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Cryoproective role of ribitol in *Xanthoparmelia somloensis*

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Abstract

Thalli of *Xanthoparmelia somloensis* with natural content of polyols (control) and polyol-free thalli (acetone-rinsed) were used to study ribitol effects at low temperatures. Thalli segments were cultivated in ribitol concentration of 32 or 50 mM for 168 h at temperatures +5, 0, and -5 °C. The chlorophyll fluorescence parameters (potential yield of photochemical reactions in PS 2 (variable to maximum fluorescence ratio, F_v/F_m), effective quantum yield of photochemical reactions in PS 2 (Φ_{PS2}), and non-photochemical quenching (NPQ) were monitored in 24-h intervals using an imaging system. The effect of 32 mM ribitol on F_v/F_m and Φ_{PS2} was apparent only at -5 °C, however, the effect was seen throughout the whole exposure. Surprisingly, 50 mM ribitol concentration treatment led to a decrease in F_v/F_m and Φ_{PS2} and to an increase in NPQ values at -5 °C, while no change was observed at 0 °C and +5 °C. Acetone-rinsing caused decrease of F_v/F_m, Φ_{PS2} and NPQ.

Additional key words: chlorophyll fluorescence imaging, fluorescence parameters, lichen thallus, low temperature, *Trebouxia*.

Introduction

Polyols (sugar alcohols) serve in lichen thalli as energy storing sugars and they are transported from algal partner to a mycobiont. In mycobiont, they serve as the source for growth and maintenance of lichen biomass and biosynthesis of fungal secondary metabolites. Polyols are also osmotically-active compounds which are involved in maintenance of physiological activity at freezing temperatures. Among polyols, mannitol and ribitol are abundant in lichen thalli (Da Silva *et al.* 1993, Chapman *et al.* 1994). Ribitol is synthesized by symbiotic green algae. It is a high-energy compound exported from alga to fungus. Obviously, its natural concentration in a lichen thallus ranges from 2 to 7 mg g^{-1}(d.m.) (Feige and Jensen 1992, Armstrong and Smith 1994, Dahlman *et al.* 2003). In fungal hyphae, ribitol is transformed into mannitol by a pentose phosphatase pathway (Lines *et al.* 1989). Mannitol serves as the energy source for maintenance and growth of fungal hyphae as well as for production of fungal-specific compounds (Palmqvist 2000), such as pigments (Solhaug and Gauslaa 2004), lichen acids (McEvoy *et al.* 2006, Takahagi *et al.* 2006, Hager *et al.* 2008), or physcion-derived compounds (Brunauer *et al.* 2007). Ribitol is considered a marker of carbon pool in a green algal photobiont, and CO_2 assimilation rate. Mannitol is considered a specific marker of fungal storage pool of carbon (Feige 1978, Sturgeon 1985). Arabitol, another polyol found frequently in lichen thalli (*e.g.* Richardson and Smith 1968, Armstrong and Smith 1994), is attributed to as a fungal partner and considered a product of fungus metabolism (Armstrong and Smith 1998). High arabitol content is typical for some fungal species (Tsai *et al.* 2008). High arabitol contents might indicate either increased demand for maintenance costs and synthesis of secondary compounds in a fungal partner or, on the contrary, inhibited consumption of arabitol in such processes. Such an inhibition may be caused by *e.g.* long-lasting exposition of lichen to stress (Rankovic *et al.* 2007).

Natural concentrations of polyols in lichen thalli are species-specific and dependent on many factors. Among them, site of collection plays a role. Roser *et al.* (1992a) reported varying ribitol and mannitol concentrations up to 10 and 19 %, respectively, of total soluble sugars in thalli of Antarctic lichen species collected in different sites.

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Abbreviations: Car - carotenoids, Chl - chlorophyll, F_v/F_m - variable to maximum fluorescence ratio (potential yield of photochemical reactions in PS 2), NPQ - non-photochemical quenching of chlorophyll fluorescence; Φ_{PS2} - effective quantum yield of photochemical reactions in PS 2.
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Concentration of polyols in lichen thalli showed also depencence on thallus orientation (Dudley and Lechowicz 1987), season (Armstrong and Smith 1994, Legaz *et al.* 1986), and day time (Aubert *et al.* 2007). It is also dependent on frequency of hydration/dehydration cycles as shown earlier by *e.g.* Farrar (1976). Amount of total polyols and sugars in lichen thalli vary in different species and might reach up to 36 mg g^{-1}(d.m.) as reported by Roser *et al.* (1992b) for *Umbilicaria decussata* grown in maritime Antarctica. Unfortunately, only few studies have yet investigated natural concentration of ribitol in green algal lichens (Armstrong and Smith 1994, Dahlman *et al.* 2003). Also a number of studies focused on their cryoprotective effect at subzero temperatures is very limited (*e.g.* Fontaniella *et al.* 2000). In our previous study (Hájek *et al.* 2009), we showed that addition of ribitol improved photosynthetic activity of two foliose lichen species (*Lasallia pustulata* and *Umbilicaria hirsuta*) at freezing temperature. In recent experiments, we have focused on utilization of ribitol in synthesis of lichen secondary metabolites. Therefore, the methods of acetone rinsing of experimental lichen thalli was used in order to remove intrathalline secondary metabolites without affecting photosynthesizing pigments. Such approach allowed us to study both the ribitol role in freezing tolerance as well as carbon source for secondary compound synthesis. Since acetone rinsing removes secondary metabolites, we expected that additional carbon source (ribitol) would be preferentially used for the secondary metabolites synthesis rather than freezing tolerance. We hypothesised that experimentally created pool for carbon in acetone-rinsed thalli would cause lower freeze resistance, in terms of inhibition of primary photosynthetic processes, in acetone-rinsed thalli than in control thalli.

