Microcalorimetric Studies of the Toxic Action of La^{3+} on *Halobacterium Halobium* R1 Growth

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A microcalorimetric technique was used to evaluate the influence of La^{3+} on *Halobacterium halobium* R1 growth. By means of LKB-2277 bioactivity monitor, ampoule method at 37 ℃, the thermogenic curves of *Halobacterium halobium* R1 growth were obtained. In order to analyze the results, the maximum power P_m and the growth rate constants k were determined, showing that values of P_m and k are linked to the concentration of La^{3+}. Addition of low concentration of La^{3+} can cause a decrease of the maximum heat production and growth rate constant. However, high concentration of La^{3+} may promote growth of *Halobacterium halobium* R1, but at much higher concentration of La^{3+}, the growth of *Halobacterium halobium* R1 is inhibited again. For comparison, the shapes of *Halobacterium halobium* R1 cell were observed by means of transmission electron microscope. According to the thermogenic curves and TEM photos of *Halobacterium halobium* R1 under different conditions, it is clear that metabolic mechanism of *Halobacterium halobium* R1 growth is changed with the addition of La^{3+}.

Keywords *Halobacterium halobium* R1, microcalorimetry, thermokinetics, metabolism, La^{3+}

Introduction

The rare-earth elements are characterized with their physiological functions and biological effects on some organisms. Under the normal environment, it has been reported that appropriate concentration of their ions can stimulate the growth and development of some plants.[1] However, there is no report concerning biological effects of rare-element on organism in the extreme environment, such as temperature, pH values and salt concentration.

Many environments, which are considered as the extreme, are colonized by some microorganisms. These microorganisms not only survive but also grow actively under such conditions. According to their respective original habitats, four parameters are commonly used to classify these so-called "extremophiles": (1) high and low temperatures, defining thermophiles and psychrophiles, respectively; (2) high and low pH values, defining alkaliphiles and acidophiles, respectively; (3) high salt concentrations, defining halophiles; (4) high pressure, defining barophiles.[2] Extremophiles have unique genome type, special physiological mechanism and particular metabolic production. They give new enlightenment in the origin of life phylogeny, biodiversity and open up a new concept of life, also give a new chance to develop biotechniques.[3] In recent years, extremophiles have become an interesting field of science research. Halobacterium halobium, which is a species of extremely halophilic bacteria, thrives at the concentration of 4—5 mol/L. They are classified as archaebacteria because they are similar to archae-organism in cell walls, components of membranes, rRNA, tRNA and so on. They are gram-negative bacteria as *Escherichia coli*.[4-6] In this paper, *Halobacterium halobium* R1 is used as "test animal" to evaluate physiological effects of rare-element (La^{3+}) on the microorganism which can colonize the extreme environment.

Microcalorimetry is a non-destructive and non-invasive technique. Therefore, it is valuable for monitoring a variety! of processes, such as metabolism of microorganism. The thermogenic curves of the metabolism of *Halobacterium halobium* R1 and the effect of La^{3+} on it were studied by using LKB-2277 Bioactivity Monitor. Compared to the metabolic thermogenic curves, we obtained some interesting results. All the experimental results are very important and significant to the study of the bioeffect of rare-earth element.

Experimental

Materials

Halobacterium halobium R1 was provided by the Chinese Center for Type Culture Collections, Wuhan Universi-

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The La_2O_3 has been roasted at 550 ℃ for 4 h and weighted 1 g. Then it was changed into $LaCl_3$ with 0.1 mol/L HCl, and made into 1 L solution with sterile deionized water. So, the concentration was 3.07×10^{-3} mol/L.

Halobacterium halobium R1 grew on a Halo-8 medium. Halo-8 medium consists of NaCl (250 g), $MgCl_2 \cdot 6H_2O$ (30 g), K_2SO_4 (2 g), yeast extract (2 g), lactalbumin hydrolysate (2.5 g) per liter at natural pH. The medium was sterilized by autoclaving for 30 min at 120 ℃.

Epuipment

An LKB-2277 Bioactivity Monitor (Thermometric AB, Sweden) was used, the performance of this and the details of its construction have been described previously.[7-9]

Initially, *Halobacterium halobium* R1 was inoculated in the prepared Halo-8 culture medium, which was put into the ampoule at once. The metabolic thermogenic curves of mitochondria were recorded using ampoule method. One sealed ampoule contained a reference solution such as the cultural medium, and the other ampoule contained the sample. The sample normally occupied position A in the monitor and reference occupied position. All calorimetric experiments were conducted at 37 ℃.

