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RESEARCH ARTICLE

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Study on eruption of heat for *Escherichia coli* B aroused by lanthanum nitrate and its mechanism

Abstract Biological effect of rare-earth lanthanum nitrate on the growth of *Escherichia coli* B was studied using the calorimetric method. There were exceptional changes on the growth thermogenic curves for high concentrations of lanthanum nitrate. For example, the peak high, the total quantity of heat (Q) of cultures and the growth rate constants (k) are evidently increased when compared with normal *E. coli* B cultures. When the concentration of lanthanum nitrate was at 300 mg/L and 500 mg/L, the Q of the cultures reached 3.89 and 2.54 times of normal cultures, respectively. The survivability of cells and the biomass of the cultures were measured using biological methods and the results show that the growth and multiplication of cells were inhibited and that the biomass decreased at high concentration of lanthanum nitrate. These revealed that the inhibiting cells discharged more quantity of heat than the normal growing cells. We named this phenomenon as "eruption of heat". It was suggested that the mechanism for the eruption of heat was that La^{3+} ion damages the outer cell membrane and increases its permeability and the proton-electron potential energy across the cell membrane was reduced or couldn't even be initiated. Energy could not be translated into ATP effectively in the course of oxidative phosphorylation resulting in heat release. So, the growth of the cells was inhibited due to scarceness of energy ATP.

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Microcalorimetric technology is a useful technique that has been widely used in the life sciences [1], in clinical [2] and in pharmacological analysis [3] because of its high sensitivity, high accuracy and automaticity [4,5]. It is applicable to different levels of biological study, such as a molecule, a cell, tissues, individual and ecological systems etc [6]. In the past twenty years, people used the microcalorimetric method to study the growth and metabolism of microbes and obtained a lot of useful data. They simultaneously analyzed the process of microbial development using the thermokinetic equation [7].

It is well known that the rare-earth elements (REE) have physiological functions and biological effects on some life-forms. It has been used extensively in many fields, especially in agriculture (fertilizer). At the same time, the biochemical effect and pharmacological reaction of REE has attracted much attention from biologists and pharmacists and has been studied further [8,9]. Research studies reported that REE could combine with other biologic molecules, participate in vital process and activated or inhibited the enzyme or zymogen. In this paper, we studied the biological effect of rare earth lanthanum nitrate on the growth of *Escherichia coli* B by using the calorimetric method. There was an abnormal "eruption of heat" phenomenon in high concentration of lanthanum nitrate. Further more we used biological method to study the biochemic and physiological effects of La^{3+} on cells, in order to search for the reason of the "eruption of heat" and the mechanism of La^{3+}.

1 Materials and methods

1.1 Equipment

The heat conduction microcalorimeter, LKB-2277 Bioactivity Monitor, which had been made in Sweden, was used to obtain the thermogenic curves of the phage-host interaction [10,11].

1.2 Bacterial strains, reagents and culture medium

The bacterial strain *E. coli* B and bacteriophage T4 were obtained from the China Center for Virus Collection (Wuhan Institute of Virology, Chinese Academy of Sciences).

LBG medium consists of 0.5% NaCl, 1% Bacto tryptone, 0.5% Bacto yeast-extract and 5% glucose with a pH of 7.0–7.2. The medium was sterilized by autoclaving for 20 min at 0.1 MPa [12].

Analytical reagent grade $La(NO_3)_3$ dissolved with distilled water which were prepared by the reaction between La_2O_3 and HNO_3.

1.3 Preparation of stock solution of *E. coli* B cells

E. coli B was stored in 20% glycerol solution at −20°C and cultivated at 37°C with LB culture medium. The stock of host cells was prepared as follows: (1) a single colony of *E. coli* B from LB plates was inoculated into a 50-mL LB liquid culture medium and cultivated at 37°C in a rotary shaker (200 r/min); (2) the cells were centrifuged at 5000 r/min for 10 min and resuspended in sterile 0.01 mol/L $MgSO_4$; (3) the amount of viable cells were determined using the spread-plate technique.

