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NITROGEN-FIXING (C_2H_2-REDUCING) CYANOBACTERIA EPIPHYTIC ON MOSS COMMUNITIES IN THE ALPINE ZONE OF MT. FUJI

Takayuki NAKATSUBO[1] and Shuji OHTANI[2]

[1]*Department of Biology, School of Education, Waseda University, 6–1, Nishiwaseda 1-chome, Shinjuku-ku, Tokyo 169*
[2]*National Institute of Polar Research, 9–10, Kaga 1-chome, Itabashi-ku, Tokyo 173*

Abstract: Epiphytic cyanobacteria were observed on the moss communities growing in the alpine zone of Mt. Fuji (3776 m alt.). Nitrogen-fixing activities of these cyanobacteria were investigated by the acetylene reduction method. Weak or no nitrogen-fixing activity was detected for moss communities growing in the xeric habitat on the southwestern slope. The dominant cyanobacteria in these moss communities were *Stigonema minutum* and *Gloeocapsa magma*. *Nostoc* sp. was also observed for some communities. Moss communities growing on the moist northeast-facing cliff showed high nitrogen-fixing activities. *Nostoc* sp. was the dominant epiphyte on these moss communities. These moss-cyanobacteria associations were similar to those of the continental Antarctic in several points.

1. Introduction

In the recent two decades, intensive researches have been carried out on the nitrogen cycling in polar terrestrial ecosystems. One of the most important findings in this field is that nitrogen-fixing cyanobacteria, especially those epiphytic on mosses, contribute a significant portion of nitrogen input to the ecosystems (GRANHALL and SELANDER, 1973; ALEXANDER *et al.*, 1978; DAVEY and MARCHANT, 1983; NAKATSUBO and INO, 1987; CHRISTIE, 1987). It has been pointed out that moss communities are of special importance as a habitat of nitrogen-fixing cyanobacteria in polar regions, because dark-colored mosses provide a particularly warm environment which promotes the nitrogen-fixing process (ALEXANDER *et al.*, 1978). This suggests that similar moss-cyanobacteria associations may be found in alpine regions. In fact, PORTER and GRABLE (1969) reported nitrogen fixation by cyanobacteria associated with mosses in wet mountain medows of Colorado. For subalpine zone, LAMBERT and REINERS (1979) reported the nitrogen-fixing activities of various moss communities. However, information about the moss-cyanobacteria associations in the alpine zone is scarce, although nitrogen-fixing activities associated with moss communities have been reported from some alpine tundras (ALEXANDER and SCHELL, 1973; WOJCIECHOWSKI and HEIMBROOK, 1984).

At the summit of Mt. Fuji (3776 m), epiphytic cyanobacteria were often found on the moss communities. In this study, we examine nitrogen-fixing activities and species composition of these epiphytes. The similarities between these moss-cyanobacteria associations and those of the continental Antarctic are discussed.

2. Materials and Methods

Mount Fuji is a dormant volcano with an altitude of 3776 m. The timberline is around 2400 m. Above 3200 m, mosses and lichens are the dominant vegetation. At the summit, 27 species of bryophytes have been recorded (TAKAKI, 1971).

Observation and sample collection were carried out at Kengamine, the highest point of Mt. Fuji. Kengamine is situated in the southwestern part of the lip of the summit crater (Fig. 1). Annual mean air temperature, recorded at the Meteorological Observatory of Kengamine, is −6.5°C. Monthly mean air temperature of August and January are 5.8°C and −18.9°C, respectively (NATIONAL ASTRONOMICAL OBSERVATORY, 1989). Snow cover lasts for about 9 months, usually from October to June. The southwestern side of Kengamine is an open slope. This side is relatively dry because of the direct sunshine and the prevailing wind from the west. The northeastern side of Kengamine is a cliff. This cliff provides shaded and moist habitats for bryophytes. Sample collection was carried out at both sides of Kengamine in June 1987. Some moss colonies are blackish green or gray green with epiphytic algae and cyanobacteria but the others are vivid green without visible epiphytes. The amount of these epiphytes

