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EFFECTS OF YTTERBIUM ION ON THE GROWTH, METABOLISM AND MEMBRANE FLUIDITY OF *TETRAHYMENA THERMOPHILA*

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Power–time curves and metabolic properties of *Tetrahymena thermophila* BF_5 exposed to different Yb^{3+} levels were studied by ampoule method of isothermal calorimetry at 28°C. Metabolic rate (*r*) decreased significantly while peak time (PT) increased with the increase of Yb^{3+}. These results were mainly due to the inhibition of cell growth, which corresponded to the decrease of cell number obtained by cell counting. Compared with cell counting, calorimetry was sensible, easy to use and convenient for monitoring the toxic effects of Yb^{3+} on cells and freshwater ecosystem. It was also found that cell membrane fluidity decreased significantly under the effects of Yb^{3+}, which indicated that Yb^{3+} could be membrane active molecules with its effect on cell membranes as fundamental aspect of its toxicity.

Keywords: *cell growth, isothermal calorimetry, membrane fluidity, metabolism, Tetrahymena thermophila* BF_5, Yb^{3+}

Introduction

China has the richest rare-earth element resources. And the rare-earth element fertility has been used widely in China. So more and more rare-earth elements have entered the environment and then got into biological body through food chain. Recently, growing concern has been expressed about their possible effects on the environment and potential threats to human health [1]. Therefore, it is urgent for us to study their biological function, predict their impacts on the living organisms and further investigate the mechanism of toxic effects.

Tetrahymena species belong to ciliated protozoa. They distribute widely and perform key functions in energy flow and elementary cycling in freshwater ecosystem. Their ubiquitous distribution and ecological significance place them at the front rank of ideal early-warning indicators of aquatic ecosystem deterioration. They are eukaryotic unicellular organisms, which makes them sensible to the pollutants. And they can grow rapidly and easily in axenic condition [2, 3]. Therefore, *Tetrahymena* well suit for toxicant screening studies in environmental fields and have been used to detect water quality [4]. There have been a few studies about the effects of rare-earth elements on the cell growth, nucleus and conjunction of *Tetrahymena* [5–7]. However, their effects on the metabolism of *Tetrahymena* have never been reported.

After a few decades of calorimetric investigation, biological calorimetry is attracting more attention. Calorimetry has demonstrated its power as a universal, integral, non-destructive, good reproducibility and highly sensitive tool for detecting the overall metabolism of the whole living system [8, 9]. The effects of rare earth ions on the metabolism of prokaryote cells and mitochondria of animals have been studied by calorimetry [10–12]. And calorimetric measurements enable a rapid and accurate determination of toxic effects against *Tetrahymena* [13]. In this paper, the power-time curves of *Tetrahymena thermophila* BF_5 exposed to different ytterbium ion levels were studied by ampoule method of calorimetry at 28°C. Furthermore, in order to understand further its toxic effects, cell density and cell membrane fluidity of *T. thermophila* BF_5 were also investigated under the stress of ytterbium ion.

Experimental

Species and culture medium

Tetrahymena thermophila BF_5 was provided by East China Normal University. The cells were cultured at 28°C in a liquid medium containing 2% (*w*/*v*) proteose peptone (Oxoid), 0.1% yeast extract (Oxoid) and 0.5 mM $FeCl_3$.

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Calorimetric measurements

The calorimeter is an eight-channel TAM Air isothermal heat conduction calorimeter 3114/3236 (Thermometric AB, Sweden). The calorimetric channels are in a single removable block contained in an air thermostat that keeps the temperature within ±0.02°C. Each channel consists of a sample and a reference vessel. The limit of detection is 2 μW and the baseline deviation over 24 h is ±5 μW. All the calorimetric measurements were performed in 20 cm^3 glass ampoules at 28°C.

