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Photosynthetic electron transport at low temperatures in the green algal foliose lichens *Lasallia pustulata* and *Umbilicaria hirsuta* affected by manipulated levels of ribitol

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Abstract

In lichens, ribitol is known as a carbon storage compound, an osmotic agens involved effectively in cell compartments protection during dehydration of lichen thalli and as a cryoprotective compound. In our study, we investigated the effect of ribitol on photochemical processes of photosynthesis in foliose lichens [*Lasallia pustulata* (L.) Mérat*., Umbilicaria hirsuta* (Sw. ex Westr.) Hoffm.] at low temperature. The effects of three concentrations of ribitol, added externally to thalli segments on several chlorophyll (Chl) fluorescence parameters, were evaluated. The 72 h exposition to 8, 16, and 26 mM ribitol led to a concentration-dependent increase in F_V/F_M, decrease in non-photochemical quenching (NPQ) but no change in quantum yield of photosystem II photochemistry (Φ_{PSII}) values at –5 °C). At higher temperature (0, +5 °C), no effect of ribitol addition on the photosynthetic parameters was apparent.

Additional keywords: chlorophyll fluorescence imaging; freezing tolerance; photosystem II; polyols.

Introduction

Lichens are organisms with a high tolerance to subzero temperatures, which helps them to thrive and survive in high mountains and polar regions (Kappen 2000). Mechanisms of lichen tolerance to freezing temperature are recently a subject of many studies targeting to *e.g.* re-crystallization inhibitor activity (Doucet *et al.* 2000). Due to their osmotic activity, polyols (sugar alcohols) are reported as effective cryopreserving (Wynn 1990) and protective compounds during lichen thalli desiccation (Wasley 2004). In higher plants, similarly, polyols act as cryoprotectants, as osmotic regulators in drought and salt stressed plants, and as antioxidants (Popp and Smirnoff 1995).

Polyols help lichens to maintain their physiological activity at sub-zero temperatures. Natural concentrations of polyols in lichen thalli are species-specific and dependent on site of collection (Roser *et al.* 1992a). The amount of polyols and sugars might reach up to 36 mg g^{-1} (DM) as reported by Roser *et al.* (1992b) for the Antarctic species *Umbilicaria decussata.* Some studies report seasonal dynamics in polyol contents in lichens (Legaz *et al.* 1986, Armstrong and Smith 1994). Actual amount of ribitol, as well as other polyols, depends also on thallus orientation and leaching due to rainfall (Dudley and Lechowicz 1987). Mannitol, ribitol, and arabitol are abundant in large quantities in lichen thalli (da Silva *et al.* 1993, Chapman *et al.* 1994, Haranczyk *et al.* 1998). Ribitol is the main high-energy compound exported from a symbiotic producer (alga) to a consumer (fungus) and its natural concentration in a lichen thallus ranges between 2 and 12 μg mg^{-1}(DM) (Armstrong and Smith 1994; Dahlman *et al.* 2003). In green plants, there are several possible biosynthetic pathways to polyols, including synthesis from the intermediates of the Calvin-Benson cycle and cytoplasmic synthesis from triose phosphate exported from chloroplast. It is, however, unclear which of these pathways is responsible for the production of ribitol in lichen symbiotic alga *Trebouxia* (Lines *et al.* 1989). It is known that ribitol is produced from sugar alcohols by *Trebouxia* (Richardson and Smith 1968, Armstrong and Smith 1994, Dahlman *et al.* 2003). In fungal hyphae, ribitol is transformed into mannitol,

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Abbreviations: Chl – chlorophyll; DM – dry mass; DRY – dry pre-treatment; F_V/F_M – potential quantum yield of PSII photochemistry; NPQ – non-photochemical quenching; WET – wet pre-treatment; Φ_{PSII} – effective quantum yield of PSII photochemistry.
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which serves as energy source for maintenance and growth of fungal hyphae, as well as production of fungal-specific compounds (Palmqvist 2000), such as *e.g.* pigments (Solhaug and Gauslaa 2004). Limited number of experiments that focused on cryoprotective effects of ribitol on physiological processes of symbiotic algal cells has been made so far (Fontaniella *et al.* 2000).