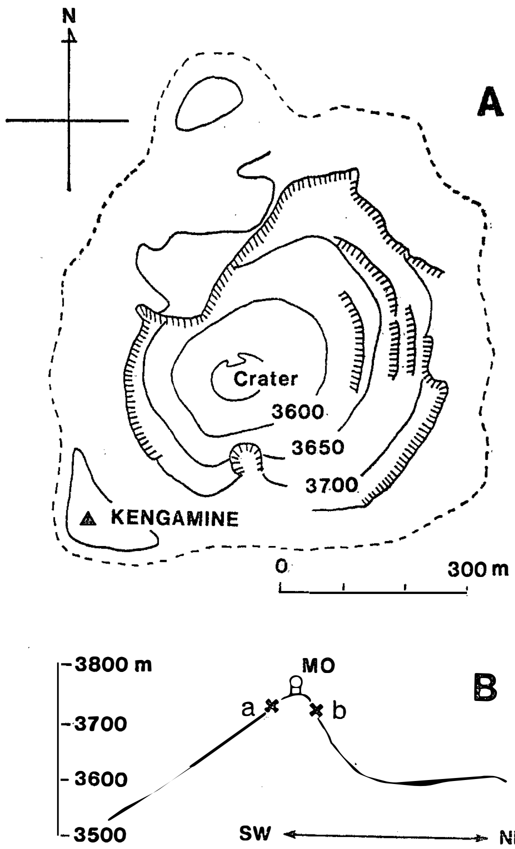

Fig. 1. (*A*) *Map of the summit crater of Mt. Fuji.* (*B*) *The profile of Kengamine. Sample collection was carried out on the southwestern slope* (*a*) *and the northeast-facing cliff* (*b*) *of Kengamine. MO shows the Meteorological Observatory.*

seems to be depending on microscale topography and water availability of the moss communities. Since this study mainly deals with the relation between the species composition and the acetylene-reducing activities of the epiphytic cyanobacteria, moss communities with epiphytes were collected principally.

Samples were brought to the laboratory and kept in a growth box at 10°C until the measurements. The samples were prepared for the use by cutting out cores of the moss community 1.3 cm^2 in surface area. Three core samples were taken from each moss community.

A modified form of the acetylene reduction method described by STEWART *et al.* (1967) was used for the measurement of the nitrogen-fixing activities of the samples. Each core sample was placed in a 23-m*l* Erlenmeyer flask and each flask was capped with a rubber stopper. Part of the air within a flask was replaced with acetylene gas generated from calcium carbide to make 0.1 atm C_2H_2. All flasks were incubated in a water bath at 20°C and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD for 3 hours.

The production of ethylene was determined using a gas chromatograph equipped with a flame ionization detector (Shimadzu GC-8A). Hydrocarbon separation was achieved using a 2.0 m $\times$ 2.6 mm glass column packed with 80/100-mesh Porapak R. Acetylene gas samples were also checked for potential ethylene contamination. Control flasks containing core samples without acetylene addition were employed to detect the endogenous ethylene production. Ethylene was not detected in any cases. The acetylene-reducing activities were expressed as nanomoles of ethylene produced per square centimeter of the moss community in one hour.

After the measurements of acetylene-reducing activities, cyanobacteria in these samples were identified in living condition. For making a microscopic preparation, a small piece of moss was taken from outer layer (5 mm) of moss samples, and the epiphytes were scrapped from the piece by a needle under the binocular. Samples were examined using the light microscope at magnification up to $\times$1000.

3. Results and Discussion

At the summit of Mt. Fuji, cyanobacteria are the main components of the epiphytes on moss communities. Among them, *Nostoc* sp., *Stigonema minutum* and *Gloeocapsa magma* were relatively abundant and often observed in moss samples examined (Fig. 2). *Aphanocapsa elachista* var. *conferta*, *Aphanothece saxicola*, *Chroococcus minutus*, *Synechococcus aeruginosus*, and two species of *Lyngbya*, *Calothrix* sp. were rarely found. Associated algae with cyanobacteria were coccoid green algae, desmids (*Cylindrocystis*, *Mesotaenium*) and pinnate diatoms (*e.g. Pinnuralia borealis*, *Hantzschia amphioxys*). These associated algae were always poor in quantity.