The stationary-stage *Tetrahymena* cells were counted at first. Then appropriate amount of cell suspension, $Yb(NO_3)_3$ solution and sterilized culture medium were added into the sterilized ampoules. Cell suspensions were adjusted to 1000 cells mL^{-1} with a volume of 5 mL. The final concentration of Yb^{3+} is 0, 3.33, 10, 30, 50, 75, 100, 125 and 150 mg L^{-1} in different experimental groups, respectively. Then, the sample and reference ampoules were hermetically sealed and put into the different channels. Finally, the power-time curves of *T. thermophila* at 28°C were recorded every minute by use of the Picolog software supplied with TAM Air. The measurement of each power-time curve was repeated twice or three times. After the calorimetric measurements, cell numbers in sample ampoules were counted by Leitz microscope.

Cell membrane fluidity determination

The stationary-stage *Tetrahymena* cells were prepared for cell membrane fluidity determination. Fluorescence probe was 1,6-diphenyl-1,3,5-hexatriene (Sigma, USA). Fluorescence depolarization method was used to measure values of fluorescence anisotropy by Perkin-Elmer LS 55 luminescence spectrometer [14]. After the fluorescence depolarization value of the control cells were measured, appropriate Yb^{3+} solution was adding into the control cells suspension. Then, the fluorescence depolarization value of cells under the stress of Yb^{3+} was also measured immediately.

Statistical analyses

The data are given as the arithmetic mean ±standard derivation. The one-way ANOVA statistical method was used to assess the significance of differences in measured parameters among the experimental groups at $P \leq 0.05$. Correlations among parameters were also analyzed at $P \leq 0.05$.

Results and discussion

Power-time curves and metabolic properties of Tetrahymena exposed to Yb^{3+}

Calorimetry has proven a useful tool for measuring the energy. The advantage of calorimetry is that it measures the total energy flow. The environmental changes, especially pollutants of different biological toxicity to be distributed to the environment, can influence the living activities of organisms. Therefore, they certainly cause the changes of the heat produced by metabolism, which can be easily detected by calorimetry [15]. The power-time curves of *T. thermophila* BF_5 exposed to different Yb^{3+} levels at 28°C were shown in Fig. 1. From the power-time curves, it was apparent that the metabolism processes of cells had been changed by adding into different concentrations of Yb^{3+}. Generally, the population growth curve accords with logistic model. Therefore, as for the power-time curves, the metabolism kinetic characteristics were also simulated by the classical logistic model [16]:

$$\ln\left(\frac{P_{max}}{P_t}-1\right)=\alpha-rt$$

where P_t is the power output at time t, r is the metabolic rate, P_{max} is the potential maximum power output, that is to say, the power output when *Tetrahymena*'s number gets to environmental carrying capacity (maximum number in specific environment). α is a constant which stands for the orientation of logistic curves relative to origin.

From Table 1, it was apparent that all of the correlation coefficients, R, were greater than 0.99, indicating a good correlation relationship and reproducibility. The values of metabolic properties were revealed in Table 2. The data showed that there was no significant difference in Q_T. Under the high concentration Yb^{3+}, there were significant decreases for P_{max} and P_m but significant increases for Q_{log}. Metabolic rate, r, showed a significant decrease and PT showed a significant increase with the increase of Yb^{3+} concentration even under the stress of 3.33 mg L^{-1} Yb^{3+}. The values of r and PT showed that Yb^{3+} delayed the growth metabolism in the log phase, which caused the decrease of metabolic rate. From Fig. 2, it was also found that r and PT had both significant correlations with the concentrations of Yb^{3+}. Then according to the inhibitory ratio of r and increase ratio od PT, the half inhibition concentrations (IC_{50}) of Yb^{3+} were calculated as about 126 and 90 mg L^{-1}, respectively. Previous reports about the effects of rare earth elements showed that at low concentrations, they could stimulate the proliferation of