In the present study, we tested a range of ribitol concentration added to lichen thalli exposed to low and subzero temperature. Using Chl fluorescence technique, we detected early response of lichen photobiont, its primary photosynthetic processes in particular, to addition of ribitol. The aim of the study was to address the inter-specific differences between two foliose lichens [*L. pustulata* (L.) Mérat*., U. hirsuta* (Sw. ex Westr.) Hoffm.] in response to the ribitol addition. We hypothesised that apart from general inter-specific differences, the two species grown at and collected from the same location and thus resembling environmental conditions, may exhibit similar response of photosynthetic parameters to the addition of polyols. We tested the hypothesis that the added ribitol maintains primary photochemical processes active at subzero temperatures in lichen thalli, while the untreated thalli exhibit temperature-dependent inhibition. Since temperature was one of the experimental factors, the choice of temperature-sensitive parameters (F_V/F_M, Φ_{PSII}, NPQ) was made according to our previous studies (Hájek *et al.* 2001, Roháček and Barták 1999, Hájek *et al.* 2006).

Materials and methods

Samples collecting and handling: Thalli of foliose lichen species *L. pustulata, U. hirsuta*, both with *Trebouxia* as a photobiont, were collected from granitic rocks in the valley of the river Chvojnice in a close vicinity of Ketkovice, 35 km W of Brno. In the field, both species undergo a wide range of temperatures typical by minimum in January (Ketkovice station: long-term month mean air temperature of –3.1 °C; source: Amet.cz). Collected thalli samples were stored at +5 °C and low irradiance of 5 μmol m^{-2} s^{-1} provided by a *NARVA LT 36W/640-020* (Brand-Erbisdorf, Germany). Before the experiment, thalli of both studied species were placed between two sheets of filter paper sprayed regularly for 24 hours to reach optimally-hydrated state. The hydration status was checked by a *FluorCam 700MF* fluorometer (*Photon Systems Instruments*, Czech Republic) using a Chl fluorescence imaging (CFI) – for application in lichens *see* Barták *et al.* (2000). When a particular thallus reached maximum F_V/F_M, the thallus was considered optimally-hydrated. After hydration, circular segments (1.5 cm^{-2}) were cut from the thalli and used for the experiments.

Application of ribitol: The segments exhibiting highest F_V/F_M were randomly divided into two groups used for the experiment. To test the effect of thallus dehydration/hydration on the uptake and the impact of ribitol on function of photosynthetic apparatus of symbiotic *Trebouxia*, we applied water-dissolved ribitol into dry and wet lichen thalli. The first group, denoted as DRY in the following text, was first dried out in a silicagel-based desiccator for 8 h to reach constant dry mass, and then exposed to the below-specified concentrations of water-dissolved ribitol. The second group, denoted as WET, remained in the optimally-hydrated state. Into both groups, water solutions of ribitol in concentrations of 0 (control, demineralized water), 8, 16 and 26 mM were applied and left for 3 h before the temperature treatment to allow a penetration of the respective solutions into the thalli segments. The three ribitol concentrations were selected according to naturally-occurring (Roser *et al.* 1992b) and experimentally used concentrations (Solhaug and Gauslaa 2004). The thalli segments of *U. decussata* and *L. pustullata* were then placed on a filter paper (wetted with a particular ribitol solution) into Petri dishes and treated at +5, 0 and –5 °C (cultivator *LABIO*, Czech Republic) for 60 h. They were exposed to 15 μmol m^{-2} s^{-1} PAR (source: 100 W bulbs). During exposition, the thalli segments were aerated by regular opening of Petri dishes (each 12 h for 2 min) to assure availability of ambient CO_2 and O_2 concentration. Constant air temperature and relative humidity, were maintained and monitored by *HOBO* data loggers (*OnSet Computers*, Pocasset, MA, USA).

