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The adaptability of three Arctic microalgae to different low temperatures

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Abstract In order to study the adaptability of Arctic microalgae to different environmental temperatures, the growth curves and antioxidase system of three microalgae (*Skeletonema marinoi*, *Chlorella* sp. and *Chlamydomonas* sp.) that were separated from the Ny-Ålesund, the high Arctic, at different low temperatures (0 ℃, 4 ℃ and 8 ℃) were determined. The result showed that the adaptability of the microalgae to temperatures depended on the species. The growth rate, SOD and CAT activities of *Skeletonema marinoi* were the highest at 4 ℃, but MDA content was the lowest. The growth rate and enzyme activity of *Chlorella* sp. were the highest at 8 ℃, while the lowest MDA content presented at 0 ℃. The growth of *Chlamydomonas* sp. at the different temperatures was not so significant, the lowest MDA content presented at 8 ℃. The change of antioxidase system also depended on species and temperatures. Three indexes of antioxidase system of *Skeletonema marinoi* between 0 ℃ and 4 ℃ showed extremely significant difference ($p < 0.01$). SOD activity of *Skeletonema marinoi* and *Chlorella* sp. between 0 ℃ and 8 ℃ showed significant difference ($p < 0.05$), and the other two indexes of them differed insignificantly. Antioxidase systems of *Chlamydomonas* sp. at the three temperatures differed insignificantly. In conclusion, the three microalgae had good adaptability to the three temperatures; their MDA content presented a low level, and had unique physiological mechanism to adapt to the environment with different low temperatures.

Key words Arctic microalga, adaptability, MDA, SOD, CAT.

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1 Introduction

Low temperature is an important geographical feature in the polar regions, an-

nual mean temperature in Ny-Ålesund, Svalbard, is generally slightly above 0 ℃[1], the seawater temperature is about 5 ℃ in summer[2]. But as a result of global warming, the Arctic environment including the sea ice is experiencing remarkable changes[3,4], such as decreasing of the sea ice cover, thinning of the sea ice, increasing of water temperature and fresh water influx, and aggravating pollution. Those changes would have a profound influence on the Arctic marine ecosystems, especially the microalgae as the important primary productivity in the polar ecosystem[5]. Therefore, physiological adaptability and mechanism of microalgae to the polar environmental change become one of the hotspots of scientific research. At present, related studies are focused on the adaptive mechanism of the Antarctic algae to their habitats[6-9], and the study on antioxidase system (e. g. SOD, POD, CAT and MDA) is an important part of it[10,11].

Comparatively, studies on relations between light and the Arctic algae[12,13] are more than that on their adaptability to temperatures, which has regulating effect on the growth and development of algae; and has different influences on the enzyme activity, assimilation and utilization efficiency of nutrients and cell division cycle and many others[14]. In this study, we use three microalgae separated from Ny-Ålesund region, Svalbard (high Arctic), to analyze their growth characteristics and antioxidase system at different low temperatures (0 ℃, 4 ℃ and 8 ℃), and the relations of their growth and physiological metabolism features with the ambient temperature, to provide theoretical basis for discussing the adaptation mechanism of Arctic microalgae to low temperatures.

2 Material and methods

2.1 Materials and cultivation

The Arctic microalgae that were used in the experiment were *Skeletonema marinoi*, *Chlorella* sp. and *Chlamydomonas* sp.. The *Chlorella* sp. was a freshwater green algae species and separated from the glacial melting water in the Ny-Ålesund area in 2006, and *Skeletonema marinoi* and *Chlamydomonas* sp. were sea water species and separated from Kongsfjorden (Kings Bay) in Ny-Ålesund. They were preserved in the Polar Microalgae Pool of the Polar Research Institute of China. Triplicate cultures of each species were grown simultaneously, each in 3 L Erlenmeyer flasks containing 2 L of sterile f/2 (*Skeletonema marinoi* and *Chlamydomonas* sp.) or B (*Chlorella* sp.) culture medium with the inoculation quantity of 20%. The algae were grown at 0 ℃, 4 ℃, and 8 ℃, respectively, under a 12:12 light-dark cycle with a light intensity of 27～40 μmol · m^{-2} · s^{-1}. Flasks were shook 3 times a day. Sampling was made every other day for the determination of the growth curve, and samples were taken every 4 days to determine the antioxidase activity and MDA content.