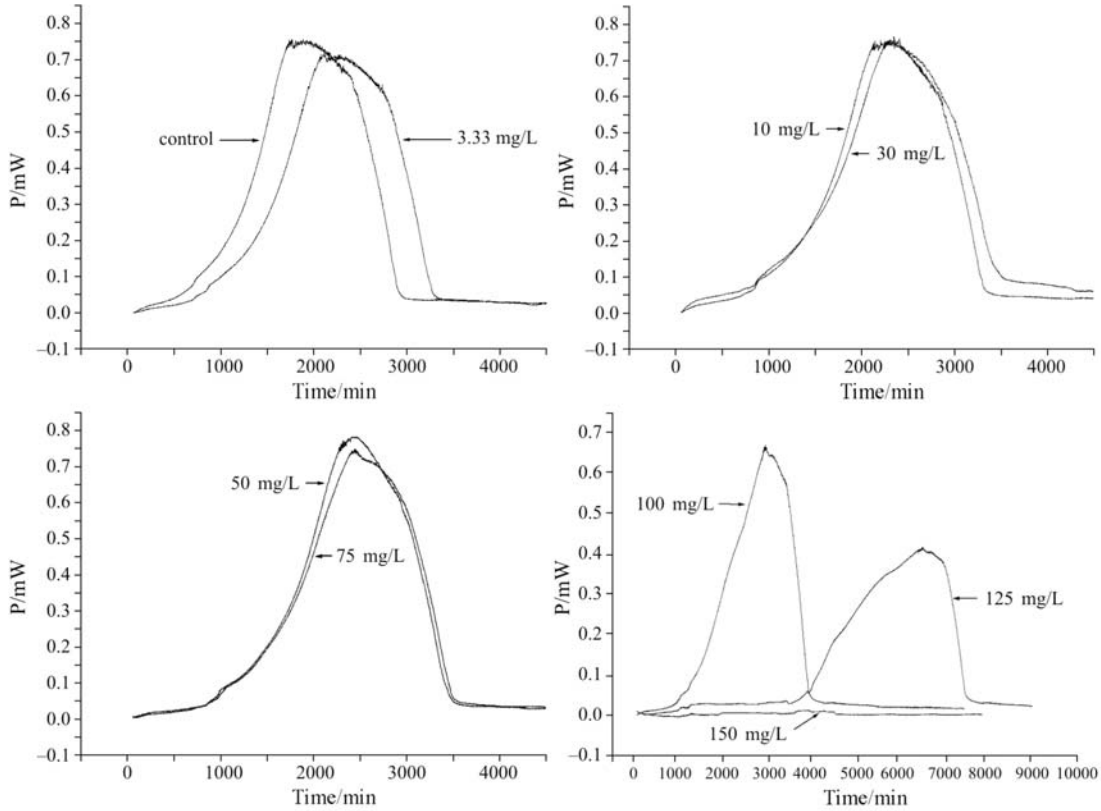

Fig. 1 Power-time curves of *Tetrahymena thermophila* BF_5 under the effects Yb^{3+} at 28°C

Table 1 Logistic equations and coefficients of *Tetrahymena thermophila* BF_5 at 28°C

Sample	Logistic equation	Coefficient
Control 1	In $(0.90/P_t-1)=4.97-0.00325t$	0.999
Control 2	In $(1.03/P_t-1)=4.98-0.00334t$	0.997
Control 3	In $(0.97/P_t-1)=4.32-0.00335t$	0.999

Table 2 Metabolic parameter values[a] of *T. thermophila* BF_5 under the effects of Yb^{3+}

Yb^{3+}/mg L^{-1}	$r/10^{-3}$ min^{-1}	P_{max}/mW	Q_T/J	Q_{log}/J	P_m/mW	PT/min
0	3.31±0.05	0.97±0.07	63.81±6.81	25.42±3.17	0.72±0.05	1795±123
3.33	2.62±0.02*	1.19±0.18	63.03±7.76	24.82±0.91	0.68±0.06	2286±240*
10	2.69±0.03*	1.07±0.02	67.26±10.01	29.32±6.50	0.71±0.06	2378±89*
30	2.19±0.00*	1.06±0.16	73.40±13.77	32.10±2.65	0.70±0.10	2558±292*
50	2.19±0.14*	1.52±0.33*	67.57±3.26	30.38±4.75	0.73±0.08	2608±202*
75	2.20±0.14*	1.30±0.07	67.78±2.08	31.23±0.42	0.73±0.03	2456±4*
100	2.39±0.04*	0.66±0.06	62.78±10.46	37.54±0.04*	0.62±0.08	3374±570*
125	1.80±0.06*	0.50±0.11*	65.53±7.31	44.38±4.66*	0.47±0.07*	6176±494*
150	0*	0*	0*	0*	0*	0*