1.4 Calorimetry setup and heat measurement assay

The sample cells of the calorimeter were cleaned and sterilized in turn with 0.1 mol/L HCl, 0.1 mol/L NaOH, 75% alcohol and sterilized distilled water. Once the baseline had been stabilized, a certain concentration of La^{3+} reagents and *E. coli* B (cfu = 10^5) were pumped into the microcalorimeter at a flow rate of 50 mL/h by LKB 2132 MicroPerpex pump. Then, the monitor was opened and the heat production rate was recorded at 60-s intervals.

1.5 Biological assay

1.5.1 The survivability mensuration of *E. coli* B affected by $La(NO_3)_3$

0.2 mL *E. coli* B cells were spread on plates which contained LB sterilized solid culture medium and different concentrations of $La(NO_3)_3$. The concentrations were 0, 50, 100, 200 and 500 mg/L, cultivated at 37°C. Every essay was repeated thrice. The number of colonies on the plates were mensurated after 20 h.

1.5.2 The biomass measurement of *E. coli* B growth affected by $La(NO_3)_3$

Simulating the condition of LKB-2277, we inoculated *E. coli* B cells (10^5 cfu/mL) in the sterilized liquid culture medium which contained different concentrations of $La(NO_3)_3$. The concentrations were 0, 20, 100, 200 and 500 mg/L, cultivated at 37°C. We took out 20 mL culture using an asepsis injector culture at a certain time and collected cells by centrifugation, and then measured the wet weight and dry weight of the samples.

2 Results

2.1 Effect of $La(NO_3)_3$ on the thermokinetic process of *E. coli* B growth

We used the stop-flow method to measure the thermograph of *E. coli* B grown in LBG culture medium at 37°C. The result is credible with high repeatability. The curves are identical with the results which we obtained before [13] (Fig. 1). But a remarkable change took place when adding $La(NO_3)_3$ to the culture medium. The value of the 3 peaks increased markedly, especially for the third peak, and the value increased with increasing $La(NO_3)_3$ concentrations. A dose-effect relationship existed. The growth rate constant of *E. coli* B could be calculated according to the equation [14]:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

Where P_t is the power output at time t, P_0 is the initiative power output and k is the growth rate constant. Making use of the data of the ascending portions of the peaks to fit linear equations, growth rate constants (k) can be obtained. The thermokinetic equations,k, R and other parameters are shown in Table 1. While the first peak power output (P_{peak1}) and its time of appearance (t_{peak1}), maximum of power output (P_{max}) and its time of appearance (t_{max}), and total quantity of heat (Q) are list in Table 1.

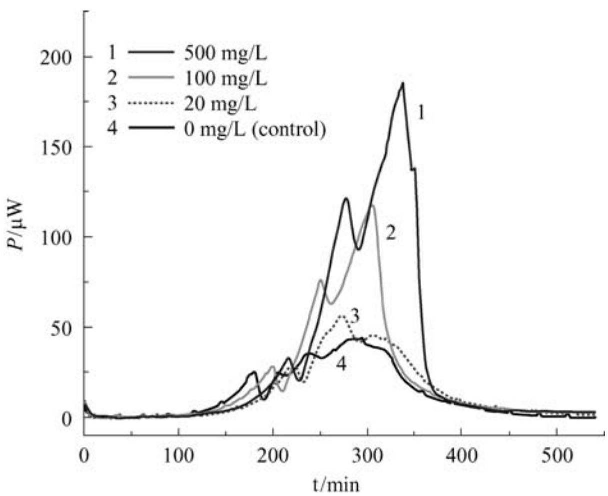

Fig. 1 The thermogenic power-time curves of growth for *E. coli* B and the effect of lanthanum nitrate

Table 1 Data of metabolic parameters of the thermogenic power-time curves of growth for *E. coli* B and the effect of lanthanum nitrate

