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ESTABLISHMENT OF EXPERIMENTAL MODEL OF BACTERIAL GROWTH UNDER INHIBITORY CONDITIONS
Study of optimum growth temperature

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Abstract

The thermal curves of *B. subtilis* and *P. atruginosa* were determined by using a 2277 Thermal Activity Monitor (Sweden). Under inhibitory conditions, an experimental model of bacterial growth was established. The growth rate constant (μ), deceleration rate constant (β) and optimum temperature (T) of bacterial growth were calculated.

Keywords: bacterial growth, experimental model, inhibitory conditions, microcalorimeter, optimum growth temperature

Introduction

In any living system, the various metabolic events occurring within the cells are all heat-producing reactions. We can study the metabolic processes of living cells by continuous measurement of the heat effect of the growing cells with a calorimeter, which reveals the thermal curve of bacterial growth. Generally, the metabolism of bacteria is very complicated. We have studied only part of the metabolic processes.

In this paper, the thermal curves of *B. subtilis* and *P. atruginosa* were determined by using a 2277 Thermal Activity Monitor (Sweden) under inhibitory conditions. An experimental model of bacterial growth was established. These thermal curves allow calculation of the growth rate constant, deceleration rate constant and obtimum growth temperature.

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Experimental

Instrument

A new type of heat-flow microcalorimeter, the 2277 Thermal Activity Monitor (Sweden), was used in this experiment. The Monitor can be applied at 10–80°C, the working temperature range of the thermostat. The Monitor is maintained at a given temperature constant to within $\pm 2 \times 10^{-4}$ deg.

This system is very sensitive: the detection limit is 0.15 μw and the baseline stability (over a period of 24 h) is 0.2 μw. The 2277 Thermal Activity Monitor has three operating modes: ampoule mode, flow-through mode and flow-mix mode.

In this experiment, the flow-through mode was used. The sample is pumped through the flow cell by a Micropepex pump.

Experimental method

The complete cleaning and sterilization procedure for the flow tubing was as follows:

(1) Sterilized distilled water was pumped through the system for 30 min at a flow rate of 30 $ml \cdot h^{-1}$.

(2) 0.1 *M* HCl was pumped through the system for 30 min at a flow rate of 30 $ml \cdot h^{-1}$.

(3) Alcohol solution (75%) was pumped through the system for 30 min at a flow rate of 30 $ml \cdot h^{-1}$.

Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 $ml \cdot h^{-1}$ for 30 min and the baseline was determined. After a stable baseline had been obtained, the bacterial sample was pumped into the flow cell system and the Monitor recorded the thermal curve of continuous bacterial growth. When the recording pen returned to the baseline and became stabilized, the process of bacterial growth was complete.

Materials

The following bacteria were employed: *B. subtilis* and *P. atruginosa*. A soluble medium (pH = 7.2–7.4) was used, containing 1 g NaCl, 2 g peptone and 1 g beef extract in every 200 ml.

Establishment of experimental model of bacterial growth

For non-inhibitory conditions, the model of bacterial growth follows an exponential law [1]:

$$dN(t) / dt = kN(t) \tag{1}$$

In the growth phase, the number of bacteria is an accordance with the following law for inhibitory conditions [2].

$$dN(t) / dt = \mu N(t) - \beta N^2(t) \tag{2}$$

where μ is the growth rate constant, β is the deceleration rate constant, and $N(t)$ is the number of bacteria at time t.

If the bacterial growth power is P_o, the

$$P(t) = P_o N(t) \tag{3}$$

Accordingly:

$$d(P(t)/P_o) / dt = \mu(P(t) / P_o) - \beta(P(t) / P_o)^2$$

$$\text{or } dP(t) / (dt) = \mu P(t) - (\beta / P_o)P^2(t) \tag{4}$$

The integral of Eq. (4) is

$$P^{-1}(t) = e^{-\mu t}(P_o^{-1} - \beta / \mu P_o) + \beta / \mu P_o \tag{5}$$

$$a = P_o^{-1} - \beta / \mu P_o \qquad b = \beta / \mu P_o$$

$$\text{or } P(t)^{-1} = ae^{-\mu t} + b \tag{6}$$

The $P(t)$ and t data obtained from the bacterial growth curve fitted a nonlinear equation. The growth rate constant (μ) and deceleration rate constant (β) can be obtained.