[a]P_{max} is potential maximum power output, r is growth rate, Q_T is total heat, Q_{log} is total heat in the increasing period, and P_m is measured maximum power output. PT is the time when the power output is P_m. The values are given as mean ±S.D. (n=2–3). The values marked with '*' are significant at $p<0.05$ compared with control group

Tetrahymena, while at the certain high concentrations, they could inhibit the cell growth [5, 6]. In the present study, it was surprising that no stimulation but inhibition was found even at the lowest concentration Yb^{3+}.

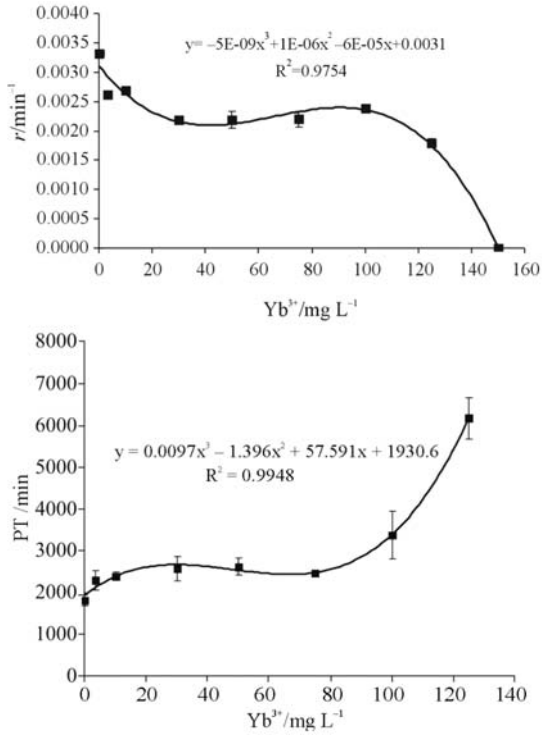

Fig. 2 Correlation between metabolic rate (*r*), peak time (PT) and Yb^{3+} concentration

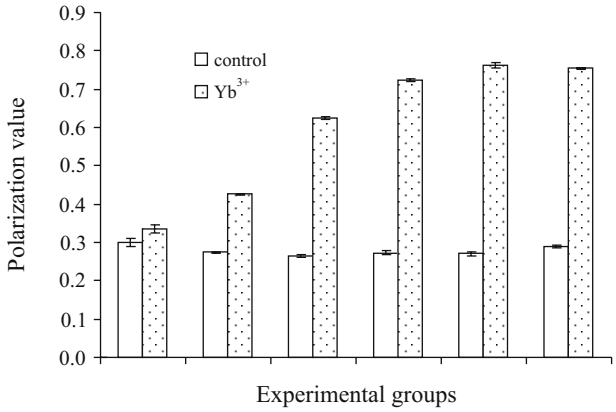

Fig. 3 Fluorescence polarization values of cells exposed to different Yb^{3+} levels (3.33, 10, 30, 50, 100, 150 mg L^{-1} from left to right groups, *n*=10)