Chl fluorescence measurements: The effects of ribitol addition and temperature treatments on primary photosynthetic processes of lichens, their algal photobionts, respectively, were evaluated using Chl fluorescence parameters measured with a portable Chl fluorescence imaging system (*HFC–010, Photon Systems Instruments*, Brno, Czech Republic). For each segment, background fluorescence (F_0), maximum fluorescence on dark-adapted sample (F_M), maximum fluorescence on irradiance-adapted sample (under irradiance; F_M') and steady-state fluorescence during irradiation (F_S) were measured (Roháček 2002). The measurements were done each 12 h for the following 3 days using routine protocols (Hájek *et al.* 2006) and instrumental set up (measuring irradiance: 5 μmol m^{-2} s^{-1}; actinic irradiance: 210 μmol m^{-2} s^{-1}; saturation pulse: 1 850 μmol m^{-2} s^{-1}). For the evaluation of the effects of ribitol addition and temperature treatment on primary photosynthesis, potential quantum yield of PSII photochemistry [$F_V/F_M = (F_M - F_0)/F_M$; Krause and Weis 1991], effective quantum yield of PSII photochemistry [$\Phi_{PSII} = (F_M' - F_S)/F_M'$;

Genty *et al.* 1989] and non-photochemical quenching [NPQ = ($F_M - F_M'$)/F_M'; Schreiber *et al.* 1995] were used.

Statistical analysis: The statistically significant effects of individual factors involved in the experiment (ribitol concentration, application of ribitol into DRY and/or WET thallus, respectively, experimental temperature) were evaluated by a multivariate analysis of variance. Statistically significant differences between treatments were tested by Scheffé test ($\alpha = 0.05$) with *Statistica v 6.0* (*Jandel Scientific*, San Francisco, USA).

Results

Photosynthesis in response to temperature: Temperature effect on F_V/F_M was not apparent, except of samples with no addition of ribitol at low temperature of –5 °C (Fig. 1). Throughout the experiment, F_V/F_M was constant, reaching a value over 0.8, which indicated unaffected functioning of PSII. It was true for both *L. pustulata* and *U. hirsuta,* however, slight difference between the species was seen in F_V/F_M values recorded at the end of the experiment (data not shown).

Contrastingly to F_V/F_M, Φ_{PSII} increased with rising temperature, from about 0.25 recorded at –5 °C to 0.47 (*L. pustulata*) and 0.41 (*U. hirsuta*) recorded at +5 °C (Fig. 2). Irrespective of ribitol treatment, the Φ_{PSII} values recorded for a certain experimental temperature remained more or less constant throughout the time of exposition. However, statistically significant differences in Φ_{PSII} were found between the samples treated with different ribitol concentrations.

Effect of ribitol on Chl fluorescence parameters: Addition of ribitol increased F_V/F_M values above untreated control only in thalli segments exposed to –5 °C. No significant effect of ribitol on F_V/F_M, Φ_{PSII} and NPQ was observed at 0 and +5 °C, respectively. At –5 °C, presence of 26 mM ribitol maintained F_V/F_M at control level, while thalli treated with low ribitol concentration

