Adaptations of cyanobacterium *Nostoc commune* to environmental stress: Comparison of morphological and physiological markers between European and Antarctic populations after rehydration

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Abstract

Availability of water may influence activities of all living organisms, including cyanobacterial communities. Filamentous cyanobacterium *Nostoc commune* is well adapted to wide spectrum of ecosystems. For this reason, *N. commune* had to develop diverse protection strategies due to exposition to regular rewetting and drying processes. Few studies have been conducted on activities, by which cyanobacteria are trying to avoid water deficit. Therefore, the present study using physiological and morphological parameters is focused on comparison between European and Antarctic ecotypes of *N. commune* during rewetting. Gradual increase of F_V/F_M ratios, as the markers of active PS II, demonstrated the recovery processes of *N. commune* colonies from Europe as well as from Antarctica after time dependent rehydration. During the initial hours of rewetting, there was lower content of soluble proteins in colonies from Antarctica in comparison to those from Europe. Total content of nitrogen was higher in European ecotypes of *N. commune*. Significantly higher frequency of occurrence of heterocysts in Antarctic ecotypes was observed. The heterocyst cells were significantly longer in Antarctic ecotypes rather than European ecotypes of *N. commune*.

Key words: Antarctica, soluble proteins, cyanobacteria, chlorophyll fluorescence, heterocysts, nitrogen, James Ross Island

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Introduction

Nostoc commune is a filamentous cyanobacterium which forms macroscopic colonies. Hydrated colonies have an olive-green colour with the consistency of a solid gel, while in dry state they resemble black crusts (Novis et al. 2007). In *N. commune* several cell types can be differentiated: vegetative cells, which are photosynthetic cells that fix carbon (Meeks et Elhai 2002) and heterocysts, which are specialized nitrogen-fixing cells containing nitrogenase enzymes. They perform many functions, but one of the main roles is to provide nitrogen and nutrients (phosphorus *etc*.) to cells in filament. Cells of *N. commune* are ringed by gelatinous matrix, consisting primarily of polysaccharides. These polymers are responsible for protection against protozoan predation, antimicrobial agents, but mainly against desiccation (Potts 1994, De Phillippis et Vincenzini 1998). In general, *Nostoc* can survive for 100 years in almost complete dehydration in the dark (Cameron 1962).

N. commune is well adapted to terrestrial ecosystems from the tropics to polar regions of the Earth. Activities of cyanobacterial communities are influenced by the availability of water, and pressure of a water deficit apparently affected the cells at the early stage of their evolution (Potts 1994). Some species of cyanobacteria developed the way for desiccation tolerance (Kennedy et al. 1994). Filamentous cyanobacterium *N. commune* developed the protective strategies against water deficit because of exposition to regular rewetting and drying processes. Water content of desiccated (anhydrobiotic) cyanobacteria cells is very low. In this dry state, *N. commune* as photosynthetic organism must have strategy to avoid not only desiccation but photoinhibition as well. For photosynthesis, the light is essential, but under dry conditions when photosynthesis is inhibited, the light becomes harmful. Deactivation of photosystem II and photosynthetic activities would be appropriate because when photosynthesis is stopped while photochemical reactions are active due to inhibition of Calvin- Benson cycle by dehydration, strong reductants or oxidants could be created by PS I or PS II (Hirai et al. 2004). Physiological changes in *N. commune* during the cycles of wetting and drying were investigated in several studies. According to Scherer et al. (1984) *N. commune* can perform photosynthetic CO_2 fixation and nitrogen fixation under wet conditions. Satoh et al. (2002) measured recovery processes during rewetting in colonies of *N. commune*, focused on energy transfer and photochemical reactions. It has been shown that phycobiliproteins and photosystem I (PS I) complexes activate their forms very quickly as soon as 1 min after rehydration, but recovery of the photosystem II (PS II) was slower. Respiration processes recovered 30 minutes after initiation of rehydration. However, the details of these processes of recovery and activation of photosynthesis remain unknown.

