MICROCALORIMETRIC STUDY OF THE SCREENING SPECIFIC PROMOTER BACTERIA OF NUTRIENT DRUG

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Abstract

The power *vs.* time curves of the promoter bacteria of a nutrient drug were determined by using a 2277 Thermal Activity Monitor (Sweden). A new experimental model of bacterial growth were established. The growth rate constant, heat output and optimum concentration of specific promoter bacterial of nutrient drug were calculated.

Keywords: Escherichia coli, microcalorimeter, nutrient drug, optimum concentration, optimum promoter action

Introduction

In previous papers [1-3], we reported the study of the optimum fungistatic action of a synthetic medicine and establishment of the microorganism growth model at an inhibitory condition and the study of promoter action of a ginseng, for bacteria.

In this work, we determined the power vs. time curves of the promoter bacteria of Astragalus, Ganoderma lucidum, Lycium chinense for Escherichia coli. The growth rate constant (μ) and heat output (Q) at different concentrations (C) of nutrient drug have been calculated. From μ vs. C and Q vs. C curves. The optimum concentration of nutrient drug has been established.

Experimental

Instrument

A new type of heat-flow microcalorimeter, the 2277 Thermal Activity Monitor (Thermo Metric AB, Sweden) was used in this experiment. The sample was pumped through the flow cell by a 2132 microperspex peristaltic pump. This system is very sensitive. The instrument can be used within the range 10–90°C. It was maintained at a given temperature constant to within $\pm 2 \cdot 10^{-40}$ C. The detection limit is $1.5 \cdot 10^{-7}$ W, and the baseline stability (over a period of 24 h) is $2 \cdot 10^{-7}$ W.

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Method

First, the flow tubing was cleaned and sterilized. Sterilized distilled water, 0.1 mol L^{-1} HCl, 0.1 mol L^{-1} NaOH and ethanol solution (75 vol%) were pumped through the system for every 30 min at a flow rate of 30 ml h⁻¹.

Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 ml h⁻¹ for 30 min and the baseline was determined. After a stable baseline had been obtained, the bacterial sample, medium and nutrient drug of different volume were pumped into the flow cell system and the monitor began to record the power *vs.* time curves of continuous growth for *Escherichia coli*. When the recording pen had returned to the baseline and became stabilized, the process of bacterial growth was complete. The bacteria employed in this study were *Escherichia coli*.

A soluble medium (pH 7.2–7.4) was used, containing NaCl (1 g), peptone (2 g) and beef extract (1 g), in each 200 ml. The soluble medium also contained nutrient drug of different volumes as *Astragalus, Ganoderma lucidum* and *Lycium chinense*.

Every nutrient drug was decocted for 30 min and filtered to obtain the drug solution. The concentration of *Astragalus, Ganoderma lucidum* and *Lycium chinense* is 1.02 g ml^{-1} , 0.535 g ml⁻¹ and 1.268 g ml^{-1} , respectively. The bacterial number was 10^7 cells.

Establishment of new experimental model of bacterial growth

For non-limited condition, the model of bacterial growth follows the Malthus law [4]

$$dN(t)/dt = KN(t)$$
(1)

where K is the rate constant follow from Malthus law, N(t) is the bacterial numbers at time t.

In the inhibitory condition, microorganism growth model follows the Logistic equation of Verhults [5].

In the growth phase, bacterial numbers and time are related according to

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

where μ is the growth rate constant, β is the promoter growth rate constant. If the power produced by every bacterium is P_{o} , then

$$P(t) = P_{\rm o}N(t) \tag{3}$$

and accordingly

$$dP(t)/dt = \mu P(t) - (\beta/P_0)P(t)^2$$
(4)

The integral Eq. (4) is given by

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

or

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

where $a = 1/P_{o} - \beta/\mu P_{o}$, $b = \beta/\mu P_{o}$.

Experimental results and calculation of growth rate constant, heat output and optimum concentration

The power vs. time curves were determined at 310 K with different concentrations of nutrient drug for *Escherichia coli*. The part of curves are seen in Fig. 1.