$La(NO_3)_3$ mg/L	500	200	100	50	20	control
k/min^{-1}	0.0339	0.0344	0.0330	0.0323	0.0322	0.0324
R	0.9990	0.9983	0.9972	0.9984	0.9970	0.9937
G/min	19.52	19.57	20.78	19.45	20.25	21.26
$P_{peak1}/\mu W$	32.47	31.26	28.43	31.41	28.85	26.38
t_{peak1}/min	215	210	199	216	221	180
$P_{max}/\mu W$	185.29	175.26	117.65	85.70	56.12	45.01
t_{max}/min	337	298	305	291	272	283
Q/J	0.0978	0.1272	0.0653	0.0509	0.0443	0.0501

k, growth rate constant; R, correlation coefficient; P_{max}, maximum of power output; t_{max}, appearance time of P_{max}; Q, total quantity of heat for the peak

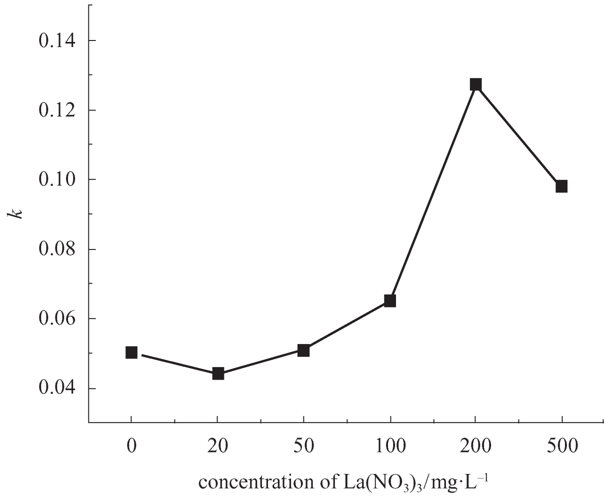

Fig. 3 Plot of k for the growth of *E. coli* B versus concentration of lanthanum nitrate

The results show that the effect of $La(NO_3)_3$ to the thermogenic curves is distinct. The change is more obvious along with the increment of $La(NO_3)_3$ concentration. The time of appearance of the three thermokinetic peaks were delayed and the height increased markedly, especially for the second and third peak values which were 2–4 times of the normal condition. When the concentration of lanthanum nitrate was at 200 mg/L, P_{max} and Q of the cultures reached up to 3.89 and 2.54 times of normal cultures respectively (Fig. 2).

Curve 1, control without lanthanum nitrate, and curve 2 to 4 with 20, 100, and 500 mg/L $La(NO_3)_3$, respectively. The curves for 200 mg/L and 50 mg/L are not plotted in the graph.

Otherwise, growth rate constants (k) fitted to equation (1) increases with the rising of the concentration of lanthanum nitrate (Fig. 3). In normal conditions, the metabolite rate and the growth rate of cells is in correlation with k. k and P_{max} will rise with the increase of the metabolite rate and growth rate. When adding lanthanum nitrate, does the abnormal heat that had erupted above and k relate to the metabolism and growth of cells? Does the metabolic rate increase relate factually to the growth and reproduction of cells? From the analysis of the thermokinetic equation, the question could not be answered with certainty.

2.2 The effect of $La(NO_3)_3$ on *E. coli* B growth

2.2.1 The effect $La(NO_3)_3$ on *E. coli* B livability

In order to analyze the reason that the eruption of heat and k rose, the experiment was done as follows: *E. coli* B was spread on a LBG solid culture medium plates which contained $La(NO_3)_3$. The concentrations were 0, 50, 100, 200 and 500 mg/L, and cultivated at 37°C. Every assay was repeated thrice. At a certain time, the colonies on the plates were counted and we calculated the survivability of cells (Fig. 4).