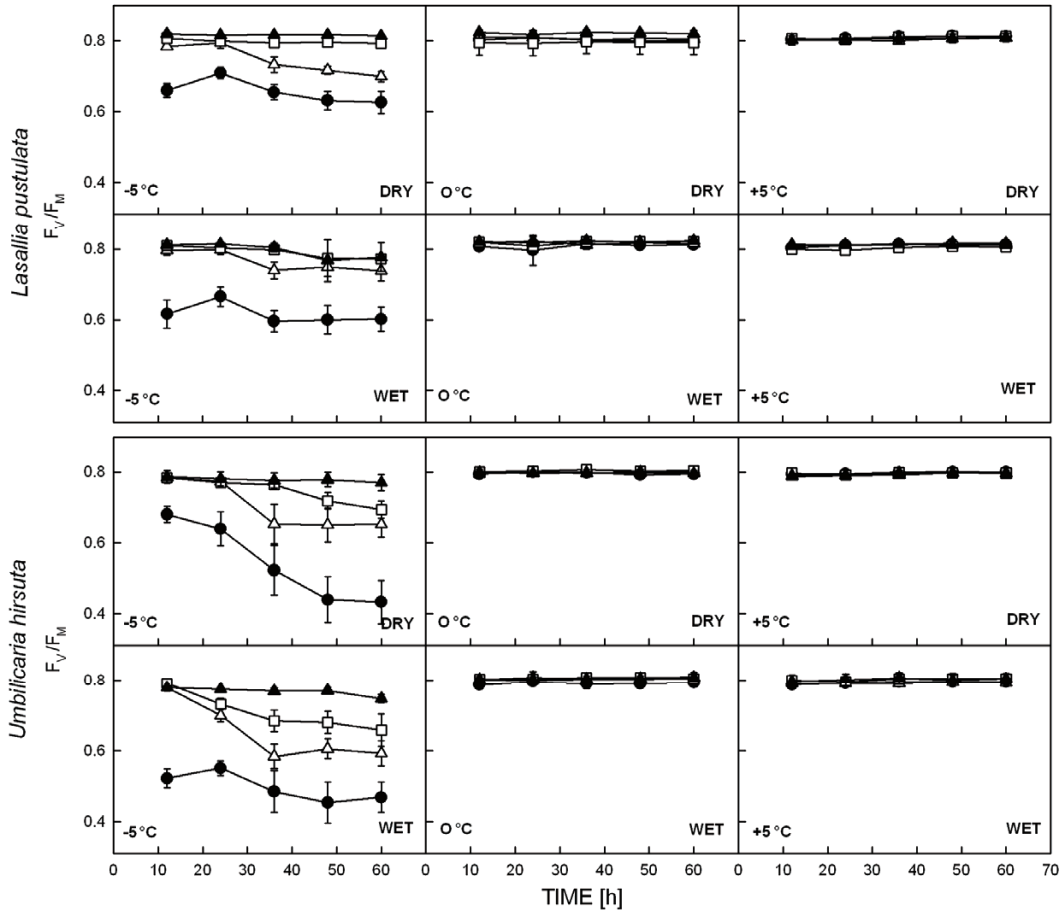

Fig. 1. Time courses of potential quantum yield of PSII (F_V/F_M) in *Lasallia pustulata* (*upper panels*) and *Umbilicaria hirsuta* (*lower panels*) in control: ●, and affected by ribitol concentration of 8 mM: △, 16 mM: □, 26 mM: ▲. The concentrations were applied onto the DRY and WET thalli segments at temperature of –5 °C, 0 and +5 °C. Data points are means of at least 5 replicates ± SD.

