Proc. NIPR Symp. Polar Biol., 8, 194–199, 1995

TEMPERATURE ACCLIMATION AND THE FATTY ACID COMPOSITION OF AN ANTARCTIC GREEN ALGA *CHLORELLA*

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Abstract: Antarctic green alga *Chlorella vulgaris* strain SO-26 cells cultured at 20°C photosynthesize between 0–35°C, with a maximum rate at 20°C, while cells cultured at 10°C show a shift of the optimum to about 2 to 3°C lower and show higher activity than cells cultured at 20°C. Mesophilic alga *Chlorella sorokiniana* strain C-133 cells cultured at 20°C photosynthesize between 5–45°C with a maximum rate at 35°C, while cells cultured at 10°C photosynthesize between 0–40°C with a maximum rate at 30°C, in a temperature range about 5°C lower than cells cultured at 20°C. Strain C-133 cells cultured at 20°C show higher photosynthetic activity than cells cultured at 10°C. In both strains tested the major fatty acids are palmitic, linoleic and linolenic acids.

When culture temperatures were changed from 20 to 10°C, the increase of the ratio of unsaturated fatty acid to total fatty acid in strain SO-26 was considerably greater than that in strain C-133. These results indicate that in photosynthesis the properties of an Antarctic *Chlorella* SO-26 are more psychrophilic than those of mesophilic *Chlorella* C-133, and both strains can be acclimated by the culture temperature, at least partly because of fatty acid unsaturation.

1. Introduction

The Antarctic presents an extremely harsh environment for terrestrial and fresh water algae with very low temperatures, frequent and rapid fluctuations from freezing to thawing, severe winds, low humidity, and long periods of light and darkness (BECKER, 1982). Many studies have been carried out on algal distribution and habitat in terrestrial Antarctic environments (*e.g.*, PARKER *et al.*, 1982; OHTANI, 1991). It is an interesting problem how microalgae can grow and survive in such extreme low temperatures. SEABURG *et al.* (1981) assayed for growth of 35 taxa of Antarctic algal isolates over a range of 2–34°C. There have been several studies of physiological properties such as photosynthesis on terrestrial algae iso-

lated from Antarctica. TOMINAGA and FUKUI (1981) observed the effects of chloride concentration and temperature on photosynthesis of phytoplankton in saline lakes. BECKER (1982) showed that Antarctic *Prasiola* and *Nostoc* can photosynthesize below $-5°C$.

However, few studies on the unicellular green alga *Chlorella* isolated from Antarctica have been reported until now (SHIN *et al.*, 1986). This alga may be suitable to compare the properties of Antarctic and mesophilic algae of the same species, because *Chlorella* are distributed all over the world. On the other hand, it has been reported that unsaturation of fatty acid composition of cyanobacterium may be related to low temperature adaptation (WADA and MURATA, 1990). Similar mechanisms of adaptation to low temperature may occur in the alga *Chlorella.*

We have previously isolated some *Chlorella* strains from terrestrial areas of Antarctica and observed some properties of the algae (NAGASHIMA *et al.*, unpublished data). In this experiment, we compared the effect of temperature on the photosynthesis of an Antarctic green alga, *Chlorella vulgaris* strain SO-26 and a mesophilic alga, *C. sorokiniana* strain C-133 (formerly classified as *C. vulgaris*). The effects of culture temperature on photosynthesis and fatty acid compositions were also examined, because there is a possibility that both algae may be acclimated by the temperature at which they were cultured previously.

2. Materials and Methods

Chlorella vulgaris strain SO-26 was isolated by S. OHTANI, Shimane University, from a wet moss surface, near Syowa Station, Antarctica. *C. sorokiniana* strain C-133 was provided by IAM (Institute of Applied Microbiology, the University of Tokyo). These *Chlorella* were cultured in medium C (ICHIMURA, 1971) with a stirrer at 10 or 20°C under alternating 12 h light (150–180 $\mu Em^{-2}s^{-1}$) and 12 h dark periods for 1 month. The photosynthetic O_2 evolution of the algae was measured in a 2 m*l* reaction mixture containing 0.01 M phosphate buffer (pH 7.0), by using an oxymeter (RANK BROTHERS LTD, England) with a control unit for temperature (COOLNIT CL-80, TAITEC, Japan) under 210 $\mu Em^{-2}s^{-1}$ illumination at different temperatures. Chlorophylls of the algae were extracted with hot methanol and the amounts were quantified by the use of a spectrophotometer (Shimadzu UV-300, Japan). Fatty acid analysis was carried out by the methods of MATSUMOTO *et al.* (1990). Briefly, fatty acids were extracted with ethyl acetate after saponification (0.5 M KOH/MeOH, 70°C, 4 h) and acidification, and purified by silica gel column chromatography. Fatty acid methyl esters were analyzed by the use of a JEOL Automass 150 GC-MS system connected with a fused silica capillary column (J&W Sci, DB225, 30 m × 0.32 mm i.d., film thickness 0.25 μm).