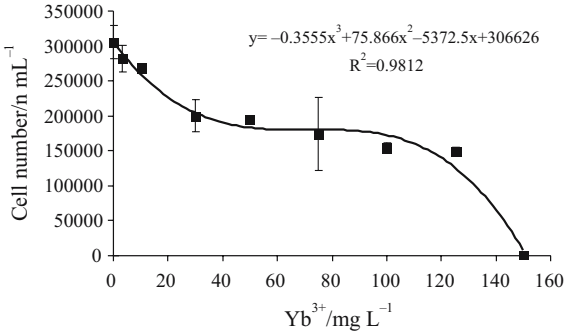

Fig. 4 Correlation between cell density and Yb^{3+} concentration (*n*=2)

Membrane fluidity and cell number of Tetrahymena exposed to Yb^{3+}

Membrane fluidity is an important physical character of cell membrane. Many cell functions, including energy transformation, nutrients transportation and transferring signals, are all tightly relevant with cell membrane fluidity. Therefore, stability of membrane fluidity plays an important role in keeping normal cell functions and resisting various environmental stresses [17]. In cells, DPH is distributed within the hydrophobic region of lipid membranes [18] and DPH polarization reflects the average fluidity of all cellular membrane lipids [19]. And an inverse relationship exists between membrane fluidity and polarization. In our study, TBT caused an increase in the fluorescence polarization of DPH, reflecting a significant decrease in membrane fluidity of *Tetrahymena*, which had also been confirmed in red blood cell [20]. Rare earth ions mainly react with lecithoid group. The reaction between rare earth ions and lecithoid polarity head group caused that the alignment of lecithoid molecular was closer, the sport of the fat chain were restricted and the rigidity of the whole membrane increased [21, 22]. All these facts indicated that rare earth ions could be membrane active molecules with its effect on cell membranes as fundamental aspect of its toxicity.

When Yb^{3+} concentration was 30–150 mg L^{-1}, cell number decreased significantly, which reflected that cell growth was inhibited by Yb^{3+}. This fact accorded with the inhibition of metabolic heat. Cell number had significant correlation with the Yb^{3+} concentration, which was shown in Fig. 4. And it was found that the figure of effects of ions on cell number was similar to that of inhibition effects on metabolic rate. They are both cubic polynominal equations. The half inhibition concentration (IC_{50}) of Yb^{3+} according to cell density was calculated as about 117 mg L^{-1}, which was similar with 126 mg L^{-1} by metabolic rate, but higher than 90 mg L^{-1} by peak time. Apparently, the inhibition of metabolic heat was mainly caused by the decrease of cell number. However, metabolic heat was affected by not only cell number but also every cell metabolic heat. Cell number obtained by counting only presented the cell number changes caused by toxic substances, not every cell physiological level. In addition to that, cell counting usually had relatively high experimental errors due to uneven sampling and counting some dead cells. In the present study, the lowest effective concentration of Yb^{3+} (3.33 mg L^{-1}) obtained by metabolic rate was much lower than that (30 mg L^{-1}) obtained by cell number, which suggested that calorimetry was more sensible than cell counting. Furthermore, because calorimetry could monitor metabolism of the living cells automatically

and each power depended on total cell number and metabolic level of every cell, the power-time curve was relatively accurate, easy to use, and could provide the complete information about the effects of the toxic substances on the cells. Therefore, calorimetry could be useful for monitoring the toxic effects of Yb^{3+} on cells and ecosystem.

Conclusions

The power-time curves of *T. thermophila* BF_5 had been changed when exposed to Yb^{3+}. Metabolic properties obtained by curves quantitatively showed the effects of Yb^{3+} on the metabolism. Metabolic rate and PT changed significantly even exposed to 3.33 mg L^{-1} Yb^{3+}, which suggested that Yb^{3+} could still inhibit the cell metabolism at the lower concentration. Cell number obtained by cell counting decreased with the increase of Yb^{3+}, which was consistent with the inhibition of metabolism. Compared with cell counting, calorimetric method was sensible, easy to use and convenient for monitoring the potential effects of Yb^{3+} on cell and freshwater ecosystem. Yb^{3+} could also reduce the membrane fluidity of *T. thermophila*. This fact suggested that it might be membrane active molecules with its effect on cell membranes as fundamental aspect of its toxicity.

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