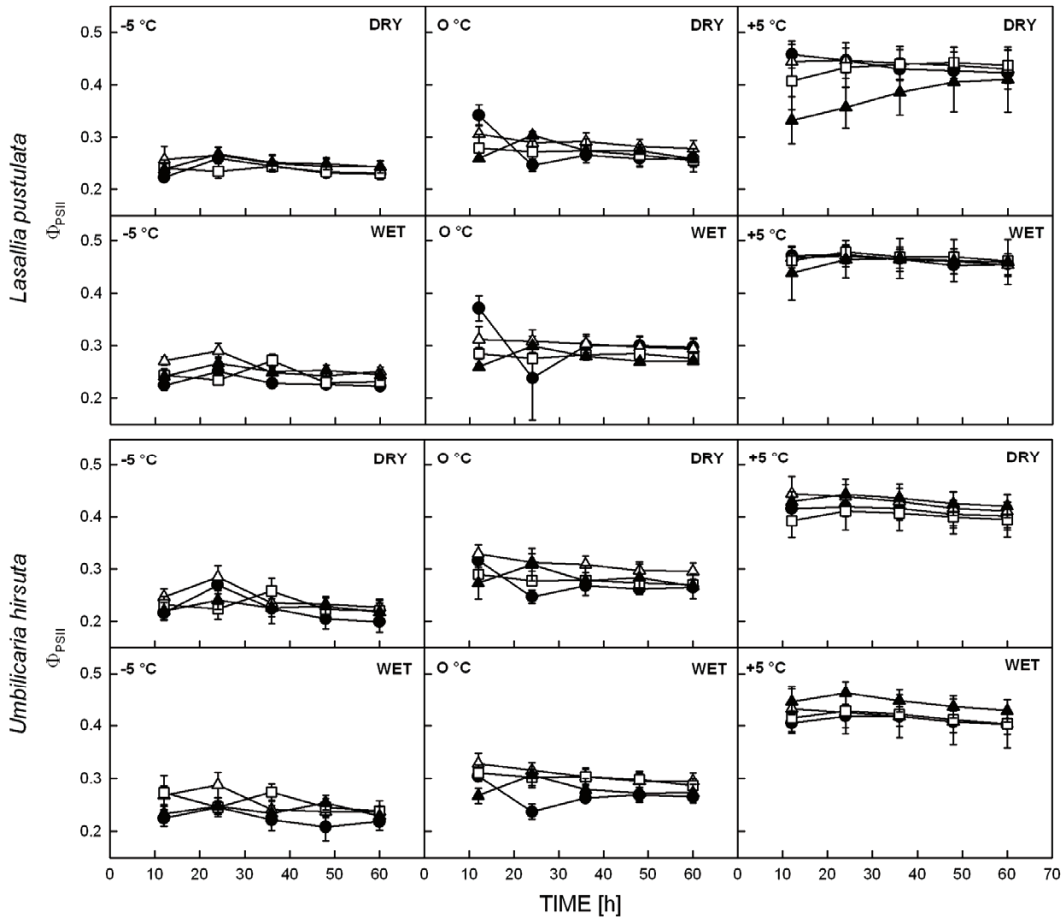

Fig. 2. Time courses of effective quantum yield of photosystem II (Φ_{PSII}) in *Lasallia pustulata (upper panels*) and *Umbilicaria hirsuta* (*lower panels*) in control: ●, and affected by ribitol concentration of 8 mM: △, 16 mM: □, 26 mM: ▲. The concentrations were applied onto the DRY and WET thalli segments at temperature of –5 °C, 0 and +5 °C. Data points are means of at least 5 replicates ± SD.

exhibited decrease in F_V/F_M with time of exposition (Fig. 1). This response was similar for both species, since the highest ribitol concentration caused no change in F_V/F_M (0.81 for *L. pustulata.* and 0.78 for *U. hirsuta*). The first signs of F_V/F_M decline in 8 and 16 mM treatment (Fig.1 A) were apparent after 36 h of exposition. The decline was more pronounced with time of exposition. There were, however, no significant differences in Chl fluorescence parameters between ribitol treatments applied in DRY and WET thalli. Contrastingly to F_V/F_M courses, no effect of added ribitol on Φ_{PSII} was found (Fig. 1,2).

The effect of ribitol application on changes in non-photochemical quenching (NPQ) was apparent particularly at –5 °C (Fig. 3). In the control, we recorded NPQ values of 2.2 (*L. pustulata*) and 4.1 (*U. hirsuta*) for fully-hydrated samples. NPQ values for pre-dried samples of *L. pustulata* were slightly lower (1.93). For samples treated with 26 mM ribitol, lower values of NPQ (1/4 and 1/40 of the control for *L. pustulata*, and *U. hirsuta*) were recorded.

Discussion

Addition of ribitol led to an increase of F_V/F_M in both experimental species only at –5 °C, while no response was apparent at other temperatures. The effect might be associated with a cryopreserving function of sugar alcohols in plants and symbiotic organisms from cold habitats. It is well established that sugar alcohols are found in *e.g.* Antarctic lichens. Chapman *et al.* (1994) detected them in *Candelariella hallettenensis* and *Usnea antarctica* with dominating amount of arabitol, mannitol and ribitol. Their cryoprotective action is based on blocking the formation of ice crystals by steric mismatch of hydrogen bonds which may be formed between them and water. Obviously, the overall concentration of polyols in lichen thalli is not sufficient to decrease