The results show that the survivability of *E. coli* B was reduced inconspicuously at low concentrations of La

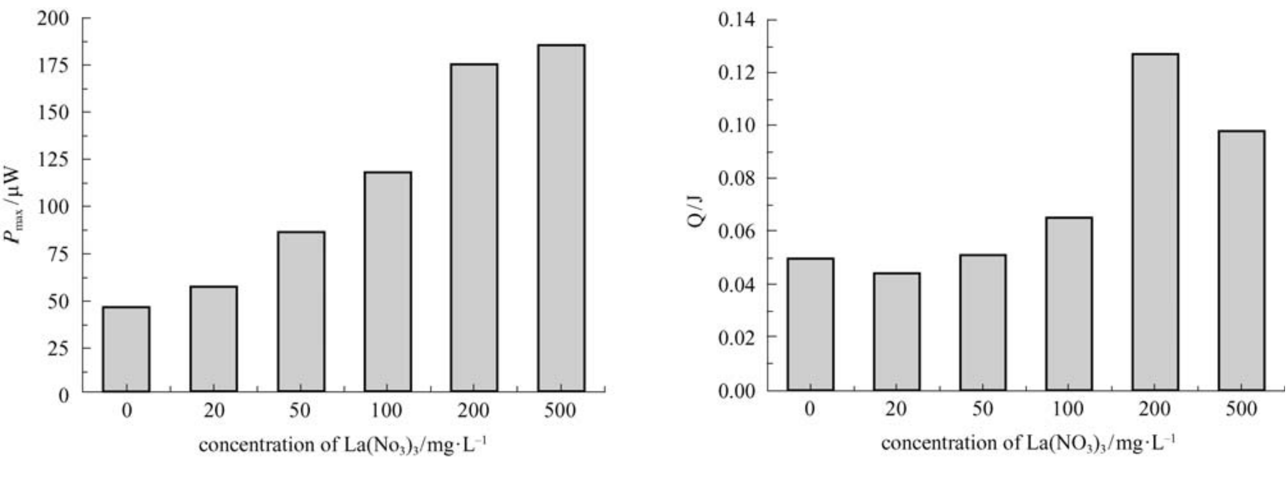

Fig. 2 Plot of P_{max} and Q for the growth of *E. coli* B versus concentration of lanthanum nitrate

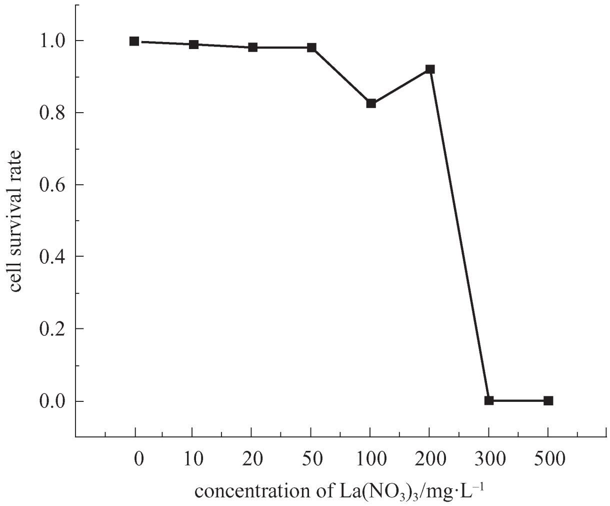

Fig. 4 The effect of $La(NO_3)_3$ on the livability of *E. coli* B on the solid culture medium

$(NO_3)_3$ (10–50 mg/L). It was about 99%–98%. When the concentration came to 100 or 200 mg/L, the survivability decreased markedly. Furthermore, when it reached 300 mg/L, the survivability decreased to 0. These revealed that high concentrations of $La(NO_3)_3$ (≥300 mg/L) shows strong inhibitory effects to *E. coli* B cells. Cell growth would stop or the cell may die. But the thermogenic curves show that the culture produced more heat in 300 mg/L and 500 mg/L $La(NO_3)_3$ than in low concentrations of $La(NO_3)_3$ and normal culture. So the conclusion could be made that the *E. coli* B cells do not grow and reproduce in conditions of high $La(NO_3)_3$, but produce more quantity of heat than cells in normal culture conditions. This phenomenon is different from cell growth that is promoted and subsequently produces more heat. We named this abnormal phenomenon as an "eruption of heat". Heat Eruption is not caused by the flourishing growth and multiplication, but by the fact that the energy metabolism of cells was disturbed by La $(NO_3)_3$. The proton transmembrane current was counteracted and the current energy ATP could not be synthesized effectively. The energy had to be released as heat which resulted in a useless consumption of energy.

