542.7	2271	0.0
0.05	1.34	0.9996	422.2	2300	27.6
0.10	2.20	0.9985	473.5	2329	–18.9
0.21	0.94	0.9991	396.7	2340	49.5
0.32	1.34	0.9983	423.1	2940	27.6
0.41	1.15	0.9988	436.1	2372	37.9
0.51	1.80	0.9994	714.9	4407	2.70
0.61	0.00	–	–	–	100.0

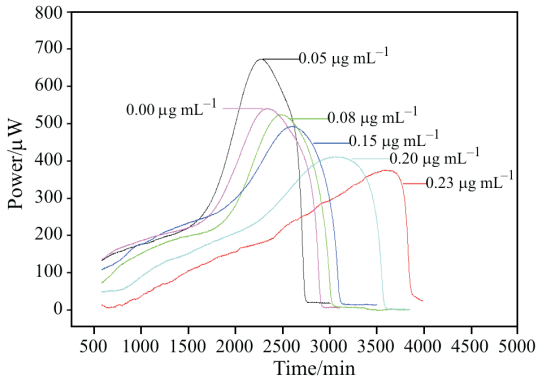

Fig. 1 The metabolic power–time curves of growth of *E. coli* DH5α in LB medium containing various concentrations of the PIP at 37°C

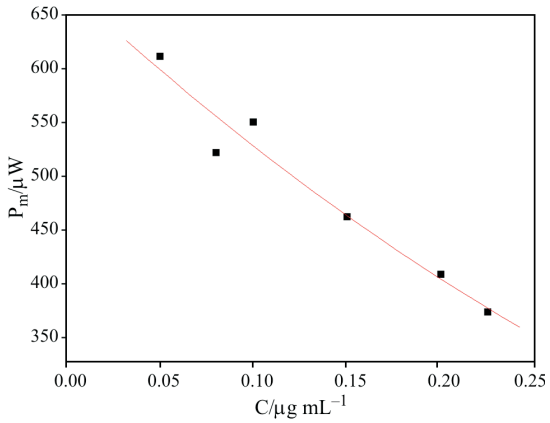

Fig. 2 Relationship between P_m and C (the concentration of PIP)

From Table 1 and Fig. 2, one can see that the maximum power output P_m of growth phase decreases with the increasing of the PIP concentration. The relationship between P_m and C is as follows:

$$P_m=1253C^2-1617C+676.5$$
$$R^2=0.95435\ (0.05–0.225\ \mu g\ mL^{-1})$$

Figure 3 shows the time (t_m) corresponding to maximal power output P_m for growth phase is delayed gradually with the increasing of the PIP concentration. It indicates that the growth of *E. coli* DH5α is inhibited.

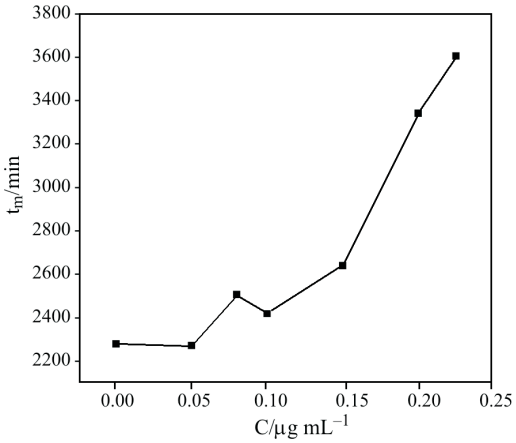

Fig. 3 Plot of t_m *vs.* C (the concentration of PIP)

The effect of composite preparation (PIP–SBT) on E. coli DH5α growth

Table 2 shows the relationship between k and C is not linear which was ascribed to the addition of SBT. When the concentration of the PIP–SBT is 0.1 μg mL^{-1}, the rate constant k shows a higher value. It demonstrates that the PIP–SBT can promote clearly the growth of *E. coli* DH5α at this time. When the concentration reaches 0.21 μg mL^{-1}, the value of rate constant k becomes very low, and the inhibitory ratio (I) is 49.4. The IC_{50} of the PIP–SBT is about 0.21 μg mL^{-1}. Hereafter, there is an interesting phenomenon that the rate constant k begins to increase again with the augment of concentration of the PIP–SBT. However, the PIP–SBT still has inhibitory activity. When the concentration reaches 0.6 μg mL^{-1}, *E. coli* DH5α could not grow any more.