In this study, we tested populations of cyanobacterium *N. commune* from Antarctica and from Europe. We have focused on the physiological parameters as well as the morphological differences of the studied species after time dependent rehydration. We evaluated the chlorophyll fluorescence measurements for assessment of changes in photosynthetic processes, content of soluble proteins, frequency and size of heterocysts and nitrogen content in rehydrated colonies of *N. commune*.

Material and Methods

Plant material

Antarctic populations of *N. commune* colonies were collected randomly (during January 2017) at James Ross Island, Antarctica (Fig. 1) in shallow streams where the appearance of colonies is widespread (Skácelová et al. 2013, Komárek et al. 2015). Selected locality was close to Czech Antarctic station J. G. Mendel (63° 48′ 02″ S, 57° 52′ 57″ E). European cyanobacterial samples were collected randomly from the soil surface at Illmitz (47° 45′ 26″ S, 16° 49′ 01″ E) in the district of Neusiedl am See, Austria. The area is typical by wide open plains and many small salt lakes around.

Fig. 1. *Nostoc commune* colonies at James Ross Island, Antarctica, sampling site.

Rehydration assay

Nineteen *N. commune* colonies with dry weights around 100 mg were used. Desiccated colonies were rehydrated in 15 ml plastic tubes through the addition of distilled water (5 ml), at time intervals (30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours and 24 hours). During rehydration they were stored in laboratory conditions at 24 ± 1°C and illuminated with daylight fluorescent tubes (light intensity 25 μE m^{-2} s^{-1}). After these time intervals, water was removed by blotting *N. commune* colonies with paper tissue.

Chlorophyll fluorescence measurements

Rewetted colonies of *N. commune* were transferred on Petri dish and dark adapted at least 15 minutes prior to measurement. The chlorophyll fluorescence measurements were performed using a FluorCam MF-800 fluorescence-imaging camera (Pho-

ton Systems Instruments, Czech Republic) and processed by FluorCam 7 software (Photon Systems Instruments, Czech Republic). Chlorophyll *a* fluorescence expressed as F_V/F_M was calculated

$$(F_M - F_0) / F_M = F_V/F_M \qquad \textit{Eqn. 1}$$

- F_0 represent ground fluorescence in the dark-adapted state
- F_M represent maximum fluorescence caused by saturating radiation pulse in the dark-adapted state
- F_V/F_M – quantum yield of PS II photochemistry in the dark adapted state

For this study, we measured chlorophyll fluorescence at time intervals (30 minutes, 1 hour, 2 hours, 4 hours, 8 hours, 16 hours and 24 hours) after the rehydration. Chlorophyll fluorescence parameters were taken from ten positions on each Petri dish, and the average value was used as one observation.

Soluble proteins

N. commune colonies were homogenized in an ice- cold mortar with added 2 ml of 50mM phosphate buffer (pH 6.5). Homogenized suspension was then centrifuged at 15 000 x g at 4 ºC for 20 min. After centrifugation, soluble proteins content was measured according to Bradford (1976) method. Using the 900 µl Bio-Rad protein assay kit (Bio- Rad Laboratories Inc., Hercules, California) and 100 µl of supernatants we obtained a solution of soluble proteins. Absorbance of samples was spectrophotometrically measured at wavelength 595 nm with the multi-detection microplate reader The Synergy HT, BioTek. Bovine serum albumin was used as a calibration standard.

CHNS analysis

Dry thalli of *N. commune* around 100 mg were homogenized in an ice cold mortar. Then they were transferred to 2 ml eppendorfs and used for measurement. Elemental analysis for carbon, hydrogen, nitrogen and sulphur was performed by elementary analyzer Vario MACRO cube (Elementar Analysensysteme GmbH, Germany) equipped with a thermal conductivity detector. Helium and oxygen (purity 99.995%) were used as the carrier combusting gases. Combustion tube was set up at 1150°C and reduction tube at 850°C. Sulfanilamide (C ¼ 41.81%, N ¼ 16.26%, H ¼ 4.65%, S ¼ 18.62%) was used as a CHNS standard. This analysis was measured at Institute of Geotechnics, Slovak Academy of Sciences in Košice, Slovakia.