By using the data P(t) and t obtained from power vs. time curves for the growth phase, the corresponding non-linear equations of the new experimental model have been established as follows:



Fig. 1 Power vs. time curves of *Escherichia coli* at 310 K and different concentration of *Astragalus*: C is concentration, g ml^{-1}

When drug solution concentration of *Astragalus* is 0.0102 g ml⁻¹, $P(t)^{-1}=4.51928e^{-0.0415t}+0.0183, t<140 \text{ min}, \mu=0.0415 \text{ min}^{-1}.$

When drug solution concentration of *Astragalus* is 0.0153 g ml⁻¹, $P(t)^{-1}=1.96826e^{-0.0655t}+0.01460, t<110 \text{ min}, \mu=0.04655 \text{ min}^{-1}.$

The data for P(t), $P(t)^*$ and t are shown in Table 1.

In a similar way, we can also calculate the growth rate constants of different concentrations of other nutrient drugs.

The power *vs.* time curves of *Escherichia coli* indicate microorganism growth process on the promoter bacteria. The area (under a curve) represents the heat output and can be calculated.

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	0.0102 g ml^{-1}			0.0153 g ml^{-1}	
<i>t</i> /min	$P(t)/\mu W$	$P(t)^*/\mu W$	<i>t</i> /min	$P(t)/\mu W$	$P(t)^*/\mu W$
40	1.3	1.2	40	3.0	3.1
80	5.5	5.5	60	7.5	7.4
100	11.0	11.2	80	15.5	16.1
120	20.5	20.2	100	30.1	30.0
140	36.0	31.3	110	40.0	38.0

Table 1 P(t), $P(t)^*$ and t values with promoter for *Escherichia coli* at 310 K and different concentration of *Astragalus*

P(t) is the experimental data;

 $P(t)^*$ is the data calculated from the new model

The data of growth rate constant and heat output for *Astragalus*, *Ganoderma lucidum and Lycium chinense* are shown in Table 2.

From these growth rate constants and heat output at different concentrations of nutrient drugs, we determined the optimum concentration of a nutrient drug for promoter *Escherichia coli*, Fig. 2.



Fig. 2 Data of the growth rate constant, heat output on the different concentration of Astragalus

We extended and maintained the constant within 0.025 g ml⁻¹ at a given concentration for growth rate constant and heat output. In a similar way, we can also calculate the optimum concentration of every nutrient drug, the data are shown in Table 3.

From these data, we can establish the optimum concentration, the *Ganoderma lucidum* is good nutrient drug of promoter bacteria by three kinds for *Escherichia coli*.

Table 3 Optimum concentration of nutrient drug for promoter Escherichia coli

Nutrient drug	Optimum concentration $c/g \text{ ml}^{-1}$
Astragalus	0.025
Ganoderman lucidum	0.010
Lycium chinense	0.0325

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	Astragalus		Ga	noderma lucida	um	7	ycium chinens.	в
g ml ⁻¹	µ/min ⁻¹	Q/J	$c/{ m g}~{ m ml}^{-1}$	µ/min ⁻¹	Q/J	$c/\mathrm{g}~\mathrm{ml}^{-1}$	µ/min ⁻¹	$\widetilde{O}^{/1}$
	0.0315	0.063	0	0.0315	0.063	0	0.0315	0.063
.0102	0.0415	0.101	0.0054	0.0365	0.094	0.0063	0.0349	0.105
.0153	0.0466	0.117	0.0107	0.03816	0.152	0.0127	0.03817	0.138
.0204	0.0495	0.132	0.0267	0.03812	0.227	0.0254	0.04227	0.192
.0306	0.0518	0.138	0.0401	0.03827	0.248	0.0317	0.04342	0.214
.0510	0.0519	0.141	0.0535	0.03815	0.255	0.0380	0.04353	0.210

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Conclusions

Power vs. time curve contains a lot of information. From the curve, we have established a new experimental model of promoter bacteria, the model reflects the actual result of microorganism growth phase well. Using the data of power vs. time curve to calculate growth rate constant (μ) and heat output (Q), at different concentration (c). From μ , Q and c relation, the optimum concentration can be obtained, which is very useful for screening specific promoter bacteria of nutrient drug.

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Supported by the National Science Foundation.

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