Table 1 shows the sampling sites, the dominant moss species, relative abundance of the dominant three species of cyanobacteria and the acetylene-reducing activities of the samples examined.

The dominant moss species on the southwestern slope of Kengamine are *Racomitrium lanuginosum* (Fig. 3A) and *Grimmia elongata*. *Racomitrium lanuginosum* in this site often forms round cushion. Some of these communities were black in color. A small number of cyanobactria mainly *Stigonema minutum*, *Nostoc* sp. were observed

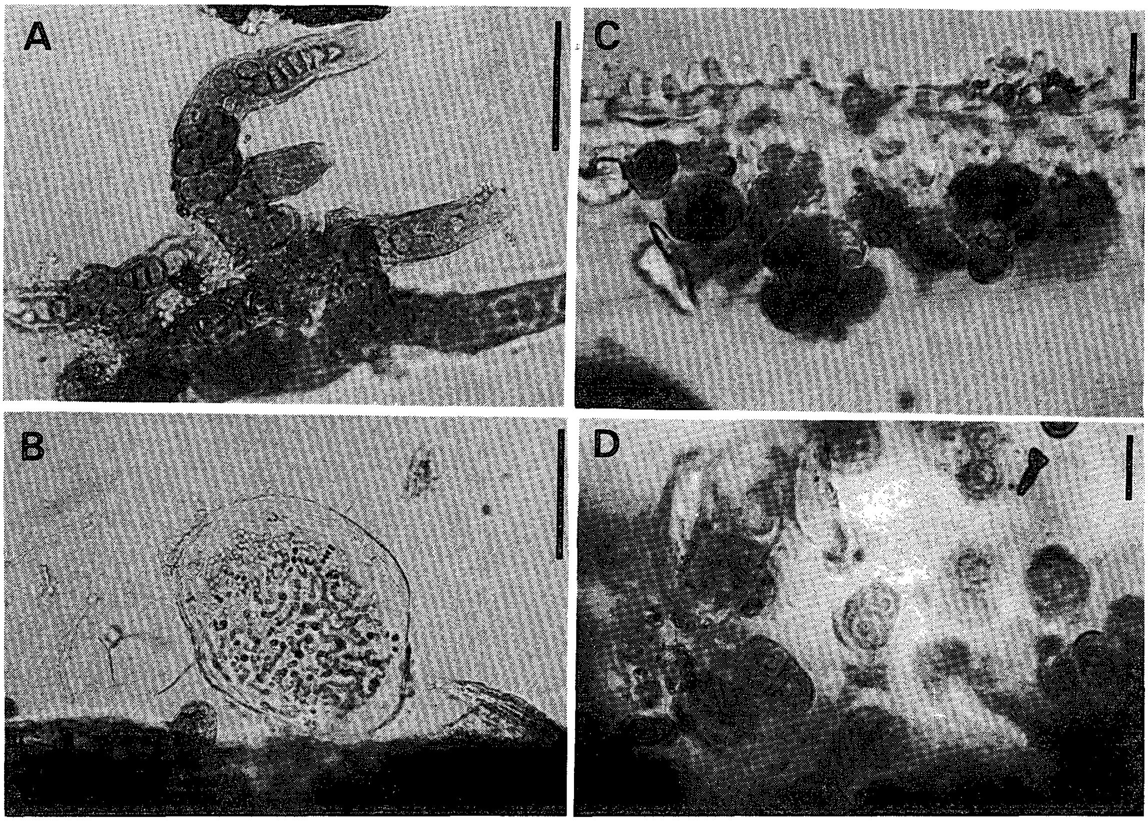

Fig. 2. Dominant cyanobacteria epiphytic on moss communities in Mt. Fuji. (*A*) Stigonema minutum. (*B*) Nostoc *sp.* (*C*), (*D*) Gloeocapsa magma *on the leaves of* Racomitrium lanuginosum. *Scale bar*: (*A*), (*B*) *50 μm*; (*C*), (*D*) *10 μm.*