Material and methods

Sample collection and handling: Thalli of foliose lichen species *Xanthoparmelia somloensis* (Gyelnik) Hale, with *Trebouxia* as a photobiont, were collected close to the Brno Lake dam, Czech Republic, in November 2006. The thalli were cleaned from debris and dried. Then, a half of the selected segments were subjected to acetone rinsing (for method see McEvoy *et al.* 2006) before experimental treatment by temperature and ribitol addition. In acetone-rinsed (AR) lichen segments, majority of secondary metabolites, such as *e.g.* usnic acids (McEvoy *et al.* 2006, Roach *et al.* 2006), barbatic acid (Elix and Wardlaw 2000), gyrophoric acid and stenosporic acid (Candan *et al.* 2006) were extracted while pigment content in lichen thallus remained unchanged (see Fig. 1). We tested the hypothesis that re-synthesis of such secondary metabolites would increase a demand for ribitol. Untreated (non-AR) and acetone-rinsed (AR) thalli were exposed to 32 mM (5 g dm^{-3}) or 50 mM (7.8 g dm^{-3}) ribitol for 10 min, left in closed Petri dishes under dim light for 24 h, and then exposed to the respective ribitol concentration for 10 min again. Such approach allowed penetration of the ribitol solutions into the thalli segments. Control (C) thalli were supplied with demineralized water.

Fig. 1. *Xanthoparmelia somloensis.* AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not.

Thalli segments of C, non-AR and AR treatments were then closed into Petri dishes using *Parafilm* and cultivated at -5, 0, and +5 °C (*LABIO*, Prague, Czech Republic) under 300 μmol m^{-2} s^{-1} of photosynthetically active radiation for 168 h. Every 24 h, parameters of chlorophyll fluorescence were measured using a *HFC-010* portable fluorometer (*Photon Systems Instruments*, Brno, Czech Republic). During cultivation, the Petri dishes were opened very shortly and lichen thalli were sprayed several times by demineralised water.

Chlorophyll fluorescence imaging: Primary photosynthetic processes of lichen segments were evaluated using chlorophyll fluorescence imaging method (for its application in lichens see: *e.g.* Barták *et al.* 2003, 2007). The method allows to measure dark-adapted background fluorescence (F_0), maximum fluorescence on dark-adapted sample (F_m), maximum fluorescence on light-adapted sample (F_m'), and steady-state fluorescence during irradiation (F_s) from Chl fluorescence image recorded by a CCD camera during light-induced Kautsky kinetics. Using the above basic data, potential yield of photochemical reactions in PS 2 ($F_v/F_m = (F_m - F_0)/F_m$; Van Kooten and Snel 1990), effective quantum yield of photochemical reactions in PS 2 ($\Phi_{PS2} = (F_m' - F_s)/F_m'$; Genty *et al.* 1989), and non-photochemical quenching of chlorophyll fluorescence ($NPQ = (F_m - F_m')/F_m'$; Schreiber *et al.* 1995) were calculated. The measurements were repeated every 24 h in order to monitor the response of photosynthetic apparatus of the experimental lichen species to low temperature and ribitol treatments.

Biochemical analyses: Contents of ribitol, mannitol and arabitol and other non-structural saccharides (NSS) were evaluated before and after the experimental treatment

using HPLC. The lichen segments were deeply frozen in liquid nitrogen and freeze dried. Soluble sugars were extracted with 80 % ethanol (15 min, 75 °C), the solvent evaporated (*SpeedVac*) and the residue dissolved in *Mili-Q* ultrapure water (*Millipore*, Bedford, MA, USA; 10min, ultrasonic water bath). After centrifugation (14 000 *g* for 10 min), supernatants were filtered through membrane filters (*Millipore*, 0.45 μm). Until the analyses the samples were kept in a freezer at -20 °C. Analyses of extracted sugars were done by a HPLC with refractometric detection (*Spectra Physics*; refractometer *Shodex RI-71*; integrator *ChromJet*; pre-column *Hema-Bio 1000Q+SB*, column *IEX Pb* form (*Watrex*, Prague, Czech Republic) using the protocol of Vojtíšková *et al.* (2006a) slightly modified for lichens (Lipavská, unpublished). Similarly to polyols, contents of chlorophyll *a*, chlorophyll *b*, and total carotenoids were measured by a spectrophotometrical method (*Spekord*, Jena, Germany) according to Wellburn (1994).

Statistics: Statistical analysis was performed by one-way analysis of variance (*ANOVA*, *Statistica* v. *6* package, *StatSoft*, Tulsa, USA)

Results

In all the examined thalli segments, an apparent drop in potential yield of photochemical reactions in PS 2 (F_v/F_m) values was found after 24-h exposure to respective temperature (Fig. 2). Maximum decrease in F_v/F_m was found at -5 °C, while the decrease was much less pronounced at 0 and +5 °C (Fig. 3). Lower F_v/F_m values were usually found in control thalli than in acetone rinsed thalli segments (AR) and in non-AR. Generally, low F_v/F_m values in thalli exposed to -5 °C were more or less constant during the whole exposure. Mean F_v/F_m values calculated for the period of 24 - 72 h (data not shown) showed that AR had no effect on F_v/F_m. At 0 °C and +5 °C, no significant effect of particular ribitol concentrations on F_v/F_m was observed. At all tested temperatures, a stepwise decrease of F_v/F_m was detected during cultivation. Application of ribitol affected F_v/F_m values only at -5 °C. While positive effect on F_v/F_m was apparent in thalli treated with 32 mM ribitol, negative effect occurred when treated with 50 mM ribitol. Such response was particularly more pronounced in AR thalli than in non-AR thalli.

