Resistance of Antarctic lichens and unicellular alga *Trebouxia* sp. to extreme temperature. Laboratory study of linear cooling and shock freezing

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INTRODUCTION

Usnea sphacelata and *Usnea aurantiaco-atra* are dominant components of Antarctic lichen flora in the South Shetlands archipelago and several other regions within maritime Antarctica. The lichens are considered to be cold resistant. They may survive long periods at sub zero temperature with no or only limited remarkable signs of damage. In majority of cases, however, the lichens face subzero temperature in dry, metabolically inactive state. When exposed to subzero temperature in wet state, lichens activate numerous protective mechanisms and reduce their photosynthetic activity. Rapid freezing of lichen thalli in wet state happens quite frequently in the field in polar regions. Consequences for lichen thallus anatomy and photosynthetic apparaus are, however, rather unknown. In our study, we focused on structural components of thallus of two Antarctic frusicose lichens (*Usnea sphacelata* and *U. aurantiaco-atra*) and their change after shock freezing (a short-term immersion of wet lichen thalli to liquid nitrogen). Anatomical properties of cross section of thalli were evaluated after the shock freezing and in untreated control. We expected species-specific differences in photosynthetic performance (monitored by several chlorophyll fluorescence parameters).

MATERIAL AND METHODS

Samples collection and handling

Thalli of experimental lichen species were collected during austral summer season. *Usnea sphacelata* samples were collected close to the Panorama Pass (63° 48´ 51´´ S, 57° 49´ 53´´ W, 242 m a.s.l.) at James Ross Island, Antarctica. Samples of *U. aurantiaco-atra* were collected at the La Cruz Plateau in the Fildes Peninsula, King George Island (62°12' S, 58°57' W, 41 m a. s. l.). The samples were dried naturally in local climate and then transported to the laboratories of the Masaryk University, Brno, Czech Republic. Before experiments, thalli were hydrated for 24 h by distilled water in Petri dishes under a wet piece of filter paper until they reached the fully hydrated state.

Rapid freezing effect on thallus anatomy

Thalli of *U. sphacelata* and *U. aurantiaco-atra* were frozen by the immersion in liquid nitrogen (-196°C) for 5 minutes. After freezing, the thalli were thawed in laboratory temperature (23°C) and moistened by distilled water. Then, anatomical characteristics were evaluated on cross sections by optical microscopy and compared to the characteristics of untreated control: thallus thickness, upper cortex thickness, medula thickness, diameter of cord.

Primary photosynthesis of Trebouxia erici in response to subzero temperature

The culture of *T. erici* was cultured on BBM liquid medium before experiment. Then, the culture was filtered through a filter paper and placed into a Kryo-Planer unit (United Kingdom) where cooled from 20 to −25°C at a constant rate of 2°C min^{-1}. During the cooling, chlorophyll fluorescence parameters F_V/F_M, Φ_{PSII} were measured by a PAM 2000 fluorometer (Walz, Germany). Repetitive saturation pulses of 5000 μmol (photons) m^{-2} s^{-1} (0.8 s) were applied each 30 s on dark-adapted sample to evaluate F_V/F_M. For Φ_{PSII} evaluation, actinic light of 30 μmol (photons) m^{-2} s^{-1} PAR was provided and combined with the repetitive saturation pulses.

RESULTS AND DISCUSSION

Rapid freezing of *U. sphacellata* and *U. aurantiaco-atra* resulted in statistically-significant changes in some anatomical parameters. Upper cortex thickness, thallus thickness and cord diameter responded most sensitively in both species. These parameters increased after rapid freezing which can be attributed to volume growth of frozen water (compared to liquid). The freezing of liquid water increased size of the thallus structures especially those formed by densely arranged hyphae. In contrast, medula, typical by large intercellular spaces did not show any freezing-induced increase the size (thickness) in both species.

In *T. erici*, F_V/F_M decreased with decreasing sample temperature forming a tri-phasic curve S curve. On the curve, the three phases were distinguished: Phase I: an initial linear decrease found at the sample temperature decreasing from 20 to 8°C, phase II: typical of more or less constant F_V/F_M value (0.32, from -11 to 8°C, and phase III: a decrease to close-to-zero values of F_V/F_M found at −25°C. Similar phases were distinguished for Φ_{PSII}, with more pronounced decrease from 20 to 8°C. The decrease in F_V/F_M and Φ_{PSII} with temperature decrease is associated with increased limitation of PSII functioning. Photosynthetic linear electron flow and utilization of ATP, NADPH in primary photosynthesis products formation (Genty et al. 1989) are limited in low temperature. Remarkable decrease in F_V/F_M and Φ_{PSII} values recorded in the range -12°C to -25°C can be explained by the formation of crystallization cores and the formation of ice crystals, which takes place in nucleation temperature. The temperature of ice nucleation is, however, species-specific. For *Trebouxia* sp., the range of -12°C to -16°C is reported by Kvíderová et al. (2013). The critical temperature for F_V/F_M and Φ_{PSII} was found similar, *i.e.* -25 °C, as for majority of polar lichens (Hájek et al. 2016) and symbiotic algae of genus *Trebouxia* (Hájek et al. 2012).

CONCLUSIONS

In conclusion, *U. sphacellata* and *U. aurantiaco-atra* are resistant to freezing even if cooled in hydrated state. However, negative changes to photosynthetic processes in symbiotic alga are apparent when the lichens are shock frozen. Shock freezing also causes voluntometric changes of lichen thallus and potential mechanical injury. During gradual cooling *U. sphacellata* and *U. aurantiaco-atra* potential (F_V/F_M) and actual (Φ_{PSII}) primary photosynthetic processes decline in a curvilinear manner with temperature fall from 20 to -30 °C (critical temperature).

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