Heterocyst counts

Measurements for quantifying heterocysts were done in a laboratory (Cell Imaging and Ultrastructure Research, University of Vienna, Austria). Colonies were maintained in laboratory conditions at 24 ± 1°C and illuminated with daylight fluorescent tubes (light intensity 25 $\mu E\ m^{-2}\ s^{-1}$). We used well- mixed culture prepared from 50 mg (DW) colonies of *N. commune* dissolved in 25 ml of distilled water. We placed a 0.5 ml drop of fluid in the centre of the slide avoiding air bubbles and we removed excess water with paper towel. Images were taken at 40x magnification with a microscope. We identified and photographed at least 200 heterocysts of both ecotypes of *N. commune*. The number of heterocysts on each filament were counted

and summed for all samples. Each sample was repeated thirty times. Filaments of *N. commune* were selected randomly trying to exclude cells of the edge of slide. The length of heterocysts were measured using ImageJ software.

Statistical analysis

The one-way ANOVA analysis of variance and Tukey´s pairwise comparisons (MINITAB 18) were used for the determination significance differences in all measured parameters. The variation about means is displayed graphically as ± the standard deviation of the means.

Results

Chlorophyll fluorescence measurements

In rewetted colonies of *N. commune*, we observed differences in F_V/F_M ratios between ecotypes from Antarctica and those from Europe (Fig. 2). Values in Antarctic ecotype were found in the range of 0.092-0.279 F_V/F_M ratio. European ecotype gained values in the range of 0.218- 0.497 F_V/F_M ratio. In both ecotypes values exhibited an increase after time dependent rehydration.

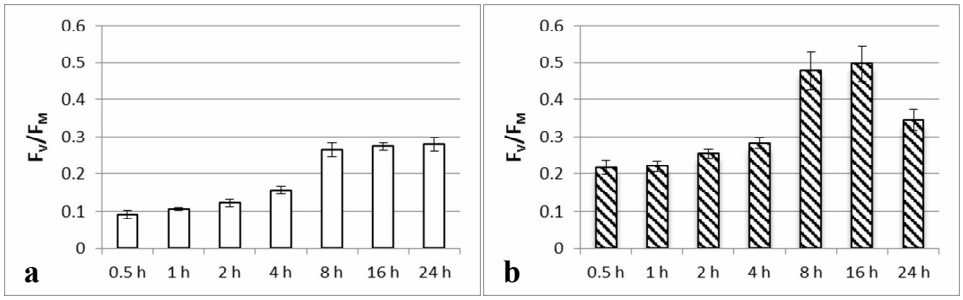

Fig. 2. Comparison in F_V/F_M ratios between tested ecotypes of *N. commune*. White columns represent Antarctic ecotype (**a**) and striped columns represent European ecotypes (**b**).

Soluble proteins

Content of soluble proteins in *N. commune* colonies from Antarctica and Europe was stable. We did not find significant changes due to rehydration in any of tested *N. commune* ecotypes. We observed lower content of soluble proteins in Antarctic ecotype in the beginning of rewetting in comparison to those from Europe (*see* Fig. 3).

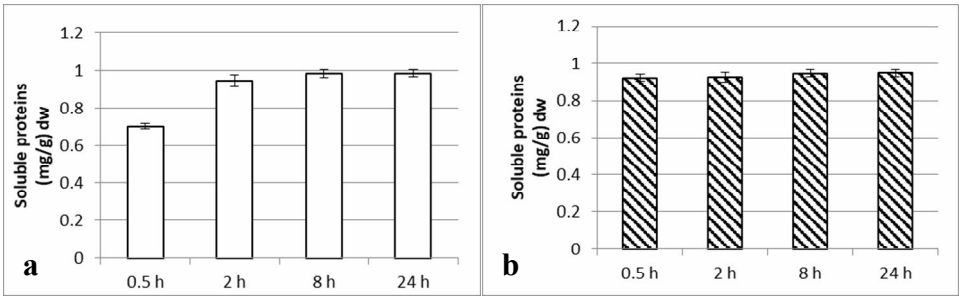

Fig. 3. Content of soluble proteins after time dependent rehydration of Antarctic (**a**) and European (**b**) *N. commune* colonies.