2.2.2 The biomass of *E. coli* B affected by $La(NO_3)_3$ in liquid culture medium

The conditions of *E. coli* B growth in a solid culture medium are different from that in the LKB-2277 biomonitor. So, the conditions in the LKB-2277 were simulated as follows: We inoculated the *E. coli* B cells (10^5 cfu/mL) in the liquid culture medium which contained different concentrations of $La(NO_3)_3$. The concentrations were 0, 20, 100, 200 and 500 mg/L, cultivated in anaerobic condition at 37°C. According to the thermograph, it took 300 min for P_{max} to appear and heat release had stopped after 500 min. We collected and measured the wet weight and dry weight of cells at 300 min and 500 min. The data are shown in Fig. 5.

When cultivation time reached 300 min, the power output almost reached the maximum. At the same time, the biomass increased for 20 and 100 mg/L of $La(NO_3)_3$, but decreased obviously when the concentrations of La $(NO_3)_3$ came to 200, 500 mg/L, the cells dry weight was 50% and 10% of normal culture, respectively. The thermogenic curve returned to baseline at 500 min, the biomass increased distinctly for 20 mg/L $La(NO_3)_3$. With $La(NO_3)_3$ concentration increasing more, the biomass decreased. The dry weight for 200, 500 mg/L $La(NO_3)_3$ are 20% and 6.7% of normal culture, respectively. The result is consistent with that of the colony amount and survivability on solid culture medium. The results above could prove that the heat release does not relate to increased biomass. In fact, the cells released more heat with decreasing cell numbers. So the reason for heat

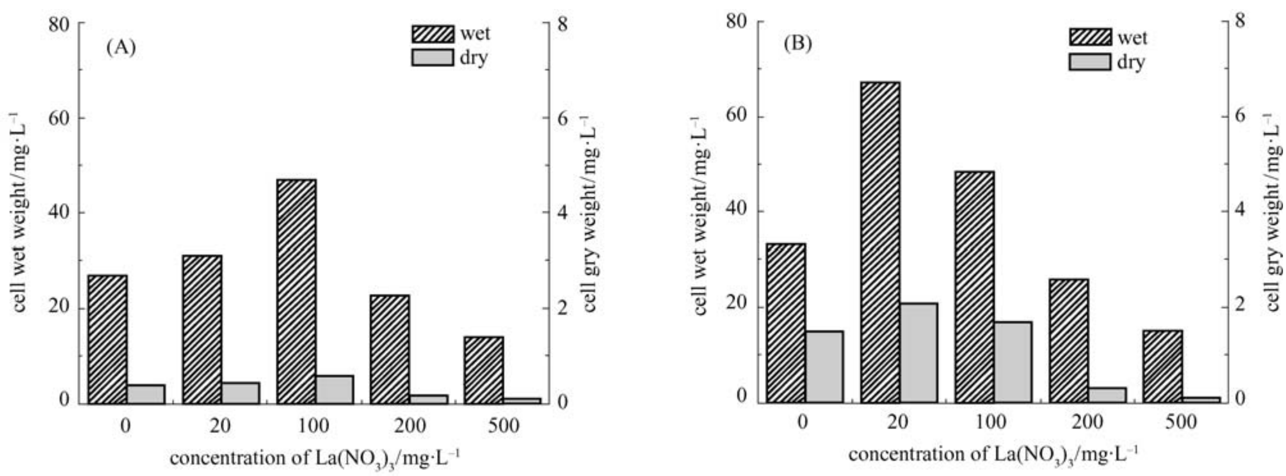

Fig. 5 The effect of $La(NO_3)_3$ on the biomass of *E. coli* B cultured in liquid culture medium
(A) 300 min; (B) 500 min

eruption and k increasing might be that the energy metabolism of cells is disturbed and the energy of catabolism resulted in heat release.