2.2 Determination of the growth curve

Samples were collected every other day from the day when inoculation was made, the corresponding methods of determining biomass were selected depending on the morphological characteristics of the algae, and the mean value of the triplicate samples of each alga was taken to make its growth curve.

The *Skeletonema marinoi* is a chain-like cell colony, and its growth curve was determined by a spectrophotometric method. The algal solution was shook up, and then the absorbency value of the algal solution at 700 nm was determined by BECKMAN DU800 spectrophotometer with medium as the control sample.

The growth curve of *Chlorella* sp., a unit cell green alga, was determined by flow cytometry. 1 mL evenly mixed algal solution was taken respectively, and the cell density was quantitatively measured by BECKMAN COULTER at 488 nm and under the excitation of laser with 22 mw power, then the statistical analysis was conducted with Cell Lab Quanta SC.

The growth curve of *Chlamydomonas* sp., growing in a form of cell conglomeration, was measured with the method of dry weight. After shaken up, 50 mL of the algal solution was taken and filtered in the filter paper that was dried to constant weight, and the dry weight of the algae was obtained by precise weighing.

2.3 Determination of the antioxidase activity

Extraction of crude enzyme: 40 mL algal solution was taken from each sample after it was shaken up, the algal solution was centrifugated at a speed of 8 000 r·min^{-1} for 10 min in a low-temperature and high-speed centrifuge to collect the algae, and the supernatant was thrown away. The cells were resuspended by adding 4 mL phosphate buffer solution (0.1 mol·L^{-1}, pH=7.8), and disrupted with the ultrasonic cell disrupter system for 30 times with the disruption power of 400 W, disruption time interval of 2 s, and ice-bath processing. The disrupted liquid was stored under 4 ℃ environment for 1 h, then centrifugated with a refrigerated centrifuge (12 000 r·min^{-1}, 4 ℃) for 20 min. The exacted supernatant, as the crude enzyme, was preserved under 4 ℃ for further analysis.

The activity of superoxide dismutase (SOD) and catalase (CAT) was determined with BECKMAN DU800 spectrophotometer referring to the methods of xanthine oxidase and ultraviolet spectrophotometry that were improved by Zuo Xiangyu [15].

2.4 Determination of MDA content

The MDA content was determined with a BECKMAN DU 800 spectrophotometer according to the thiobarbituric acid method[16].

2.5 Data analyzing

Drawing was made with SPSS 13.0 and OriginPro 7.5 softwares, and the SPSS 13.0 software was used for the significance t-test.

3 Results

3.1 Growth curves of the three algal species at different temperatures

Figure 1a shows that *Skeletonema marinoi* had the growth cycle of more than 50 days. The growth rate was the highest at 4 ℃ with no lag-phase, and achieved the highest density value in the 48th day with the light absorption value of approximately 0.55 ℃. At 0 ℃ and 8 ℃, the lag-phase was 6 days, it achieved the highest density value (light absorption value was approximately 0.36 and 0.49) on the 40th and the 48th day, respectively.

Chlorella sp. had the growth cycle of approximately 40 days (Fig. 1b), it grew faster with the increase of temperature, the lag-phase at 0 ℃ was as long as 18 days, it entered the logarithmic phase from the 20th day, and achieved the highest density (2.85×10^6 cells · mL^{-1}) on the 28th day; at 4 ℃, the lag-phase lasted about 12 days, it achieved the highest density (3.3×10^6 cells · mL^{-1}) on the 34th day; at 8 ℃, the first 18 days were the lag-phase, it achieved the highest growth density (3.74×10^6 cells · mL^{-1}) on the 34th day.

Chlamydomonas sp. had 4 obvious growth phases, but the growth curves at different temperatures had no obvious difference (Fig. 1c).