Electron microscopic observation was carried out under H-8100 transmission electron microscope. The detailed procedures were basically similar to those described by the Ref. 10.

Calculation of the growth rate constant of Halobacterium halobium

In the log phase of growth, the cell growth is exponential. If the cell number is n_0 at time 0, and n_t at time t, then

$$n_t = n_0 \exp(kt) \tag{1}$$

k is the growth rate constant. If the power output of each cell is w, then

$$n_t w = n_0 w \exp(kt) \tag{2}$$

$$P_0 = n_0 w \quad P_t = n_t w$$

$$P_t = P_0 \exp(kt) \text{ or } \ln P_t = \ln P_0 + kt \tag{3}$$

The growth thermogenic curves of the log phase correspond to Eq. (3). At the same time, in accordance with the data $\ln P_t$ and t taken from the curves to fit a linear equation, we can obtain the growth rate constant k. Thermokinetics equation and parameter were shown in Tables 1 and 2, respectively.

Results

Thermogenic curves

The growth thermogenic curve of *Halobacterium halobium* R1 in Halo-8 medium at 37 ℃ is shown in Fig. 1, and the thermogenic curves of *Halobacterium halobium* R1 growth affected by La^{3+} are shown in Fig. 2, respectively.

Table 1 Kinetic data of *Halobacterium halobium* R1 growth

c (La^{3+}, μg/mL)	Kinetic equation	k (min^{-1})	R
0	$\ln P_t = 1.44239 + 9.4656 \times 10^{-4} t$	9.466×10^{-4}	0.995
8.55	$\ln P_t = 2.17873 + 7.8203 \times 10^{-4} t$	7.820×10^{-4}	0.998
17.1	$\ln P_t = 5.97132 + 5.1305 \times 10^{-4} t$	5.131×10^{-4}	0.995
34.2	$\ln P_t = 7.11027 + 2.9380 \times 10^{-4} t$	2.938×10^{-4}	0.996
51.3	$\ln P_t = -0.59011 + 1.4100 \times 10^{-3} t$	1.410×10^{-3}	0.995
68.4	$\ln P_t = 2.38016 + 7.2367 \times 10^{-4} t$	7.237×10^{-4}	0.996
94.05	$\ln P_t = 3.95456 + 2.7363 \times 10^{-4} t$	2.736×10^{-4}	0.978
128.25	—	0	—

Table 2 Values of c (La^{3+}), k, P_m and Q

c (La^{3+}, μg/mL)	k (min^{-1})	P_m (μW)	Q (J)	c (La^{3+}, μg/mL)	k (min^{-1})	P_m (μW)	Q (J)
0	9.466×10^{-4}	239	33.41	51.3	1.410×10^{-3}	214	26.92
8.55	7.820×10^{-4}	188	23.15	68.4	7.237×10^{-4}	233	39.17
17.1	5.131×10^{-4}	188	21.99	94.05	2.736×10^{-4}	182	46.09
34.2	2.938×10^{-4}	150	26.17	128.25	0	20	5.74

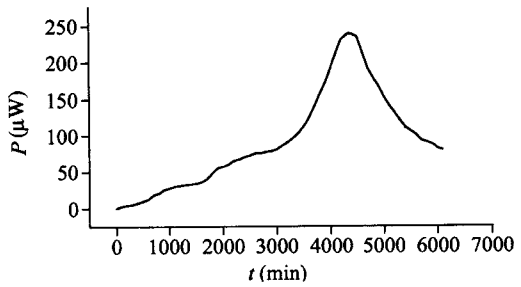

Fig. 1 Metabolic thermogenic curve of *Halobacterium halobium* growth.

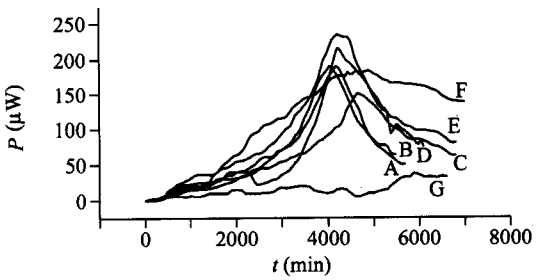

Fig. 2 Metabolic thermogenic curves of *Halobacterium halobium* growth. Effected by La^{3+}: A, 8.55 μg/mL; B, 17.1 μg/mL; C, 34.2 μg/mL; D, 51.3 μg/mL; E, 68.4 μg/mL; F, 94.05 μg/mL; G, 128.25 μg/mL.