The relationship between C and P_m is also not linear as shown in Table 2. When the concentration is in the range of 0.2–0.4 μg mL^{-1}, the P_m increases slowly. When the concentration reaches 0.5 μg mL^{-1}, the P_m increases drastically as shown in Fig. 4.

Figure 5 shows relationship between the t_m and various concentrations of the PIP–SBT. It can be seen that in the concentration range of 0.05–0.2 μg mL^{-1} for the PIP–SBT, the increase of t_m is very slow. When its concentration is 0.4 μg mL^{-1}, t_m increases drastically.

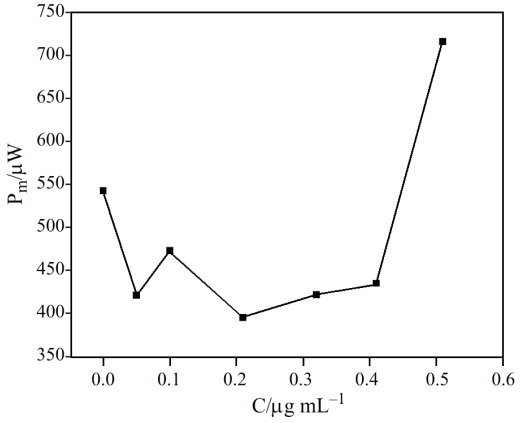

Fig. 4 Relationship between P_m and C (the concentration of PIP–SBT)

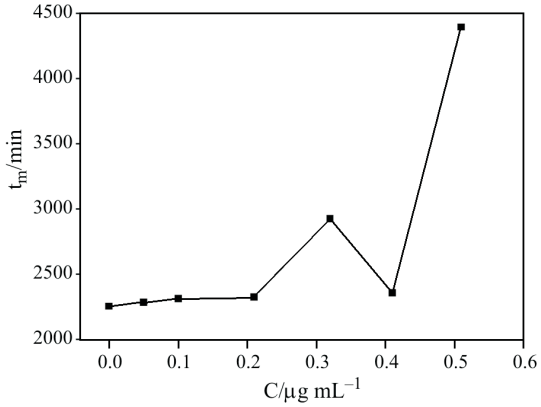

Fig. 5 Plot of t_m *vs.* C (the concentration of PIP–SBT)

Conclusions

In this study, the microcalorimetric method has been successfully used to analyze the effect of two kinds of antibiotics on the growth of *E. coli* DH5α. Comparing the experimental results, one found that when the drugs were at low concentrations (0.05 μg mL^{-1} for the PIP, 0.1 μg mL^{-1} for the PIP–SBT), they all had stimulatory effects on the growth of *E. coli* DH5α. However, they could inhibit the growth of *E. coli* DH5α when their concentrations are larger than 0.05 μg mL^{-1} for the PIP and 0.1 μg mL^{-1} for the PIP–SBT. Furthermore, when the concentration of the PIP and the PIP–SBT reached 0.25 and 0.6 μg mL^{-1}, respectively, the growth of *E. coli* DH5α could be completely inhibited.

The IC_{50} for the PIP and the PIP–SBT were 0.18 and 0.21 μg mL^{-1}, respectively. It indicates that the composite preparation of the PIP–SBT does not increase the inhibition on *E. coli* DH5α as compared with the PIP, that is, the SBT does not have inhibitory effect on the growth of *E. coli* DH5α.

Acknowledgements

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