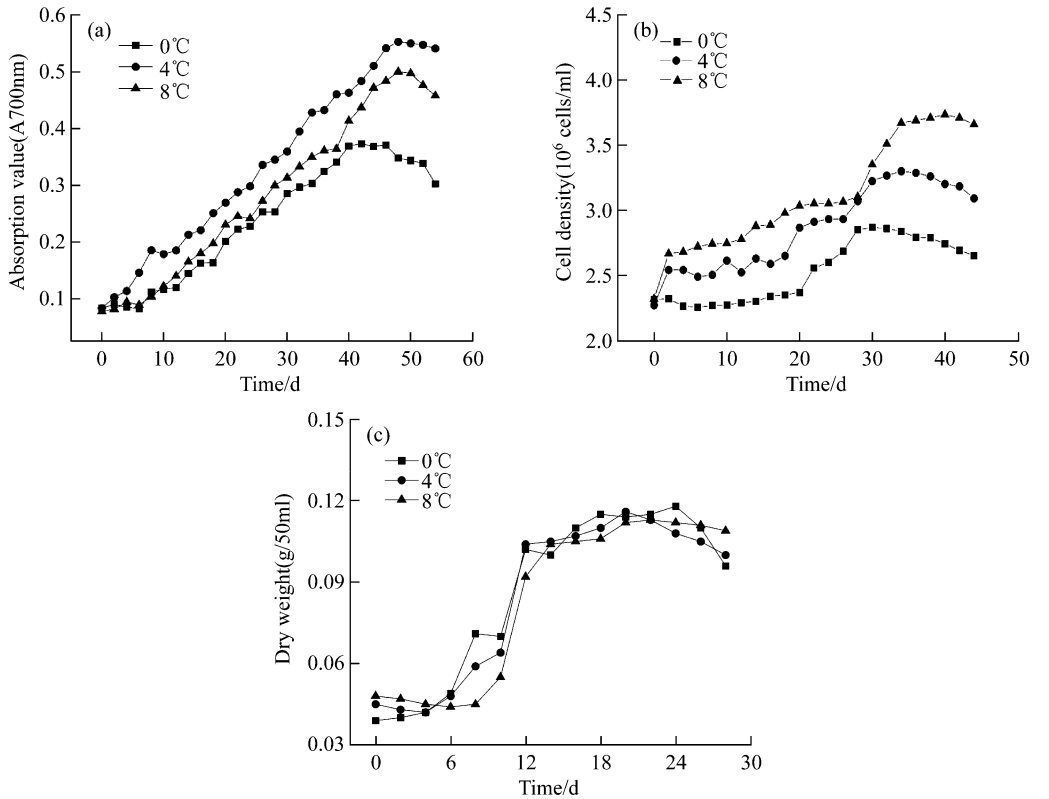

Fig. 1 The growth curves of the three algal species at different temperatures.
(a) *Skeletonema marinoi*; (b) *Chlorella* sp.; (c) *Chlamydomonas* sp.

At 0 ℃, 4 ℃ and 8 ℃, the first 2, 4 and 6 days were the lag period, respectively. It achieved the highest growth increment on the 24th, 20th and 22nd day, and the dry weight of 50 mL algal solution was 0.113, 0.116 and 0.118 g·(50 mL)$^{-1}$, respectively.

3.2 The MDA content of the three algae at different temperatures

In general, the MDA content of all three algae presented a changing trend of increase firstly and decreasing later with time (Fig. 2), but the MDA content of *Skeletonema marinoi* and *Chlamydomonas* sp. was low and did not change obviously. The MDA content of *Skeletonema marinoi* was the highest at 0 ℃ and the lowest at 4 ℃, besides, the MDA content both at 0 ℃ and 8 ℃ achieved the maximum value on the 40th day, and at 4 ℃ achieved the maximum value on the 36th day. The MDA content of *Chlorella* sp. was the highest at 8 ℃ and the lowest at 0 ℃. The maximum at different temperatures appeared on the 28th, the 32nd and the 28th day, respectively. The MDA content of *Chlamydomonas* sp. was the lowest, the content at the different temperatures were between 0.017～0.043 nmol·g^{-1}.