Effective quantum yield of photochemical reactions in PS 2 (Φ_{PS2}) showed similar response to temperature treatments as F_v/F_m. Absolute Φ_{PS2} values decreased with temperature. However, the rapidity of Φ_{PS2} decrease differed at particular temperature. While gradual Φ_{PS2} decrease was found at +5 °C, a rapid fall of Φ_{PS2} was recorded even after 24 h at -5 °C (Fig. 4). Positive effect of 32 mM ribitol on Φ_{PS2} was found at -5 °C in AR thalli while no such response was apparent in non-AR thalli. At 0 and +5 °C, no response to ribitol addition was found. Similarly to F_v/F_m, addition of 50 mM ribitol led to decrease in Φ_{PS2} when thalli were treated at -5 °C.

Ribitol addition induced significant changes of non-photochemical quenching (NPQ) at temperature of -5 °C (Fig. 5). Treatment with 32 mM ribitol (at -5 °C) did not cause any substantial changes of NPQ in both AR and non-AR thalli. Nevertheless, addition of 50 mM ribitol caused significant increase in NPQ values. AR had a significant effect on NPQ values only at -5 °C and 50 mM ribitol. For non-AR thalli, remarkably higher values of NPQ were found compared to AR ones.

In contents of chlorophyll (Chl) *a*, Chl *b*, and total carotenoids (Car) only non-significant differences among individual temperature treatments were found. However, in majority of cases, contents of Chl *a*, Chl *b* and Car increased with added ribitol in non-AR in each particular temperature treatment. In contrast, AR thalli did not show any clear response in Chl *a*, Chl *b* and Car contents to ribitol addition and temperature.

Table 1. Natural contents of polyols [mg g^{-1}(d.m.)] in different lichen species.

Lichen species	Ribitol	Mannitol	Arabitol	Source
Usnea sphacelata	2.2	3.1	26.0	Chapman
Umbilicaria decussata	2.8	9.0	15.0	*et al.* 1994
Usnea antarctica	5.3	5.0	55.0	
Xanthoria candelaria	5.5	29.0	22.0	
Candelariella hallettensis	1.4	6.0	10.0	
Pseudephebe minuscula	2.0	11.0	22.0	
Buellia frigida	8.8	2.2	31.2	Roser
Rinodina olivaceobrunnea	-	3.5	8.0	*et al.* 1992a
Evernia esorediosa	-	1.4	3.4	Hamada
Ramalina subbreviuscula	-	1.0	2.4	*et al.* 1994
Ramalina sublitoralis	-	0.4	2.0	
Hypogymnia physodes	3.7	10.8	31.6	Farrar 1976
Xanthoparmelia somloensis	2.6	4.1	35.2	this study

For each temperature, seemingly different response to addition of ribitol was seen in contents of saccharides and polyol (Table 2). In general, their contents decreased with increasing ribitol concentration at -5 °C. This was particularly typical for sucrose, glucose, fructose, ribitol and arabitol, while mannitol concentrations remained unchanged. At the temperature of 0 °C, intrathaline concentrations of saccharides and polyols remained more or less constant, irrespectively of ribitol addition. At +5 °C, however, either no significant change or slight decrease in saccharides and polyols was found. Such a decrease was more apparent for glucose. After ribitol addition and 126-h cultivation at -5 °C, polyol contents in thalli segments did not show any unambiguous response

and the mean values were similar to those determined before experiment [*cf.* 40.3 and 41.9 mg g^{-1} (d.m.)]. Ribitol content in ribitol-added thalli increased with ribitol dose only in those thalli cultivated at -5 °C (Table 2). In 0 and +5 °C, such trend was not apparent.