Relationship between k and concentration of La^{3+}

Low concentration of La^{3+} (8.55—34.2 μg/mL) causes decreasing of growth rate constant k. Furthermore, values of k are correlated to the concentration of La^{3+}, c, as

$$k = 9.24594 \times 10^{-4} - 1.9431 \times 10^{-5} c \quad (R = -0.98293) \qquad (4)$$

The linear relationship is shown in Fig. 3(a)

However, La^{3+} of concentration of 51.3 μg/mL results in increasing of growth rate constant k. When concentration of La^{3+} goes on increasing, growth rate constant declines promptly. And values of k are correlated to the concentration of La^{3+}, c, as

$$k = 0.00857 - 0.00152 \ln c \quad (R = -0.97749) \qquad (5)$$

The linear relationship is shown in Fig. 3(b).

Relationship of P_m *and c*

From Table 2, when La^{3+} in low concentration range (0—34.2 μg/mL), the maximum heat power of growth phase, P_m, decreased with the increasing of La^{3+} concentration. However, when La^{3+} in high concentration range (34.2—68.4 μg/mL), the maximum heat power increased as La^{3+} concentration increase. The relationship between P_m and c (La^{3+}) is not linear, which is shown in Fig. 4.

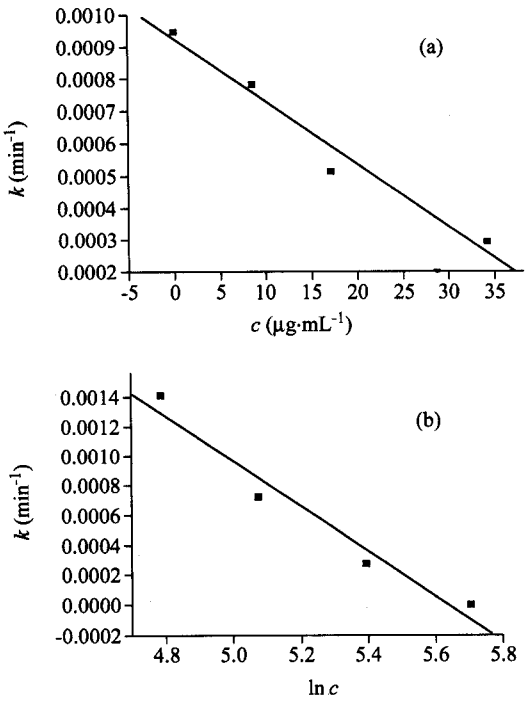

Fig. 3 Relationship between k and the concentration of La^{3+}. (a) (0—34.2 μg/mL); (b) (51.3—128.25 μg/mL).

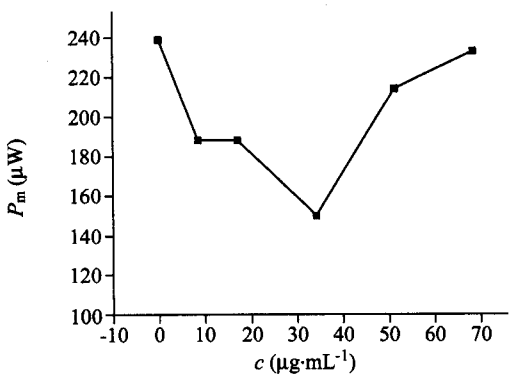

Fig. 4 Relationship between P_m and the concentration of La^{3+} (0—68.4 μg/mL).

Relationship between the total heat output (Q) and c

The total heat output is an important parameter as to metabolism of microbes, because it represents the ability of microbes to grow under particular condition. The relationship between the total heat output and concentration of La^{3+} is not linear, which is shown in Fig. 5 .

Electron microscopic observation

Electron microscopic method was used to monitor the growth of *Halobacterium halobium* R1 in the presence of La^{3+}. Fig. 6 displays the shapes of *Halobacterium halobium* R1 growth with addition of La^{3+} with different concentration.