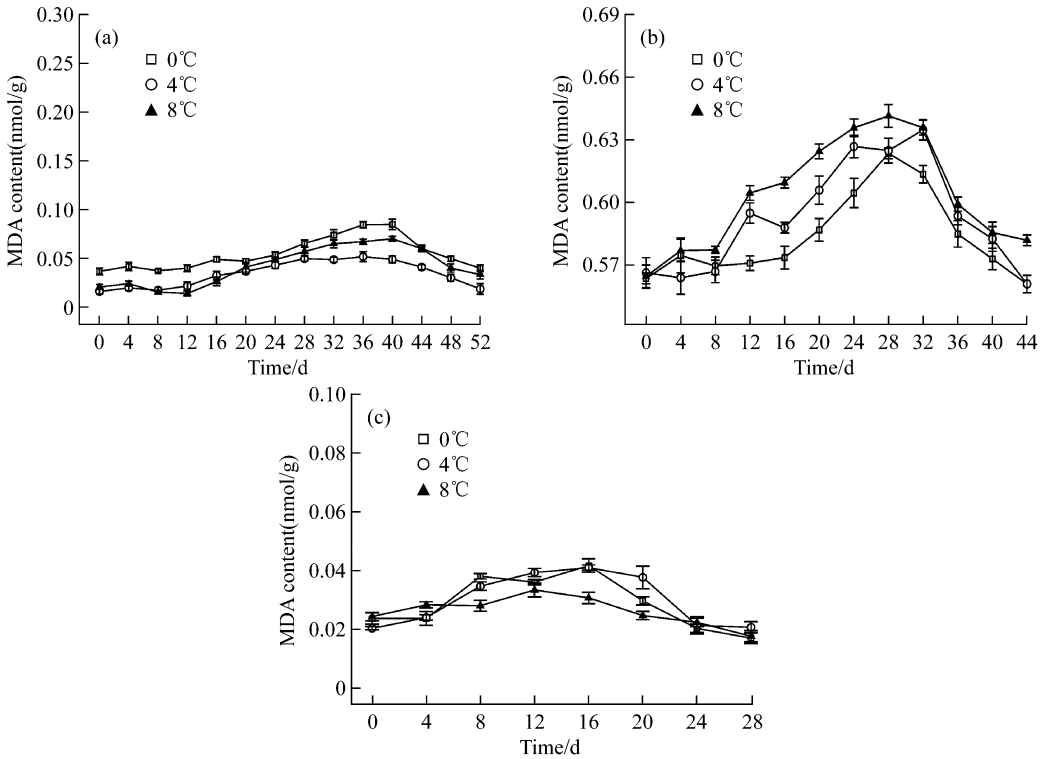

Fig. 2 The MDA contents of the three algal species at different temperatures.
(a) *Skeletonema marinoi*; (b) *Chlorella* sp.; (c) *Chlamydomonas* sp.

3.3 The SOD activity of the three algal species at different temperatures

The SOD activity of the three algae increased firstly and decreased later at all the three temperatures (Fig. 3). The SOD activity of *Skeletonema marinoi* exhibited the highest level on the 40th day, with 5.17, 8.91 and 6.79 U • g^{-1} at 0℃, 4℃ and 8 ℃, respectively. The activity exhibited the highest level at 4 ℃, while at 0 ℃, it was the lowest in each phase. The SOD activity of *Chlorella* sp. achieved the highest on the 16th day at all the three temperatures, with 2.82, 3.15 and 3.67 U • (10^6 cells)$^{-1}$, respectively. The enzyme activity decreased rapidly from the 16th to the 36th day, and during the last 8 days, the enzyme activity varied little at the three temperatures. In the anaphase of algae growth, the SOD activity declined with the decrease of the algae cell activity. The SOD activity of *Chlamydomonas* sp. at the three temperatures achieved the highest on the 20th, 24th and 16th days, with the values of 3.03, 5.07 and 3.65 U • g^{-1} at the three temperatures, respectively. On the first 20 days, the enzyme activity was the highest at 8 ℃ and the lowest at 4 ℃, and during the last 4 days, it declined at the fastest rate at 4 ℃.