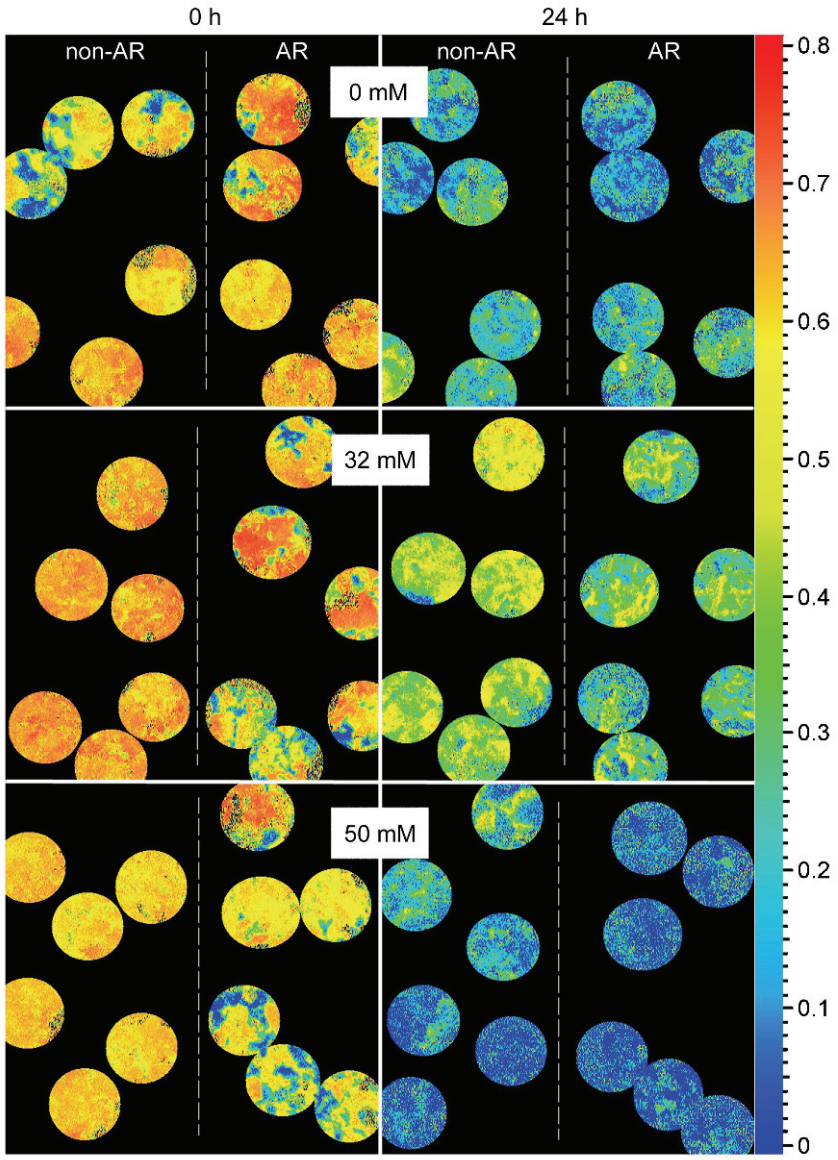

Fig 2. Heterogeneity of potential yield of photochemical reactions in PS 2 (F_v/F_m) over thalli segments of *Xanthoparmelia somloensis* before (0 h) and after 24-h cultivation after the addition of 0, 32, and 50 mM concentration of ribitol at -5 °C. AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not. F_v/F_m level is expressed in false colour scale (see a *bar on right*).

Discussion

Apart from carbon metabolism in lichens, polyols are essential compounds involved in lichen protection to freezing temperatures. This was well documented in our experiment, since positive effect of ribitol addition on F_v/F_m and Φ_{PS2} was found exclusively in thalli segments treated at -5 °C and 0 °C. However, concentration- and species-specific differences were experienced; *e.g* Hájek *et al.* (2009) found positive effect of 8, 16, and 26 mM ribitol concentration on F_v/F_m in *Lasallia pustulata* and *Umbilicaria hirsuta*. In contrast, in this study carried out on *X. somloensis* we showed that increase in ribitol concentration to 50 mM inhibited F_v/F_m in thalli treated at -5 °C. The same trend found for Φ_{PS2} (Fig. 4) may indicate that high ribitol concentrations in lichen thalli

may decrease both potential and actual photosynthetic processes in lichen symbiotic algae. The underlying mechanism for that is, however, unclear. Thus, ribitol may have positive effect on primary photosynthetic processes in lichens when added in the concentrations ranging between 8 - 32 mM. Such conclusion might be supported also by the decrease in NPQ caused by 8 - 32 mM ribitol (Hájek *et al.* 2009) while it was

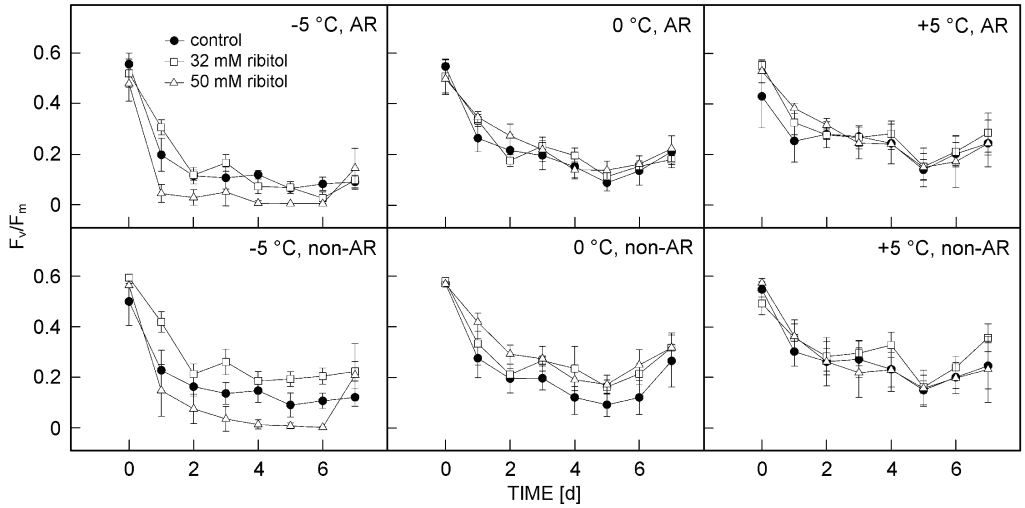

Fig. 3. Kinetics of potential yield of photochemical reactions in PS 2 (F_v/F_m) of *Xanthoparmelia somloensis* thalli segments treated with 0 (control), 32, and 50 mM ribitol solution and cultivated at -5, 0, and +5 °C. AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not. Data are mean of at least 6 replicates ± SD.

