Physiological characterization of a glacier living cyanobacterium, *Phormidesmis priestleyi* culture strain

Tabuchi, Y., Kosugi, M., Kizawa, A. and Koike, H.
Faculty of Science and Engineering, Chuo. University

Background

The cryoconite, a microbial community formed on glaciers, is a pebble like globule with a diameter of about 1 mm, and is a community of bacteria and filamentous cyanobacteria to which mineral granules are incorporated (Segawa, T. et al. 2014). The cryoconite is black and thus significantly reduces sunlight reflectance and accelerates melting of glaciers (Takeuchi, N. et al. 2001). For this reason, studies on growth and proliferation of organisms within the cryoconite is of special importance. *Phormidesmis priestleyi* is a filamentous cyanobacterium found as a dominant species of cyanobacteria that constitute cryoconites, but its physiological and ecological characteristics are not fully understood.

The nitrogen fixation activity of *P. priestleyi* maintained in our laboratory was tested using the acetylene reduction method, and the activity was detected both at room temperature and low temperatures (Yano, M. 2018). Genome sequences of *P. priestleyi* are released from several institutions, but different sequences have been reported on nitrogen fixation genes: Cole. et al. (2014) reported its presence, but Chrismas. et al. (2016) not. In order to confirm the nitrogen fixation activity of the strain maintained in our laboratory, the *nifH* gene of the strain was analyzed (Yano, M. Personal communication 2018). It was concluded that the strain does not harbor *nif* genes in the genome, and that the small activity detected is derived from a co-existing bacterium *Bradyrhizobium denitrificans*.

Tolerance against freezing and drought was analyzed by measuring photosynthetic activity of the culture strain (Yano, M. 2018). For organisms living in polar region or cold areas, freezing means that liquid water is not available by solidification at low temperatures, and organisms will face to a pseudo-drought state. Therefore, it is generally accepted that organisms occasionally exposed to a frozen state are resistant not only to low temperatures but also to drying. In previous studies, however, tolerance to freezing/drought was not observed in the culture strain (Yano, M. 2018). 1in this study, they were reexamined under different conditions.

Materials and Methods

Isolation of co-existing bacteria

Bacteria co-existing in the culture medium were isolated. The *P. priestleyi* culture solution was spread on a BG11 medium for autotrophs containing minerals or on a MAG medium for heterotrophs containing organic compounds. The plates were incubated at 30 ℃. for 1 month. Several colonies were formed and genomic DNA was extracted from colonies obtained from two agars. The 16S rRNA gene was amplified by PCR and its sequence was analyzed.

Drought tolerance

The *P. priestleyi* culture solution was centrifuged at 1,2000 xg for 2 min to spin-down the cells, and the precipitate was washed with 0.01 M TES (pH 8). The cells were spotted on a thin agar plate and air dried at 4 ℃ in the dark for 3 days. The agar plate dried together with the cells turned into a sheet. It was placed on a 0.8% agar plate or 1/10 BG11 containing 0.8% agar plate and rehydrated at 4 ℃ in the dark.

In this study, vital activity of *P. priestleyi* in the culture was measured as the presence or absence of photosynthetic activity. For photosynthetic activity, the effective quantum yield of PSII was continuously measured using a PAM chlorophyll fluorometer.

Freeze tolerance

Cells cultured in liquid BG11 medium at 4 ℃ for 3 months and fully acclimated at low temperatures were subjected for measurements. The cells were placed in an aluminum cap together with the medium, cooled and frozen at -10 ℃ over 12 hours in an incubator, and then further kept at -10 ℃ for 12 hours. Cells were then thawed at room temperature in the dark and cultured at 4 ℃ for recovering. Photosynthetic activity was measured before freezing, immediately after thawing, and after 2 days' recovery culture.

Results and Discussion

Presence of non-photosynthetic microorganisms in *P. priestleyi* culture

A total of 3 types of colonies were obtained from BG11 and MAG containing agar plates. The 16S rRNA gene sequence analysis revealed that the bacteria obtained from BG11 and MAG media are closely related each other, and it is highly possible that the two isolates belong to the genus *Bradyrhizobium*. Another isolate grown only on heterotrophic plate was found to belong to the

genus *Sphingomonas*. It was thus concluded that *P. priestleyi* culture strain in our lab contains at least two types of bacteria, and one of them (Bradyrhizobium) has potential nitrogen fixation activity.

Drought tolerance

Photosynthetic activity of *P. priestleyi* cultures was measured in wet, dried, and after rewetting for 3 days (Table 1). It was confirmed that the dried cells show almost no photosynthetic activity. Hence they do not have vital activity and are in dormant state on drying. Cells which were rehydrated by water from agar plates without medium recovered the photosynthetic activity slowly. About 30% of the activity was recovered on the first day after rehydration, while it reached to about 80% in 3 days. On the other hand, the cells that were rehydrated with 0.8% agar plate containing 1/10 BG11 medium almost recovered to the original activity after rehydration within 1 day. These results suggest that *P. priestleyi* cultures acclimated to low temperatures have drought tolerance, and that the rate of recovery of activity after rehydration is affected by the presence of inorganic nutrients.

Table 1 Effects of rehydration conditions on photosynthetic activity

Plates	dry	rewet		
		day1	day2	day3
0.8% agar	2±0.4	33±12.6	62±23.0	78±35.6
1/10 BG11+ 0.8% agar	1±0.6	87±8.5	109±9.7	118±5.2

Photosynthetic activity was measured by effective quantum yield of PSII and those of untreated cells were set to 100%. Value in the table were the averages and its standard deviations of three independent samples.

Freeze tolerance

In order to reinvestigate freeze tolerance of *P. priestleyi* cultures, cells acclimated to 4 ℃ were used for measurement. Changes in photosynthetic activity were depicted (Fig. 1). The cultured cells were active even immediately after thawing (melt, Fig. 1). The photosynthetic activity on the second day of recovery culture was even higher than that before freezing. Yano (2018) reported that *P. priestleyi* culture strain is not resistant to freezing. However, the results in the present study indicate that cells fully acclimated to low temperature acquire freeze tolerance. It was also suggested that previous results reported were obtained by cells which were not acclimated to low temperatures.

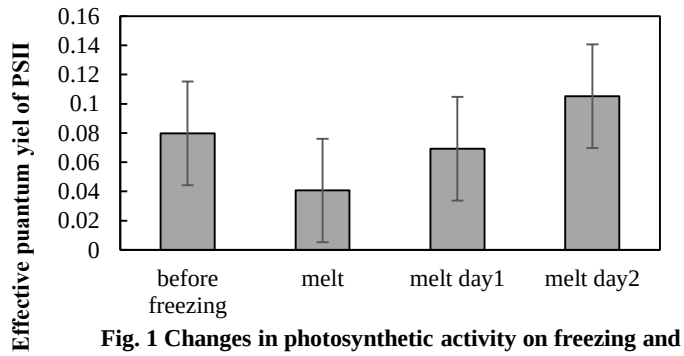

Fig. 1 Changes in photosynthetic activity on freezing and thawing

Photosynthetic activity of *P. priestleyi* culture was measured before freezing (before freezing), immediately after thawing (melt), and after 2 days of recovery culture (melt day1, melt day2). Data are the averages of three independent measurements, and error bars indicate standard deviation.

References

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