The *Halobacterium halobium* R1 permeability was changed in the presence of La^{3+}, which cause La^{3+} to get across cell membrane. Inside the *Halobacterium halobium*

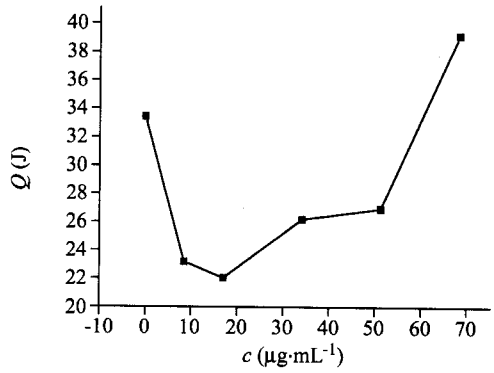

Fig. 5 Relationship between the total heat output (Q) and c (0—68.4 μg/mL).

R1 cell, appropriate amount of La^{3+} can enhance the activity of enzyme, inappropriate amount of La^{3+} would inhibit the growth of cell. Besides, La^{3+} can affect the synthesis of protein, which is one of the reasons why cell shape was changed.

Discussion

Comparing Fig. 1 with Fig. 2, it can be seen that the growth curves differ from each other when different amount of La^{3+} were added. Clearly, La^{3+} has taken part in the metabolism of *Halobacterium halobium* R1 growth. From the growth curves under different conditions, to some degrees, the metabolic mechanism of microorganism can be proved.

According to values of k, P_m and Q in Table 2, La^{3+} of low concentration (0—34.2 μg/mL) may have inhibitory effects on *Halobacterium halobium* R1; however, La^{3+} of high concentration (34.2—68.4 μg/mL) can promote growth of the bacteria; and in the presence of much higher concentration (68.4—128.25 μg/mL) of La^{3+}, the growth of *Halobacterium halobium* R1 is inhibited again. At the cellular level, many studies suggest that ion of rare-earths can activate or inhibit enzymes, and bioeffect of them lie on their concentration.[11] As for the enzyme in *Halobacterium halobium* R1 cell, may be appropriate concentration of rare-earths ion has stimulatory action on it, while lower or higher concentration will inhibit activity of enzyme. As a result, we obtained the above experimental result from the growth thermogenic curves of *Halobacterium halobium* R1 in the presence of La^{3+}. In previous research, some authors found that in nomal environment rare-element ions of low cencentration can stimulate microorganism's growth, while ion of high concentration has inhibitory effects on microorganism.[12] Clearly, biological effect of La^{3+} on microorganism in the extreme environment is different from that in normal environment because microorganism growing in the extreme environment has the unique method to adapt themselves to the environment.

According to Fig. 6(1), the shapes of *Halobacterium halobium* R1 are normal and they spread equably and densely without presence of La^{3+}. When La^{3+} (34.2 μg/mL) is added, their shapes have a little change and amount of them decreases, which is shown in Fig. 6(2). Fig. 6(3) displays the *Halobacterium halobium* R1 growing under La^{3+} of 51.3 μg/mL. Obviously, the shapes of *Halobacterium halobium* R 1 changed greatly and they