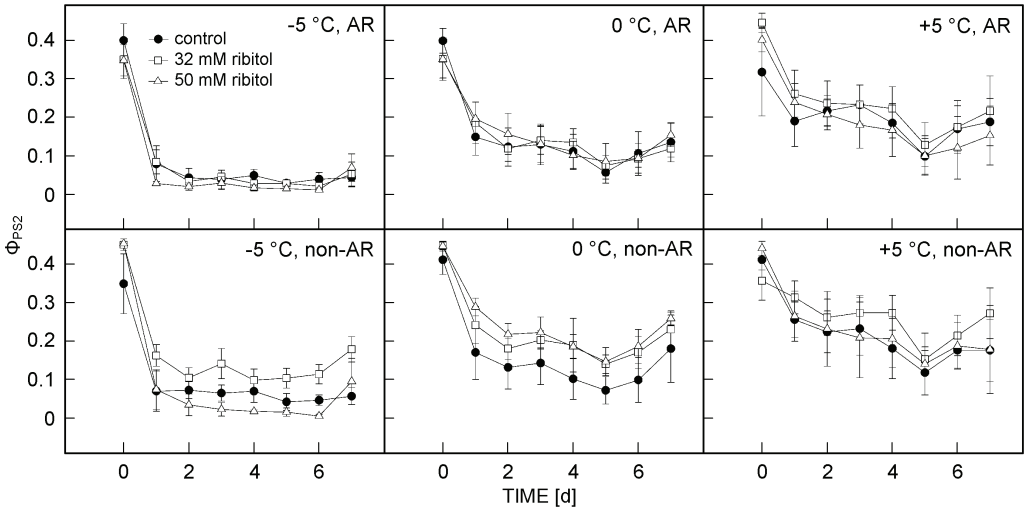

Fig. 4. Kinetics of effective quantum yield of photochemical reactions in PS 2 (Φ_{PS2}) of *Xanthoparmelia somloensis* thalli segments treated with 0 (control), 32, and 50 mM ribitol solution and cultivated at -5, 0, and +5 °C. AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not. Data are mean of at least 6 replicates ± SD.

increased by 50 mM ribitol (this study).

The sole effect of cultivation temperature on primary photosynthetic processes was manifested as an inhibition of F_v/F_m and Φ_{PS2} with temperature decrease (Figs. 3, 4). Decrease in F_v/F_m and Φ_{PS2} is well comparable results for a variety of lichen species taken from laboratory-based experiments (Hájek *et al.* 2001) and field measurements (Schlensog and Schroeter 2001). Generally, photosynthetic temperature optimum ranges in lichens within 14 to 22 °C (Hájek *et al.* 2001, Coxson and Coyle 2003) and -5 °C to -13.7 °C (Friedman and Sun 2005, Pannewitz *et al.* 2005, Reiter *et al.* 2008) for temperate and cold regions, respectively. Decrease in thallus temperature below this range causes inhibition of both photochemical (Barták *et al.* 2005, 2007) and biochemical (Lange 2003) processes of photosynthesis. Towards subzero temperature, however, many lichen species perform gradually decreasing but still substantial photosynthesis. This has been reported in gasometric field studies done in cold regions (Kappen *et al.* 1996).

Concentrations of ribitol and mannitol found in untreated thalli segments were well comparable to the values reported for variety of lichen species (Table 1). However, arabitol contents found in *X. somloensis* were higher than in the majority of species. The reason for such high arabitol content is unclear. It might be, however, speculated that it is associated with activity of fungal partner (see below). Nevertheless, total amount of sugar alcohols and soluble sugars in *X. somloensis* was always higher than 55 - 75 mg g^{-1}(d.m.) which may indicate high resistance to environmental stressors, low temperature in particular (*e.g.* Roser *et al*. 1992a,b). Similar scheme is reported for higher plants in which high content of polyols is considered a marker of plant response to abiotic stress (Stoop *et al.* 1996). It has been documented for example for wheat under salt stress that mannitol increased by the factor of more than 2 (Abebe *et al*. 2003). For fungi, specifically their fruiting bodies, even higher polyol contents are reported, *e.g.* Chang *et al.* (2001). High arabitol content found in *X. somloensis*