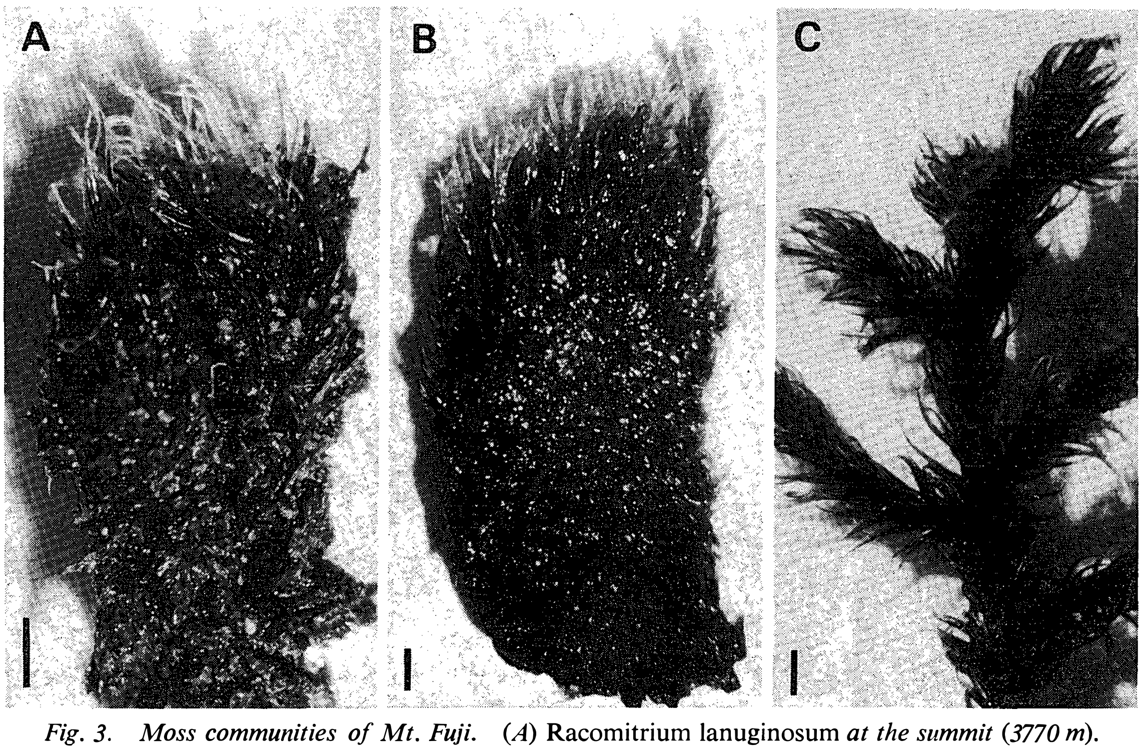

Fig. 3. Moss communities of Mt. Fuji. (*A*) Racomitrium lanuginosum *at the summit* (*3770 m*). (*B*) Ceratodon purpureus *at the summit.* (*C*) *One shoot of* Racomitrium lanuginosum *at the timberline* (*2500 m*). *Scale bar*: *1 mm.*

Table 1. Relative abundance of epiphytic cyanobacteria and acetylene-reducing activity of moss communities in Mt. Fuji.

Moss species	Cyanobacteria[1]			
	Nostoc sp.	*Stigonema minutum*	*Gloeocapsa magma*	ARA[2]
Kengamine SW (3770 m)				
Racomitrium lanuginosum	r	r	—	0.1– 0.9
Grimmia elongata-1	—	r	r	0
Grimmia elongata-2	—	+	r	0– 1.5
Kengamine NE (3770 m)				
Aongstroemia fuji-alpina	c	+	—	1.0– 6.1
Ceratodon purpureus-1	c	—	—	0.5– 2.7
Ceratodon purpureus-2	c	—	—	3.9–10.2
Timberline (2500 m)				
Racomitrium lanuginosum-1	—	r	r	0
Racomitrium lanuginosum-2	—	+	c	0
Racomitrium lanuginosum-3	—	r	r	0

[1] r: Rare, +: Common, c: Abundant.
[2] Acetylene-reducing activity ($nmol \cdot C_2H_4 \cdot cm^{-2} \cdot h^{-1}$).

in the outer layer of these communities. Low but significant acetylene-reducing activities were detected in these samples. The communities of *Grimmia elongata* were partially colonized by cyanobacteria. A small number of *Stigonema minutum* and an unicellular species *Gloeocapsa magma* were observed on the surface. Weak or no acetylene-reducing activity was detected in these communities.