3 Discussion

Thermokinetic research have shown that either low or high of concentrations of $La(NO_3)_3$ could promote the heat release of *E. coli* B. P_{max} and Q increases with the increasing $La(NO_3)_3$ concentrations. There a dose-effect relationship between $La(NO_3)_3$ and heat release. The number of colony and biomass assay show that the growth of bacteria were promoted in low concentrations of $La(NO_3)_3$ and inhibited in high concentrations. The thermogenic curves exhibited the heat release of cell growth and metabolism. In normal conditions, the faster the cells grow, the more heat is released. So, thermogenic curves could be used to describe the kinetic process of bacterial growth. But in this experiment, with high concentrations of $La(NO_3)_3$ added, the power output increased several times but the biomass decreased markedly. This abnormal phenomena reveals the physiological mechanism of $La(NO_3)_3$ acting on cells and the essential reason for $La(NO_3)_3$ inhibiting cell growth in high concentrations. Much of the energy produced in the oxidation procedure of organic substrate were transferred to heat energy and exhibited the ''eruption of heat'' phenomenon. $La(NO_3)_3$ disturbs the transformation of energy in cell metabolism. How does this happen?

In the chemical-osmotic coupling theory, the mechanism of ATP synthesis is promoted by the proton motive force (proton electric-chemical potential) in the oxidative phosphorylation process. The setting up of the proton motive force comes from the H^+ transmembrane potential. When H^+ returns to cytosol side of inner membrane, the reaction of free energy release is coupled to an ATP synthesis reaction. By eliminating the gradients of protons and electrons between two sides of membrane rapidly or accelerating the electron transfer on the electron transport chain, we can enhance the consumption of molecular oxygen and the substrate and produce a mass of heat energy instead of forming ATP. Uncouplers such as 2,4-dinitrobenzene take on this function. Some research show, that La^{3+} could promote cell growth at low concentrations but inhibits it at high concentrations [15–17]. The conclusion was proven in our experiment as well. Why does high concentration of La^{3+} restrain cell growth and produce a lot of heat energy? Surely $La(NO_3)_3$ is not an uncoupler. According to the above results, we can infer that high concentrations of La^{3+} disturbs energy metabolism and the energy is not the transferable currency energy, ATP, and had to be released as heat energy. This brings on the ''eruption of heat'' phenomena. A significant question that needs to be answered is how does La^{3+} affect energy metabolism?

E. coli are Gram-negative bacteria with an outer-membrane which is composed of lipopolysaccharide (LPS) which has a mitochondrial outer membrane. In the respiration process, electrons are transferred to the cytoplasmic membrane and protons are pumped out of the membrane. This is the protonic motive force. According to Liu's report [18], La^{3+} and Ca^{2+} have a similar atom radii and chelate characteristics [19]. La^{3+} could easily combine with the Ca^{2+} and Mg^{2+} site on the cell wall by way of replacing these ions. Different electric charges destroy the structure and stability of the outer-membrane so that its permeability is enhanced. These actions are provided on a dosage-effect and time-effect curve by the La^{3+} concentration. So, high concentrations of La^{3+} would bring increased penetrability due to the increase in small holes on the membrane. Protons leak out. The electro-chemical gradient is reduced and thus, the rate of ATP synthesis is decreased or stopped. Simultaneously, electron transfer does not stop on the cytoplasmic membrane, so, the only released is heat energy inducing the ''eruption of heat'' phenomena. Being short of ATP energy, the biomass could not be formed and the cell amount could not increase. Specifically, cell growth is inhibited.

In conclusion, the discovery and investigation of the ''eruption of heat'' is significant in the sense that it reveals the physiological effect and highlights the potential applications of rare earth elements in agriculture and in biological fields.

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