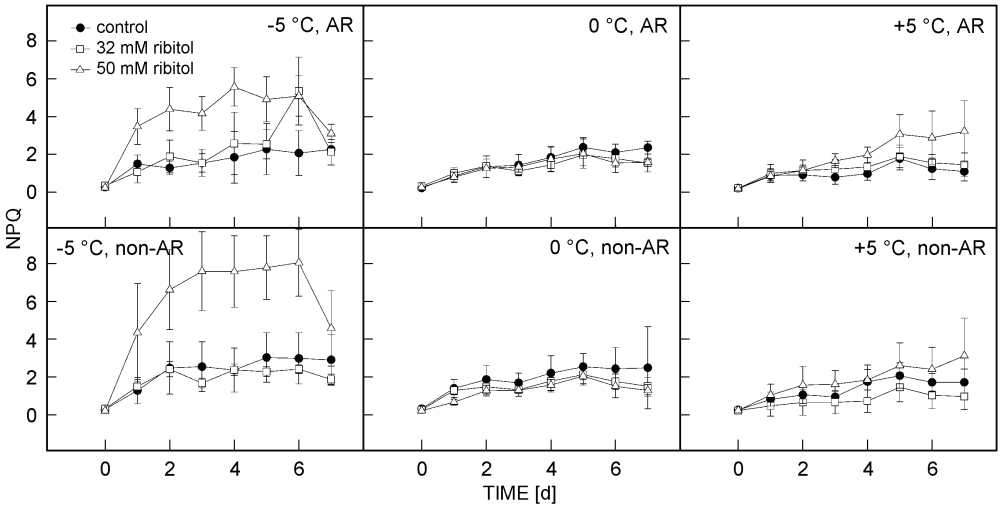

Fig. 5. Kinetics of non-photochemical quenching of chlorophyll fluorescence (NPQ) of *Xanthoparmelia somloensis* thalli segments treated with 0 (control), 32, and 50 mM ribitol solution and cultivated at -5, 0, and +5 °C. AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not. Data are mean of at least 6 replicates ± SD.

Table 2. Contents [mg g^{-1}(d.m.)] of saccharides and polyols of *Xanthoparmelia somloensis* thalli segments treated with 0, 32, and 50 mM ribitol solution and cultivated at -5, 0, and +5 °C. AR thalli underwent acetone rinsing before temperature and ribitol treatment while non-AR did not. *Abbreviations: d.e.* - detection error

Temperature	Contents	0 mM (control) non-AR	AR	32 mM non-AR	AR	50 mM non-AR	AR
-5 °C	sucrose	25.430±7.69	30.601±2.95	23.858±12.9	27.692±7.84	20.974±0.35	21.619±6.34
	glucose	9.351±3.97	13.908±6.73	7.046±5.57	10.671±7.58	16.737±8.76	9.215±4.85
	fructose	0.802±0.06	1.158±0.36	1.088±0.12	0.955±0.26	1.346±0.25	0.986±0.09
	ribitol	1.823±1.64	*d.e.*	2.640±1.09	3.623±1.95	3.195±0.91	2.175±0.37
	mannitol	2.362±1.11	5.731±1.70	4.824±1.08	5.327±0.94	5.024±0.47	5.634±0.14
	arabitol	32.404±3.10	47.800±10.8	29.394±4.83	25.158±0.50	35.484±3.73	25.676±4.55
0 °C	sucrose	16.138±1.93	28.871±7.85	17.105±4.86	24.506±9.22	12.710±0.66	27.458±5.04
	glucose	10.662±5.51	6.672±2.55	9.058±3.44	6.951±5.41	7.213±4.74	4.199±4.36
	fructose	1.297±0.13	1.253±0.12	0.973±0.20	1.060±0.38	1.144±0.16	1.106±0.07
	ribitol	1.646±0.46	2.945±0.78	2.308±0.69	2.786±1.28	1.763±0.48	2.612±0.69
	mannitol	5.990±0.80	7.472±1.29	5.613±1.15	6.502±0.53	5.667±0.55	8.673±0.87
	arabitol	30.376±3.21	31.025±6.31	31.083±4.30	26.572±4.55	25.458±5.66	28.386±3.43
+5 °C	sucrose	28.298±7.03	35.641±14.8	33.972±25.2	23.671±9.27	18.122±11.75	30.976±7.22
	glucose	22.064±6.93	13.589±5.46	10.391±5.65	11.939±6.21	13.252±5.75	10.122±3.55
	fructose	1.476±0.30	1.113±0.18	1.102±0.28	1.281±0.32	1.122±0.36	0.959±0.27
	ribitol	3.344±0.82	2.586±0.99	2.535±0.99	2.785±1.28	2.745±0.47	2.358±0.65
	mannitol	6.188±0.59	8.075±2.16	7.102±1.15	9.124±2.18	8.222±3.09	8.023±1.99
	arabitol	46.830±19.9	30.143±7.04	28.721±6.33	29.747±6.73	28.769±5.46	25.824±5.39

might be attributed to fungal partner metabolic activity. Data from our study and data available from literature showed that two groups of lichens can be distinguished: *1*) those having comparable amount of arabitol and mannitol resulting in arabitol/mannitol ratio close to 1, and 2) lichens with ten and more time higher arabitol than mannitol content.

Compared to control lichen segments, those pre-treated with AR did not exhibit any difference in their response to ribitol addition and cultivation at +5 °C and 0 °C, respectively. At freezing temperatures, however, ribitol-induced differences in F_v/F_m, NPQ were diminished and in Φ_{PS2} lacking. Such effects might be attributed to an increased demand of ribitol in AR thalli segments as a source for *de novo* synthesis of secondary compounds. In this concept, less ribitol should have been available for cryoprotection of the *X. somloensis* segments cultivated at -5 °C. Such an explanation, however, cannot be supported by ribitol content evaluation since both increase and decrease in ribitol content was found in AR compared to non-AR thalli. Such an ambiguous response to AR was apparent also for the pool of polyols and sugars (see Table 2). Therefore, further and more detailed analyses of utilization of externally added ribitol are demanded in future studies.

References

Abebe, T., Guenzi, A.C., Martin, B., Cushman, J.C.: Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity.- Plant Physiol. **131**: 1748-1755, 2003.