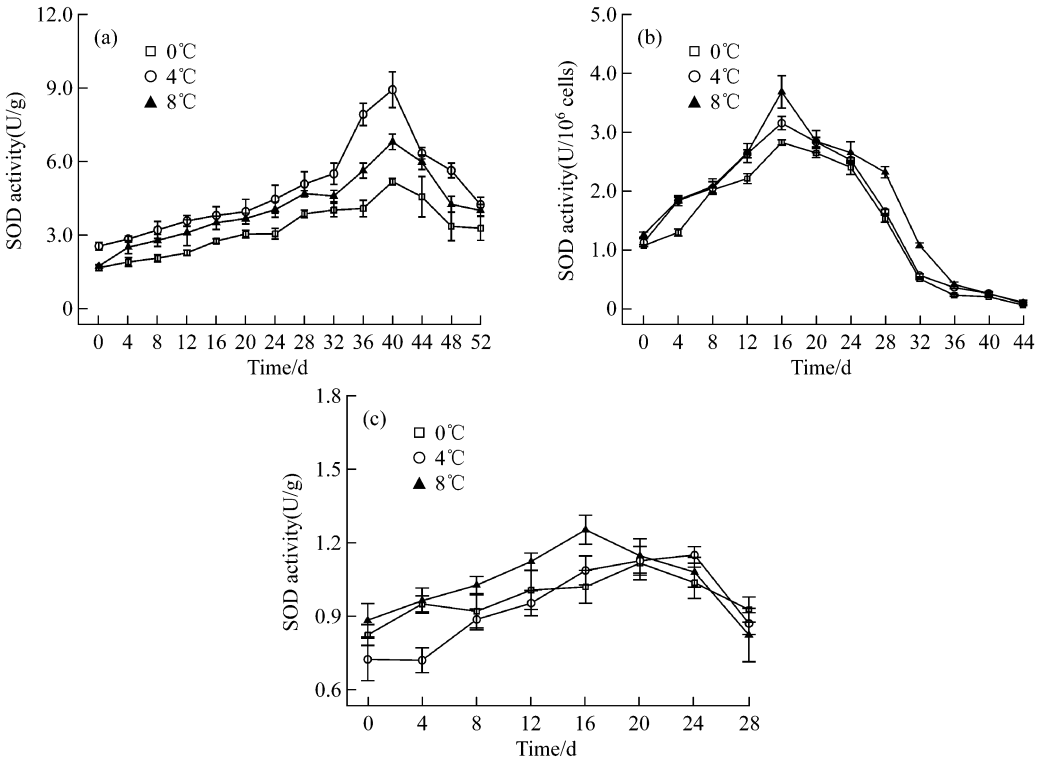

Fig. 3 The SOD activities of the three algal species at different temperatures.
(a) *Skeletonema marinoi*; (b) *Chlorella* sp.; (c) *Chlamydomonas* sp.

3.4 The CAT activity of the three algal species at different temperatures

The CAT activity of *Skeletonema marinoi* was shown in Figure 4a. Comparatively, within the entire growth cycle, the enzyme activity was the highest at 4 ℃ and the lowest at 0 ℃. The change of the enzyme activity of *Chlorella* sp. at the

three temperatures were not obvious(Fig. 4b), but the highest level of the enzyme activity appeared at 8 ℃, while that of *Chlamydomonas* sp. at the three temperatures were low, which only presented the change law of high first and low later at 8 ℃, and its highest level appeared at this temperature (Fig. 4c).