CHNS analysis

The elemental analysis of the elements C, H, N, S with the focus on nitrogen showed higher content of N in European ecotype of *N. commune*.

	N (%)
Antarctic	**2.66**
European	**3.82**

Table 1. CHNS analysis of both tested ecotype of *N. commune.* Average value was used as one observation.

Counts and length of heterocysts

We measured specialized cells, heterocysts in both of studied samples. Significant changes in the length and counts occurred between Antarctic and European ecotypes of *N. commune* colonies. In Antarctic colonies, significantly higher frequency of occurrence was observed. Mean values of length of heterocysts in *N. commune* colonies from Antarctica was 11.793 ± 0.802 µm. In European ecotype of *N. commune*, significantly smaller cells were observed 10.6568 ± 0.6677µm (*see* Figs. 4 a, b; Figs. 5 a, b).

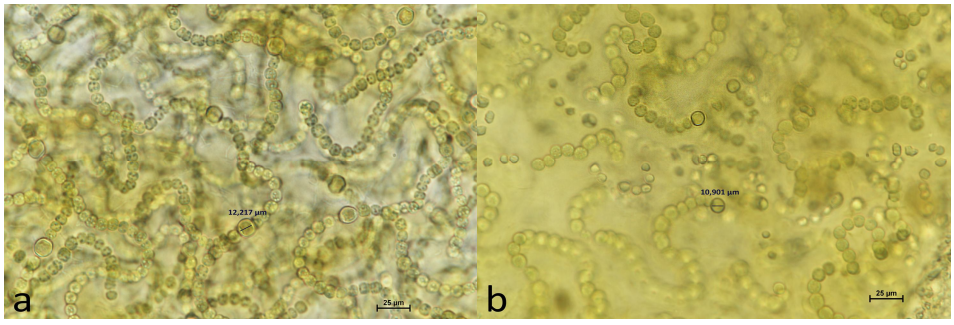

Fig. 4. Lengths measured across heterocysts in Antarctic (**a**) and European (**b**) of *N. commune* colonies.

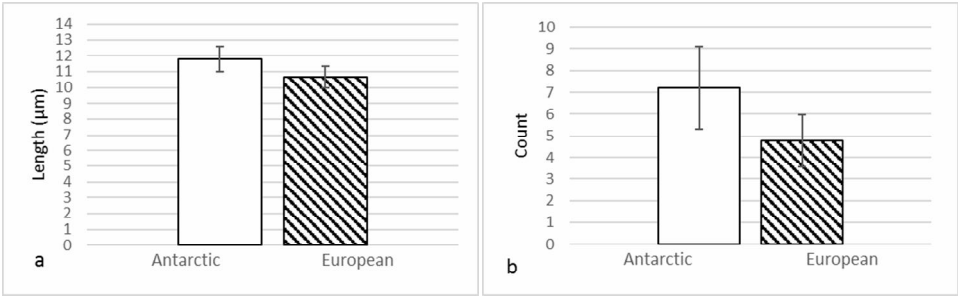

Fig. 5. Comparison in length (**a**) and count (**b**) between tested ecotypes of *N. commune*. White columns represent Antarctic ecotype and striped columns represent European ecotype.

Discussion

Antarctica is an area that has one of the most extreme environmental conditions in the world. Because of role of Antarctica in global climate, the Antarctic ecosystems are part of scientific interest in climate change research (Novis et al. 2007). In such harsh environment growth and development of plants is influenced by combination of factors such as deficiency of nutrients, substrate conditions and high dose of UV radiation. However, primitive forms of life, such as cyanobacteria, may occur in these areas. *Nostoc commune* grows either as free-living forms or on mosses as an epiphyte. Widespread occurrence is in lakes and on moist soils.

In the present study, we compared *N. commune* from Antarctica and Europe using physiological and morphological parameters during rewetting.

Barták et al. (2016) showed that gradual dehydration may cause changes in normalized difference vegetation index (NDVI), photochemical reflectance index (PRI), and primary photochemical processes of photosynthesis, effective quantum yield of photosynthetic processes (Φ_{PSII}) in photosystem II. In this study, non-photochemical quenching (qN) of absorbed light energy increased with relative water content (RWC) decrease from 100% (full hydrated state) to 0% (dry state).