Armstrong, R.A., Smith, S.N.: The levels of ribitol, arabitol and mannitol in individual lobes of the lichen *Parmelia conspersa* (Ehrh ex Ach) Ach. - Environ. exp. Bot. **34**: 253-260, 1994.

Armstrong, R.A., Smith, S.N.: Does radial growth of the lichen *Parmelia conspersa* depend exclusively on growth processes at the lobe tip? - Environ. exp. Bot. **39**: 263-269, 1998.

Aubert, S., Juge, C., Boisson, A.M., Gout, E., Bligny, R.: Metabolic processes sustaining the reviviscence of lichen *Xanthoria elegans* (Link) in high mountain environments. - Planta **226**: 1287-1297, 2007.

Barták, M., Gloser, J., Hájek, J.: Visualized photosynthetic characteristics of the lichen *Xanthoria elegans* related to daily courses of light, temperature and hydration: a field study from Galindez Island, maritime Antarctica. - Lichenologist **37**: 433-443, 2005.

Barták, M., Váczi, P., Hájek, J., Smykla, J.: Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. - Polar Biol. **31**: 47-51, 2007.

Barták, M., Vráblíková, H., Hájek, J.: Sensitivity of photosystem 2 of Antarctic lichens to high irradiance stress: Fluorometric study of fruticose (*Usnea antarctica*) and foliose (*Umbilicaria decussata*) species. - Photosynthetica **41**: 497-504, 2003.

Brunauer, G., Hager, A., Grube, M., Türk, R., Stocker-Wörgötter, E.: Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. - Plant Physiol. Biochem. **45**:146-151, 2007.

Candan, M., Yilmaz, M., Tay, T., Kivanc, M., Turk, H.: Antimicrobial activity of extracts of the lichen *Xanthoparmelia pokornyi* and its gyrophoric and stenosporic acid constituents. - Z. Naturforsch. C. **61**: 319-323, 2006.

Chang, H.L., Chao, G.R., Chen, C.C., Mau, J.L.: Non-volatile taste components of *Agaricus blazei*, *Antrodia camphorata* and *Cordyceps militaris* mycelia. - Food Chem. **74**: 203-207, 2001.

Chapman, B.E., Roser, D.J., Seppelt, R.D.: C-13 NMR analysis of antarctic cryptogam extracts. - Antarct. Sci. **6**: 295-305, 1994.

Coxson, D.S., Coyle, M.: Niche partitioning and photosynthetic response of alectorioid lichens from subalpine spruce-fir forest in north-central British Columbia, Canada: the role of canopy microclimate gradients. - Lichenologist **35**: 157-175, 2003.

Da Silva, M.D.C., Iacomini, M., Jablonski, E., Gorin, P.A.J.: Carbohydrate, glycopeptide and protein components of the lichen *Sticta* sp. and effect of storage. - Phytochemistry **33**: 547-552, 1993.

Dahlman, L., Persson, J., Nasholm, T., Palmqvist, K.: Carbon and nitrogen distribution in the green algal lichens *Hypogymnia physodes* and *Platismatia glauca* in relation to nutrient supply. - Planta **217**: 41-48, 2003.

Dudley, S.A., Lechowicz, M.J.: Losses of polyol through leaching in Sub-Arctic lichens. - Plant Physiol. **83**: 813-815, 1987.

Elix, J.A., Wardlaw, J.H.: Lusitanic acid, peristictic acid and verrucigeric acid. Three new beta-orcinol depsidones from the lichens *Relicina sydneyensis* and *Xanthoparmelia verrucigera*. - Aust. J. Chem. **53**: 815-818, 2000.

Farrar, J.F.: Ecological physiology of the lichen *Hypogymnia physodes*. II. Effects of wetting and drying cycles and the concept of 'physiological buffering'. - New Phytol. **77**: 105-113, 1976.

Feige, G.B.: Probleme der Flechtenphysiologie. - Nova Hedwigia **30**: 725-774, 1978.

Feige, G.B., Jensen, M.: Basic carbon and nitrogen metabolism of lichens. - In*:* Reisser, W. (ed.): Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored. Pp. 277-299. Biopress, Bristol 1992.

Fontaniella, B., Vicente, C., Legaz, M.E.: The cryoprotective role of polyols in lichens: effects on the redistribution of RNAse in *Evernia prunastri* thallus during freezing. - Plant Physiol. Biochem. **38**: 621-627, 2000.

Friedmann, E.I., Sun, H.J.: Communities adjust their temperature optima by shifting producer-to-consumer ratio, shown in lichens as models: I. Hypothesis. - Microbiol. Ecol. **49**: 523-527, 2005.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **990**: 87-92, 1989.

Hager, A., Brunauer, G., Turk, R., Stocker-Woergoetter, E.: Production and bioactivity of common lichen metabolites as exemplified by *Heterodea muelleri* (Hampe) Nyl. - J. chem. Ecol. **34**: 113-120, 2008.

Hájek, J., Barták, M., Gloser, J.: Effects of thallus temperature and hydration on photosynthetic parameters of *Cetraria islandica* from contrasting habitats. - Photosynthetica **39**: 427-435, 2001.

Hájek, J., Váczi, P., Barták, M.: Photosynthetic electron transport at low temperatures in the green algal foliose lichens *Lasallia pustulata* and *Umbilicaria hirsuta* affected by manipulated levels of ribitol. - Photosynthetica **47**: 199-205, 2009.

Hamada, N., Okazaki, K., Shinozaki, M.: Accumulation of monosaccharides in lichen mycobionts cultured under osmotic conditions. - Bryologist **97**: 176-179, 1994.