The corresponding nonlinear equation of the experimental model at 31°C for *B. subtilis* is

$$P(t)^{-1} = 2.28e^{-0.0208t} + 0.026t \leq 185$$

$$\mu = 0.0208 \quad \beta = 0.0012$$

and that for *P atruginosa* is

$$P(t)^{-1}=5.20\,e^{-0.022t}+0.0140 \quad t\leq 210$$

$$\mu = 0.022 \quad \beta = 0.0016$$

Similarly, the growth rate constant can be calculated at different temperatures (Table 2).

Data on $P(t)$, $\hat{P}(t)$ and t at 31°C are shown in Table 1, and the thermal curves in Fig. 1.

Table 1 $P(t)$, $\hat{P}(t)$ and t values at 31°C

B. subtilis			*P. atruginosa*		
t/min	$P(t)$/uw	$\hat{P}(t)$/uw	t/min	$P(t)$/uw	$\hat{P}(t)$/uw
50	1.2	1.20	50	0.6	0.57
75	2.0	1.98	75	1.0	0.99
100	3.2	3.21	100	1.7	1.69
125	5.0	5.11	125	3.0	2.89
150	7.8	7.88	150	4.9	4.86
175	11.8	11.62	175	8.0	8.02
185	13.1	13.37	200	13.0	12.85
			210	15.5	15.33

$P(t)$ are experimental data; $\hat{P}(t)$ are data calculated from the model

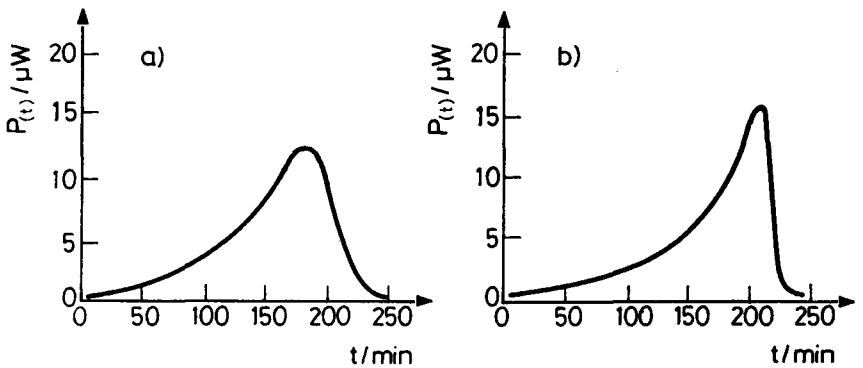

Fig. 1 Thermal curves of bacteria at 31°C

Calculation of optimum growth temperature

We have determined the growth rate constant (μ) of *B. subtilis* and *P. atruginosa* at different temperatures. From these results, a nonlinear equation can be established as $\mu = a+bT+CT^2$.

The corresponding nonlinear equation for *P. atruginosa* at $(\partial\ \mu a)/(\partial\ T_a) = 0$, $\partial\mu^2 a\ /\ \partial T_a^2 < 0$ and $T_a \approx 309.24$ K:

$$\mu a = -21.1819 + 0.1371T_a - 2.2167\times10^{-4}\ T_a^2$$

and at $(\partial\mu_b) / (\partial T_b) = 0$, $(\partial\mu_b^2) / (\partial T_b^2) < 0$ and $T_b = 309.23$ K for *B. subtilis*:

$$\mu b = -28.2312 + 0.1828T_b - 2.9557\times10^{-4}\ T_b^2$$

T_a and T_b are optimum growth temperatures.

Table 2 Growth rate constants at different temperatures

Bacteria	Rate Constant		μ / min^{-1}	
	at 31°C	34°C	37°C	40°C
B. subtilis	0.0208	0.0271	0.0298	0.0240
P. atruginosa	0.0220	0.0276	0.0290	0.0250

Conclusion

These curves contain much information concerning the kinetics of metabolic processes. From the experimental model of bacterial growth, we calculated the growth rate constant (μ) and optimum growth temperature (T). These data are very useful in studies of bacterial thermokinetic properties.

References

1 E. A. Daws, 'Quantitative Problems in Biochemistry' (6th ed), Longman, London, 1980.
2 Gao Peiji, Wang Zunong et al. 'Microorganism growth and Fermentation technology', Shandong University publication, 1990.

Zusammenfassung — Mittels eines 2277 Thermal Activity Monitor (Schweden) wurden die thermischen Kurven von *B.subtilis* und *P.atruginosa* ermittelt. Unter Inhibitionsbedingungen wurde ein experimentelles Modell bakteriellen Wachstums festgestellt. Die Wachstumsgeschwindigkeitskonstante μ, Verlangsamungskonstante α und optimale Temperatur (T) für das bakterielle Wachstum wurden bestimmt.