3. Results and Discussion

3.1. *Photosynthetic O_2 evolution*

Antarctic *Chlorella* strain SO-26 cells cultured at 20°C photosynthesize between 0–35°C with a maximum rate at 20°C. Cells cultured at 10°C showed a similar pattern, but the optimum shifted about 2 to 3°C to a lower temperature and showed higher activity than 20°C culture cells throughout the temperature range (Fig. 1). The optimum temperature of the alga for photosynthesis is similar to that of a green alga *Dunaliella* sp. (22°C) and of three diatoms (15 to 18°C) isolated from saline lakes at Syowa Oasis, Antarctica. These temperature optima are considerably higher than the environmental lake water temperature (TOMINAGA and FUKUI, 1981).

On the other hand, mesophilic *Chlorella* strain C-133 cells cultured at 20°C photosynthesize between 5–45°C with a maximum rate at 35°C. The algal cells cultured at 10°C photosynthesize between 0–40°C with a maximum rate at 30°C, showing a downward shift of about 5°C including the optimum temperature (Fig. 2). Strain C-133 cells cultured at 20°C showed higher photosynthetic activity than cells cultured at 10°C.

These results, that SO-26 has a photosynthetic activity in a lower temperature range and a maximum rate at a lower temperature than C-133, and that SO-26

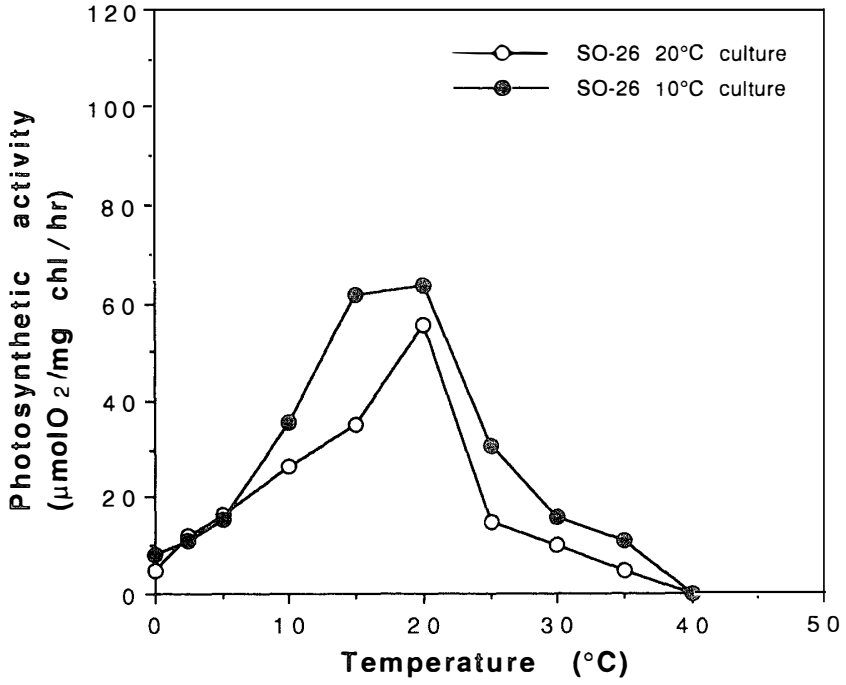

Fig. 1. Photosynthetic O_2 evolution on an Antarctic green alga Chlorella vulgaris *strain SO-26 cultured at different temperatures (10°C or 20°C). The reaction was measured in a 2 ml reaction mixture containing 0.01 M phosphate buffer (pH 7.0) by using an oxymeter under 210 $\mu Em^{-2}s^{-1}$ illumination. The photosynthetic activity per chlorophyll was calculated by average values of three experiments.*