Kappen, L., Schroeter, B., Hestmark, G., Winkler, J.B.: Field measurements of photosynthesis of umbilicarious lichens in winter. - Bot. Acta **109**: 292-298, 1996.

Lange, O.L.: Photosynthetic productivity of the epilithic lichen *Lecanora muralis*: long-term field monitoring of CO_2 exchange and its physiological interpretation II. Diel and seasonal patterns of net photosynthesis and respiration. - Flora **198**: 55-70, 2003.

Legaz, M.E., Avalos, A., De Torres, M., Escribano, M.I., Gonzáles, A., Martin-Falquina, A., Pérezurria, E., Vicente, C.: Annual variations in arginine metabolism and phenolic content of *Evernia prunastri.* - Environ. exp. Bot. **26**: 385-396*,* 1986.

Lines, C.E., Ratcliffe, R.G., Rees, T.A.V., Southon, T.E.: A ^{13}C NMR study of photosynthate transport and metabolism in the lichen *Xanthoria catcicola* Oxner. - New Phytol. **111**: 447-456, 1989.

McEvoy M., Nybakken L., Solhaug K.A., Gauslaa, Y.: UV triggers the synthesis of the widely distributed secondary lichen compound usnic acid. - Mycol. Progr. **5**: 221-229, 2006.

Palmqvist, K.: Carbon economy in lichens. - New Phytol. **148**: 11-36, 2000.

Pannewitz, S., Green, T.G.A., Maysek, K., Schlensog, M., Seppelt, R., Sancho, L.G., Turk, R., Schroeter, B.: Photosynthetic responses of three common mosses from continental Antarctica. - Antarct. Sci. **17**: 341-352, 2005.

Rankovic, B., Misic, M., Sukdolak, S.: Evaluation of antimicrobial activity of the lichens *Lasallia pustulata*, *Parmelia sulcata*, *Umbilicaria crustulosa*, and *Umbilicaria cylindrica*. - Mikrobiology **76**: 723-727, 2007.

Reiter, R., Hoftberger, M., Green, T.G.A., Turk, R.: Photosynthesis of lichens from lichen-dominated communities in the alpine/nival belt of the Alps - II: Laboratory and field measurements of CO_2 exchange and water relations. - Flora **203**: 34-46, 2008.

Richardson, D.H.S., Smith, D.C.: Lichen physiology. IX. Carbohydrate movement from the *Trebouxia* symbiont of *Xanthoria aureola* to the fungus. - New Phytol. **67**: 61-68, 1968.

Roach, J.A.G., Musser, S.M., Morehouse, K., Woo, J.Y.J.: Determination of usnic acid in lichen toxic to elk by liquid chromatography with ultraviolet and tandem mass spectrometry detection. - J. Agr. Food Chem. **54**: 2484-2490, 2006.

Roser, D.J., Melick, D.R., Ling, H.U., Seppelt, R.D.: Polyol and sugar content of terrestrial plants from continental Antarctica. - Antarct. Sci. **4**: 413-420, 1992a.

Roser, D.J., Melick, D.R., Seppelt, R.D.: Reductions in the polyhydric alcohol content of lichens as an indicator of environmental pollution. - Antarct. Sci. **4**: 185-188, 1992b.

Schlensog, M., Schroeter, B.: A new method for the accurate *in situ* monitoring of chlorophyll *a* fluorescence in lichens and bryophytes. - Lichenologist **33**: 443-452, 2001.

Schreiber, U., Bilger, W., Neubauer, C.: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of *in vivo* photosynthesis. - In*:* Schulze, E.D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 49-70. Springer-Verlag, Berlin - Heidelberg - New York 1995.

Solhaug, K.A., Gauslaa, Y.: Photosynthates stimulate the UV-B induced fungal anthraquinone synthesis in the foliose lichen *Xanthoria parietina*. - Plant Cell Environ. **27**: 167-176, 2004.

Stoop, J.M.H., Williamson, J.D., Pharr, D.M.: Mannitol metabolism in plants: a method for coping with stress. - Trends Plant Sci. **1**: 139-144, 1996.

Sturgeon, R.J.: Biosynthesis and utilization of storage sugars in algae, fungie, and lichens. - Physiol. vég. **23**: 95-106, 1985.

Takahagi, T., Ikezawa, N., Endo, T., Ifuku, K., Yamamoto, Y., Kinoshita, Y., Takeshita, S., Sato, F.: Inhibition of PSII in atrazine-tolerant tobacco cells by barbatic acid, a lichen-derived depside. - Biosci. Biotechnol. Biochem. **70**: 266-268, 2006.

Tsai, S.Y., Tsai, H.L., Mau, J.L.: Non-volatile taste components of *Agaricus blazei*, *Agrocybe cylindracea* and *Boletus edulis*. - Food Chem. **107**: 977-983, 2008.

Van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. - Photosynth. Res. **25**: 147-150, 1990.

Vojtíšková, L., Munzarová, E., Votrubová, O., Čížková, H., Lipavská, H.: The influence of nitrogen nutrition on the carbohydrate and nitrogen status of emergent macrophyte *Acorus calamus* L. - Hydrobiologia **563**: 73-85, 2006.

Wellburn, A.R.: The spectral determination of chlorophyll *a* and chlorophhyll *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. - J. Plant Physiol. **144**: 307-313, 1994.