In general, the fluorescence signal is rich in information and these parameters such as potential quantum yield of electron transfer through PS II, F_V/F_M ratio are considered indices of photosynthetic activity. The centres of photosynthetic complexes (PS I, PS II) are very similar in cyanobacteria and plants. In cyanobacteria, phycobilisomes as the light-harvesting complexes are located peripheral to the thylakoid membranes, which is a difference from the integral membrane chlorophyll a/b binding proteins occurring in higher plants. The cellular phycobiliprotein content may affect cellular fluorescence yield in cyanobacteria. The study of Satoh (2002) reports that fluorescence signals which indicate acclimation mechanisms to different intensities of light is various between cyanobacteria and higher plants.

We examined the recovery processes using chlorophyll fluorescence as a measure of activity of PS II of *N. commune* colonies after time dependent rehydration. We observed a gradual increase in F_V/F_M ratios in *N. commune* from Antarctica following short- term exposure to rehydration, the values of F_V/F_M after eight hours did not change significantly. This conclusion could be supported by Gupta et Kashyap (1995), who reported that desiccated thalli of *N. commune* from Antarctica ab-

sorb water to saturating level for at least 5 hours after initiation of rewetting. Similar rate is reported for desiccation, Kvíderová et al. (2011) showed that, F_V/F_M ratios remained unchanged even after 6 hours from the start of desiccation. In the European colonies we observed suprasaturation effect resulting in decreased fluorescence yield after 24 hours rewetting. Lange et al. (1996) reported that water suprasaturation effect could be detected at high thallus hydration in cyanolichens. Similarly, recent study of Barták et al. (2016) indicated negative effect of suprasaturation of fully hydrated *N. commune* colony.

Stable content of soluble proteins in both tested *N. commune* colonies due to time dependent rehydration has been confirmed in the present study. It is interesting that cyanobacterial ecotypes from Europe and Antarctica are differently sensitive to rewetting. Lower content of soluble proteins in Antarctic ecotype in the beginning of rewetting was observed. The Antarctic flora is composed mainly of organisms adapted to short summers and low temperatures (Putzke et Pereira 2001).

We assumed that amounts of nitrogen should be higher in European ecotype, because in Antarctic conditions, the sources of nitrogen are quite limited. As selected locality for collecting of Antarctic samples of *Nostoc commune* evaluated in this study was far from any nesting places of sea birds, there was no artificial local source of nitrogen in ecosystem. To prove this hypothesis, the total content of nitrogen using CHNS analysis was carried out.

Length of heterocyst cells was bigger in Antarctic ecotype rather than European ecotype of cyanobacterium *N. commune* as well as frequency of occurrence of these specialized cells. It depends on environment conditions, which are different in Antarctica and Europe. Heterocyst cells contain the enzyme nitrogenase, which converts N_2 and to NH_3 (Dixon et Kahn 2004) but cyanobacteria don´t produce active heterocysts in conditions where available N, such as ammonium (NH_4^+) and nitrate (NO_3^-), is a non- limiting resource (Meeks et al. 2002, Campbell et al. 2007) rather, N_2 fixation is stimulated when N is limiting in environment. Satoh et al. (2002) reported that *N. commune* can survive months of frost and drought as inactive desiccated crusts, and within few hours can reactivate nitrogen fixation when water and suitable temperatures again become available. According to this fact we might say that temperature in the environment could also play important role in recovery processes in colonies of cyanobacteria.

Conclusion

Physiological and morphological changes in both tested *N. commune* ecotypes after time dependent rehydration was confirmed. Based on the results, we conclude that there are differences between *N. commune* colonies from Antarctica and Europe. Recovery processes resulting from chlorophyll fluorescence measurements in Antarctic and European *N. commune* colonies were evident during rewetting. Higher amounts of nitrogen in European ecotype were observed. Length of heterocyst cells was bigger and frequency of occurrence of these specialized cells was higher in Antarctic ecotype.

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