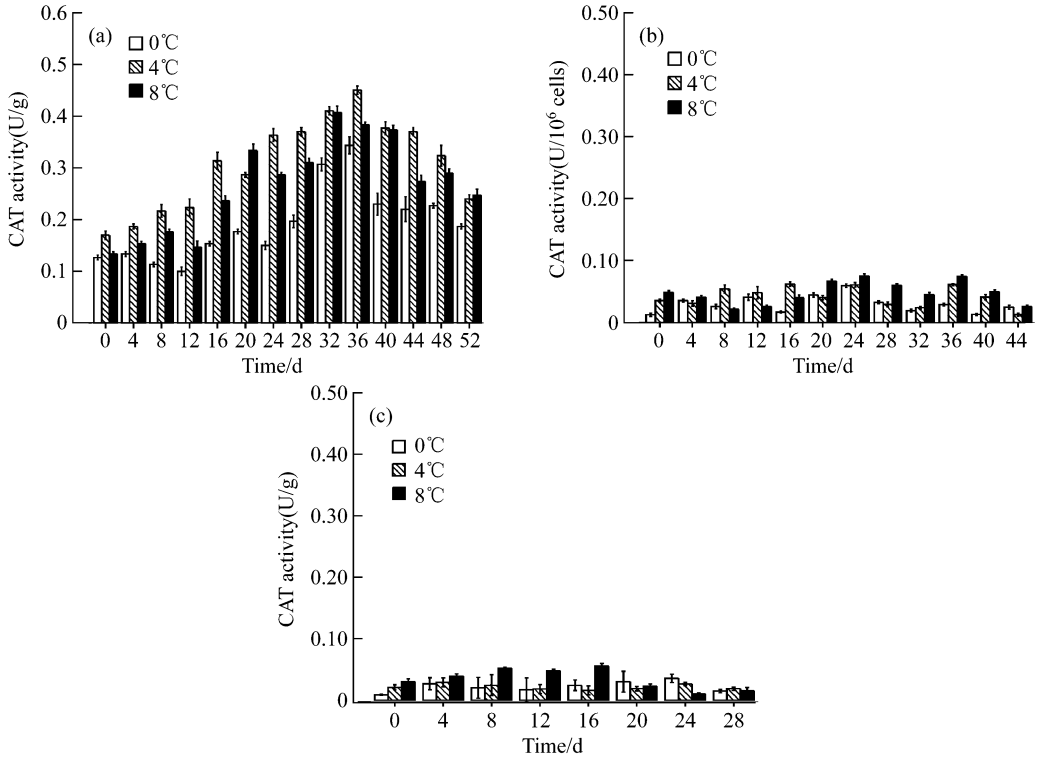

Fig. 4 The CAT activity of the three algal species at different temperatures.
(a) *Skeletonema marinoi*; (b) *Chlorella* sp.; (c) *Chlamydomonas* sp.

3.5 *Effects of temperatures on the antioxidase system*

In order to quantitatively examine the effect of different low temperatures on the antioxidase enzyme activity and MDA content of the three microalgae, analysis of variance (ANOVO) method was used in this research to analyze the significance of the difference of the effect of different temperatures, the results of which were shown in Table 1.

Table 1. Effects of different temperatures on the activities of SOD, CAT and the content of MDA of the three microalgae

	Skeletonema marinoi			*Chlamydomonas* sp.			*Chlorella* sp.		
	SOD	CAT	MDA	SOD	CAT	MDA	SOD	CAT	MDA
0 ℃－4 ℃	0.008**	0.001**	0.001**	0.25	0.858	0.810	0.688	0.061	0.347
0℃－8 ℃	0.073	0.018*	0.074	0.87	0.085	0.599	0.437	0.012*	0.052
4℃－8 ℃	0.228	0.255	0.244	0.28	0.054	0.418	0.706	0.391	0.332

* $p<0.05$, ** $p<0.01$

It could be seen that in the growth period of *Skeletonema marinoi*, the difference of the antioxidase enzyme activities and MDA content between the two groups at 0 ℃ and at 4 ℃ was extremely significant ($p<0.01$). The difference of the CAT activity of *Skeletonema marinoi* and *Chlorella* sp. between the two groups at 0 ℃ and at 8 ℃ was significant ($p<0.05$); the difference of both the enzyme activity and MDA content of the three algae between 4 ℃ and 8 ℃ was not obvious.

4 Discussion

The growth of the algae at three temperatures was different. The growth cycle of green algae was shorter than that of diatom, and that of *Chlamydomonas* sp. was shorter than that of *Chlorella* sp.. Diatom had more obvious adaptability to low temperature (4℃), but lower temperature would decrease its growth rate. *Chlorella* sp. was more adaptive to high temperature relatively; and *Chlamydomonas* sp. had broader adaptability to temperatures, and its growth at different temperatures didn't vary profoundly.