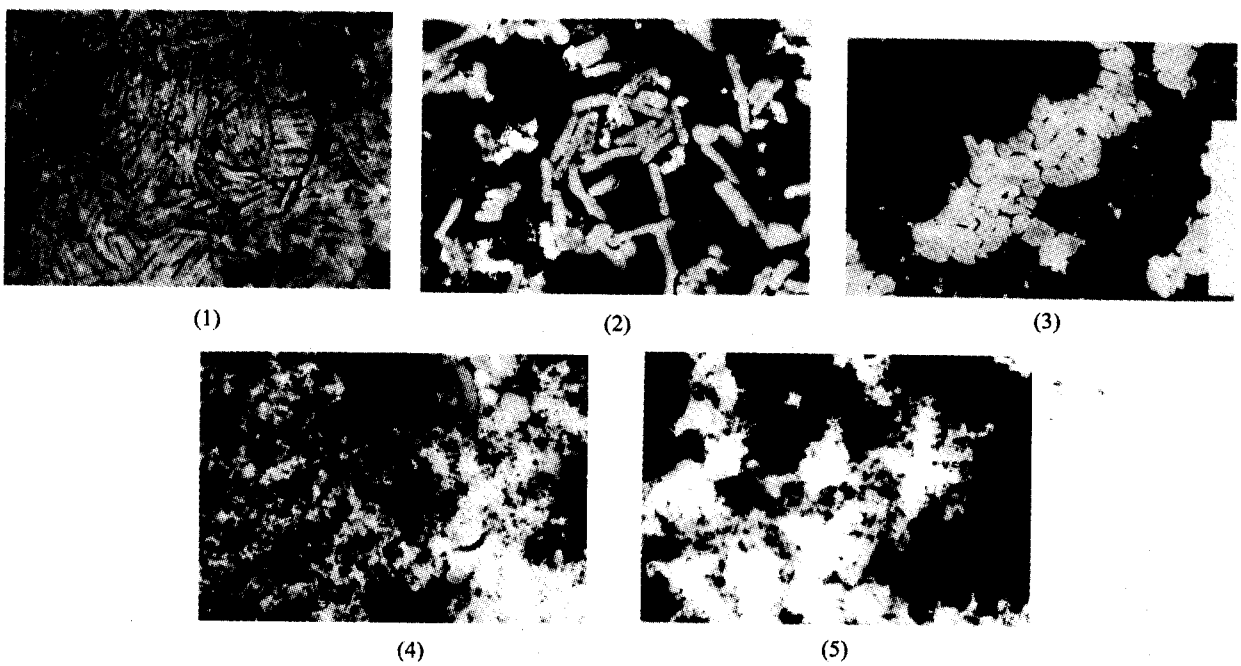

Fig. 6 Electron micrographs of *Halobacterium halobium* in the presence of La^{3+}. (1) 0; (2) 34.2; (3) 51.3; (4) 94.05; (5) 128.25 μg/mL.

congregated thickly, but amount of them seem to have increased. There is no complete thallus in Fig. 6(4) and Fig. 6(5), because *Halobacterium halobium* R1 falls into fragments in presence of La^{3+} of 94.05 or 128.25 μg/mL. To sum up, the result is identical to what we conclude from microcalorimetric method. Accordingly, the conclusion drawn from microcalorimetry proved to be right and accurate.

Due to the high sensitivity of the monitor and the fact that the whole metabolism of samples may be examined automatically and continuously, microcalorimetric method may reveal more and newer details about the metabolism than the existing methods do.[13] The microcalorimetric method requires only an observable difference between the power production in the treated and controlled incubations. Unlike many other procedures, transparent solution is not required. Colored or turbid solutions, even suspensions can be put into the calorimeter.[14] That approximates more closely the vivo state than many other techniques do. The microcalorimetric method can be used in many areas of biological sciences. Through the technique and other method, the kinetics and thermodynamics of biological sciences can be further studied, and all of these are very significant to understand biological processes.

References

1 Wang, K. *Trace Elements in Life Sciences*, Chinese Computation Science Press, Beijing, **1992**, p. 260 (in Chinese).
2 Van der Oost, J.; De Vos, W. M.; Antranikian, G. *Trends Biotechnol.* **1996**, *14*, 415.
3 Schaefer, G. *J. Bioenerg. Biomembr.* **1992**, *24*, 525.
4 Woese, C. R.; Kandler, O.; Wheelis, M. L. *Proc. Natl. Acad. Sci.* **1990**, *87*, 4576.
5 Garrett, R. A. *Nature* **1985**, *318*, 233.
6 Lake, J. A. *Proc. Natl. Acad. Sci.* **1985**, *82*, 3716.
7 Suurkuusk, J.; Wadso, I. *Chem. Scr.* **1982**, *20*, 155.
8 Xie, C. L.; Tang, H. K.; Song, Z. H.; Qu, S. S. *Thermochemi. Acta* **1988**, *123*, 33.
9 Yan, C. N.; Liu, Y.; Qu, S. S.; Shen, P. *Chemosphere* **1999**, *38*, 891.
10 Shen, P.; Fan, X. R.; Li, G. W. *Laboratory Experiments in Microbiology*, 3rd edn, High Education Press, Beijing, **1999**, pp. 52—63 (in Chinese).
11 Yi, X. W.; Huang, C. H. *Rare-earth Element*, Science Press, **1998**, pp. 266—267 (in Chinese).
12 Zhao, R. M.; Liu, Y.; Qu, S. S. *J. Biochem. Biophysi. Methods* **2000**, *46*, 1.
13 McGulnness, M. S.; Barisas, B. G. *Environ. Sci. Technol.* **1991**, *25*, 1092.
14 Wadso, I. *Chem. Soc. Rev.* **1997**, 79.

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