Several bryophytes, including *Aongstroemia fuji-alpina*, *Ceratodon purpureus* (Fig. 3B) and *Bryum* sp., were found at the northeastern side of Kengamine. Probably, the shaded and moist habitat condition are suitable for the growth of these species. Many colonies of cyanobacteria were found on these bryophyte communities. Microscopic observation revealed that closely packed microthalli ($<500\ \mu m$) of *Nostoc* sp. were dominant. These moss-cyanobacteria associations showed high acetylene-reducing activities. The maximum rate of acetylene reduction was 10.2 $nmol \cdot C_2H_4 \cdot cm^{-2} \cdot h^{-1}$.

For comparison, the communities of *Racomitrium lanuginosum* (Fig. 3C), the dominant moss species in the lava field just above the timberline (*ca.* 2500 m alt.), were also examined. Usually, algal epiphytes were not observed by the naked eye. However, microscopic-observation revealed that most of these moss communities were colonized by *Gloeocapsa magma*. A small number of specimens of *Stigonema minutum* were also observed. Acetylene-reducing activity was not detected in these samples.

There was a positive correlation between the acetylene-reducing activity and the abundance of *Nostoc* sp. This suggests that *Nostoc* is the most important nitrogen-fixing agent in this place. Although *Stigonema minutum* has heterocysts, the contribution to the activity was not clear. *Gloeocapsa* are unicellular cyanobacteria, but some species of this genus have nitrogen-fixing activities (Wyatt and Silvey, 1969). However, in this study, there was no indication that *Gloeocapsa magma* has nitrogen-fixing activities.

It is worthy of attention that the moss-cyanobacteria associations in the summit are similar to those in Antarctica. In the ice-free areas near Syowa Station in Antarctica, moss communities are often covered with cyanobacteria which have acetylene-reducing activity (NAKATSUBO and INO, 1986). Moss communities heavily colonized by these cyanobacteria are black in appearance like those at the summit of Mt. Fuji. *Nostoc* spp. are the most important epiphytic cyanobacteria in these moss communities. *Gloeocapsa magma* and *Stigonema minutum* were also found on moss communities in the vicinity of Syowa Station (OHTANI, 1986; OHTANI and KANDA, 1987). The mean rate of acetylene reduction at 20°C and 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD of the community of East Ongul Island was 19.0 $nmol \cdot C_2H_4 \cdot cm^{-2} \cdot h^{-1}$ (recalculated from the data of NAKATSUBO and INO, 1987). This value is almost twice as high as the maximum rate recorded for the moss community of Mt. Fuji. NAKATSUBO and INO (1987) concluded that nitrogen fixation by epiphytic cyanobacteria is important as a nitrogen source for the community growth on East Ongul Island. For the moss communities of Mt. Fuji, however, nitrogen fixation does not seem to be important as a nitrogen source, because a considerable amount of nitrogen is supplied by precipitation. Annual nitrogen input by precipitation measured at an altitude of 1400 m of Mt. Fuji was 1.2 $gN \cdot m^{-2}$ (HIROSE and TATENO, 1984). This value is much larger than the annual nitrogen requirement of *Racomitrium lanuginosum* in the subalpine lava field of Mt. Fuji (NAKATSUBO, 1990). The fact that some moss communities in the study sites showed no acetylene-reducing activity suggests that nitrogen fixation by cyanobacteria is not essential to the growth of these moss communities.

The abundance of the epiphytic cyanobacteria in the alpine zone and the similarities between the moss-cyanobacteria associations at the summit of Mt. Fuji and those in the continental Antarctic suggest that severe climate characteristic to these habitats may promote the formation of these associations. Further studies including culture experiments are necessary to clarify the factors that promote the formation of these moss-cyanobacteria associations.

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