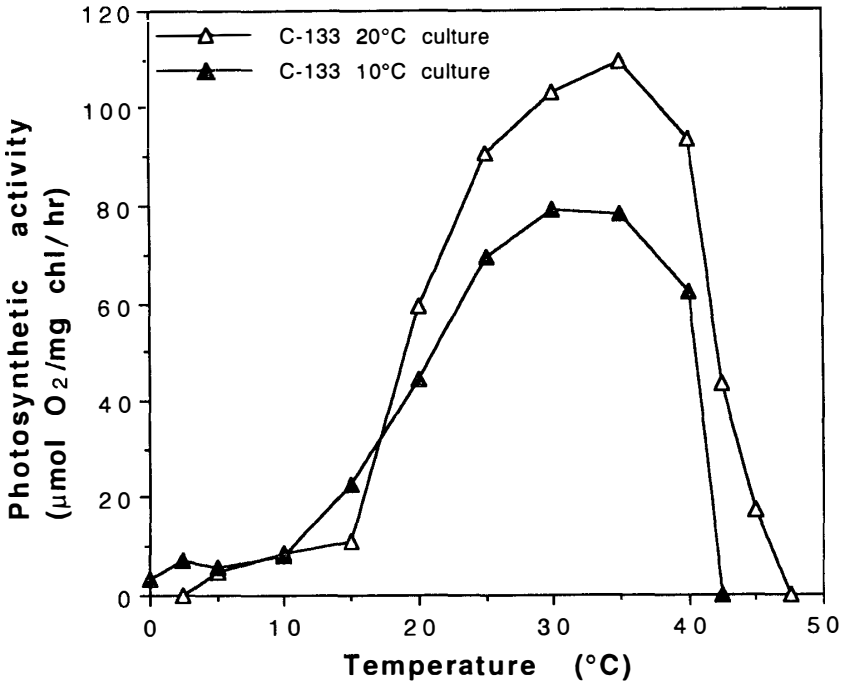

Fig. 2. Photosynthetic O_2 evolution on a mesophilic green alga Chlorella sorokiniana *strain C-133 cultured at different temperatures (10°C or 20°C). The method is the same as in Fig. 1.*

cultured at 10°C seems to activate the photosynthesis more than the alga cultured at 20°C, indicate that in photosynthesis Antarctic *Chlorella* SO-26 is more psychrophilic than mesophilic *Chlorella* C-133. SHIN *et al.* (1986) reported that Antarctic *Chlorella* sp. showed maximum growth rate at 25°C, a higher temperature than that of *Chlorella* SO-26. This may be caused by the fact that the *Chlorella* sp. was pre-cultured at 24°C, or by a property specific to this species.

3.2. Fatty acid

Fatty acids were extracted from these algae cultured at 20°C or 10°C, and were analyzed (Table 1). The results show that the major fatty acids are palmitic acid (16:0), linoleic acid (18:2) and linolenic acid (18:3) in both strains tested. Strain C-133 contains more total polyunsaturated fatty acids (TPUFA) than strain SO-26. When the culture temperatures were changed from 20 to 10°C, strain SO-26 showed a percent decrease in saturated fatty acids such as palmitic acid, and showed a percent increase in unsaturated fatty acids such as linolenic acid. As a whole, the ratio of unsaturated fatty acid to total fatty acid of strain SO-26 increased from 53.7% to 64.0%, when the culture temperature was lowered from 20 to 10°C.

Under the same lowering of culture temperature, strain C-133 showed a percent decrease in saturated fatty acid (16:0), a slight percent increase in unsaturated fatty acids (18:2, 18:3), and a slight increase from 78.5% to 82.3% in the ratio of unsaturated fatty acid to total fatty acid.

Many studies on the effect of temperature on fatty acid composition of algae indicate that decreased temperature results in increased fatty acid unsaturation

Table 1. Fatty acid composition (%) of an Antarctic green alga Chlorella vulgaris *SO-26 and a mesophilic alga* C. sorokiniana *C-133.*

Fatty acid*	*Chlorella vulgaris* SO-26		*Chlorella sorokiniana* C-133	
	10°C**	20°C	10°C	20°C
16:0	35.7	45.7	16.4	20.3
16:1	2.4	2.6	4.3	5.4
16:2	4.3	3.6	12.7	11.0
16:3	6.1	3.6	10.7	7.9
17:0	0.1	0.2	1.0	0.9
18:0	0.2	0.4	0.3	0.3
18:1	2.0	2.5	4.6	7.5
18:2	24.3	23.1	38.0	35.2
18:3	24.9	18.3	12.0	11.5
TPUFA***	64.0	53.7	82.3	78.5

* Carbon chain length: number of unsaturated bonds.
** Culture temperature. Stirring under 64–85 $\mu Em^{-2}s^{-1}$ illumination.
*** Total polyunsaturated fatty acids.

(*e.g.*, HOLTON *et al.*, 1964; THOMPSON *et al.*, 1992). Our data are consistent with the previous results. However, COHEN *et al.* (1988) pointed out that fatty acid composition of the red alga *Porphyridium cruentum* correlates clearly to growth rate. In our case, other factors in addition to temperature may affect fatty acid composition of the *Chlorella* strains.

Acknowledgments

We are indebted to Miss N. HAYASHI and Miss S. KOSUGE, Science University of Tokyo, for their technical assistance.

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(Received April 13, 1994; Revised manuscript received September 12, 1994)