MDA is not only one of the membrane-lipid peroxidation products of the cell, but also one of the peroxidation products of unsaturated fatty acid. Its content represents the peroxidation degree of membrane lipid, and is usually taken as an important symbol of the peroxidation level of the membrane lipid[17]. It was indicated from the result of the MDA content of the three microalgae that although the MDA content of *Skeletonema marinoi* was low, its contents were still different at different temperatures. The content was opposite to the growth, and the maximum MDA contents of *Skeletonema marinoi* at the different temperatures were all in the logarithmic phase. Besides, the content was low, which showed that the study of *Skeletonema marinoi* at the three temperatures had certain lipid peroxidation, but it did not obviously influence its growth. The MDA content of *Chlorella* sp. at the three temperatures were relatively higher, but the temperatures did not present remarkable influence on its life activity. Compared with that of the other two algae, the MDA content of *Chlamydomonas* sp. was the lowest, and had the fewest changes at different temperatures, which did not consistent with the three growth curves. At the different temperatures, the MDA content of the three algae all increased within their early growth periods, which may be caused by certain lipid peroxidation under the pressure of temperatures in their initial growth periods, but later the activity of protective enzyme was also stimulated and tended to be stable as a possible result of the regulatory effect of some stress-activated enzymes in vivo[18]. Therefore, the lipid peroxidation degree reduced, and the MDA content gradually decreased with the enhanced protective enzyme function and recovered to the level which could be tolerated by the cell. In a word, temperatures had less influence on the growth of three algae.

The superoxide dismutase (SOD) is one important protective enzyme on organism; it can eliminate the active oxygen in the plant cell and protect the plant from being injured. Their activity is related with the antipollution and resistance of radiation, low-temperature and disease etc. of the organism[19-21]. The SOD activity and

its highest level of the three algae appeared at the optimum temperature, and the highest level at each temperature was in the steady growth period, which indicated that SOD played a better protective role than temperature in the adaptability of microalgae. The SOD activity of the three algae had the overall changing trend of increasing first and decreasing later, which may be caused by certain lipid peroxidation under the pressure of temperature in the initial growth period of the algal cell, thus the SOD activity increased correspondingly. Later the lipid peroxidation was gradually balanced by the free radical scavenging effect of SOD, thus the SOD activity decreased gradually. Many researches indicated that the SOD activity had certain correlation with the adaptability of the organism. When it was induced by moderate environment, the SOD activity increased, which could enhance the organism adaptability[22,23].

CAT, an enzyme containing Ferrum, can decompose H_2O_2 that is produced in disproportionate by SOD to H_2O without the need of substrate. Similarly, the CAT activity of the three microalgae had different responses to the change of the temperatures. As to *Skeletonema marinoi*, its highest CAT activity appeared at the optimum growth temperature (4 ℃). As to the two green algae, the three cultivation temperatures had not enabled their CAT to show obvious enzyme activity; however, their enzyme activity had the tendency of corresponding enhancement with the temperature increased. All these were consistent with the growth curves of the three algae. This indicated that CAT played an indistinct role in the habitat adaptability at the three temperatures in this research. Under normal conditions, the synergistic effect of SOD and CAT can maintain the biological free radical at a low level and play a protective role in the biology[24].

The variance analysis result indicated that the two temperatures of 0 ℃ and 4 ℃ had much effect on the antioxidase system of *Skeletonema marinoi*. Compared with the SOD activity and MDA content, the temperatures had more effect on the CAT enzyme activity of *Skeletonema marinoi* and *Chlorella* sp. than that on the CAT activity of *Chlamydomonas* sp..

The change trend of CAT activity of the three microalgae was indistinct and their activities were very low, so we could conclude that CAT had less effect on eliminating the active oxygen under the temperatures conditions in this research, but the SOD activity was high. Therefore, the SOD activity could still maintain stronger ability in eliminating the active oxygen, which was consistent with the research result of Kan[25]. As to *Chlorella* sp., the changing trend of MDA content with temperatures was the same as that of the growth curve. The antioxidase system could counteract certain lipid peroxidation, thus *Chlorella* sp. could grow well at higher temperatures, which also showed that the metabolism of active oxygen in plants was the synergistic effect of many antioxidases. The three microalgae could achieve a new equilibrium of protective enzymes in the organism cell and maintain normal metabolism and growth of the cells by their own unique physiologically metabolic mechanism at different temperatures, thus they had good adaptability to different temperatures in our research.

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