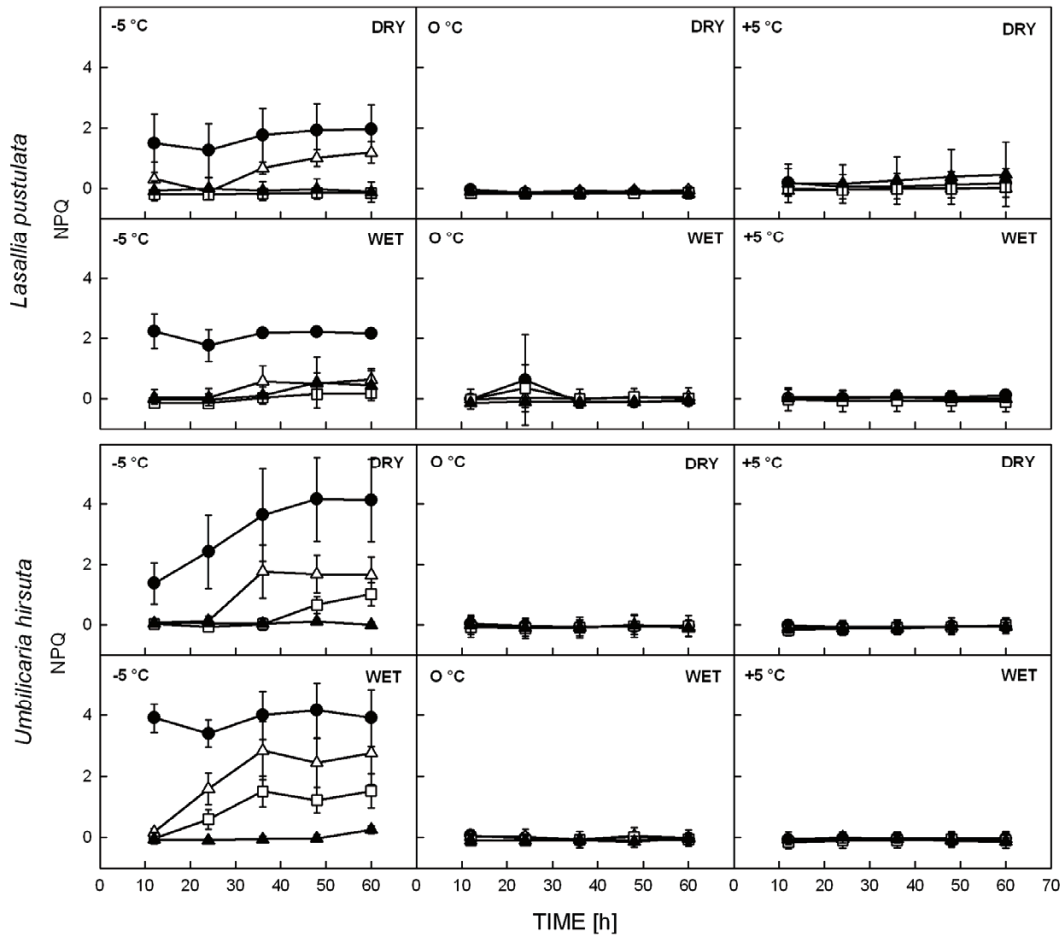

Fig. 3. Time courses of non-photochemichal quenching (NPQ) in *Lasallia pustulata* (*upper panels*) and *Umbilicaria hirsuta* (*lower panels*) in control: ●, and affected by ribitol concentration of 8 mM: △, 16 mM: □, 26 mM:▲. The concentrations were applied onto the DRY and WET thalli segments at temperature of –5 °C, 0 and +5 °C. Data points are means of at least 5 replicates ± SD.

a freezing point of cellular aqueous medium, if they are localized mainly in intracellular spaces. They may, however, contribute to the frost protection mechanism in lichens (Haranczyk *et al.* 2003).

Due to their molecular size, sugar alcohols are considered extracellular cryopreservatives (Pichugin 1993). In lichens, ribitol present in a chloroplast stabilizes structure of PSII and perhaps also promotes availability of dissolved sugars that may serve as energy source for maintenance- and repair-related synthesis of PSII components (Palmqvist 2000). Our experimental design, however, did not allow us to evaluate the amount, if any, of the added ribitol that reached chloroplast and/or photobiont cells. We may, however, expect that added ribitol at least improved availability of ribitol for fungal partner's carbon metabolism. On the other hand, the effects of administered ribitol on photo- and mycobiont, respectively can not be distinguished. Therefore, the effects of ribitol addition on Chl fluorescence parameters can not be attributed exclusively to ribitol-affected photobionts.

Experimental addition of ribitol in DRY *versus* WET thallus played only a minor role and did not affect primary photosynthetic processes significantly. However, the temperature-induced changes in Chl fluorescence parameters were slightly more pronounced if ribitol was applied into a WET thallus (Fig. 1,3). This was probably due to altered morphology of a lichen thallus in dry/wet state. In a WET thallus, faster transport of polyol molecules to the photobiont cells might be expected. From the results follows that potential quantum yield of PSII (F_V/F_M) was affected by ribitol addition (Fig. 1). On the other hand, actual photochemical processes (Φ_{PSII}) reflecting the rate of electron flow through PSII and consequent electron carriers remained unaffected. This might be attributed to an indirect effect of polyols on photosynthetic processes in thylakoid membrane. Added polyols have osmotic effect and keep cell protoplast alive under freezing temperature. Polyol molecules also stabilize membranes at freezing temperature and thus allow functioning of pigment and protein components of photosynthetic linear electron transport chain. In our study, however, the lack of direct effect on Φ_{PSII} was apparent. It seems that potential photochemical processes

in PSII were affected, while the actual processes were not. This is not a general response. In many poikilohydric organisms (lichens, mosses) experiencing low temperature stress, F_V/F_M and Φ_{PSII} show different response. Hájek *et al.* (2001) and Deltoro *et al.* (1999) *e.g.* reported no change in F_V/F_M accompanied by a progressive decrease in Φ_{PSII} with fall in temperature towards –10 °C for *Cetraria islandica* and *Leucodon sciuroides*. In our study, there might be several possible reasons for different response of F_V/F_M and Φ_{PSII} to ribitol addition at below-zero temperature. Among them, regulation of PSII functioning by the actual rate of ATP and NADPH consumption by dark reactions of photosynthesis must be mentioned. Such an explanation considers the above rates unaffected by ribitol concentrations. In this concept, low temperature represents the major factor limiting light-adapted processes of photosynthesis irrespective of ribitol treatment. However, no effect of ribitol on Φ_{PSII} might be also related to the action of ribitol in the exposed thalli. Perhaps, too short time of exposition or too low ribitol dose might be the reason for no effect on Φ_{PSII}. Therefore, the highest ribitol concentration used in this study (26 mM) might not be necessarily effective on primary photochemical processes of photosynthesis and thus inducing no change in Φ_{PSII}. The question arises what the effective concentration of polyols in thalli under natural conditions is. In laboratory experiments, individual control thalli may differ in their polyol content due to field conditions they experienced before collection. In our experiment, control thalli treated solely with demineralized water could potentially experience *e.g.* osmotic stress and/or be depleted of polyols leaching from intrathalline extracellular spaces.

From Fig. 3 follows that at –5 °C addition of ribitol decreased NPQ values in contrast to control untreated thalli segments that showed gradual increase with the time of exposition. Such effect might be explained by stabilizing role of ribitol for all compartments of symbiotic algal cells and thus maintenance of low NPQ values typical for non-stressed lichen thalli.

Multifunctional role of polyols in a lichen thallus might be a cause of difficulties in the effort to find out effective concentrations. Polyols serve for example as an energy source for synthesis both in photobiont and mycobiont, which may alleviate the direct effect, if any, on photosynthesis. Altered rate of pigment synthesis, such as parietin, in ribitol-treated lichens (Solhaug and Gauslaa 2004) and other secondary metabolites, such as antraquinones (Brunauer *et al.* 2007) may support multifunctional role and utilization of polyols in a lichen thallus. The other reason for no change in Φ_{PSII} in ribitol-treated lichens might be the interaction of low temperature effects with osmotic effects. This could be another cause of the above-described response of F_V/F_M and Φ_{PSII} to ribitol addition at low temperature.

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