

# Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*

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**Abstract** Temperature response curves of chlorophyll *a* fluorescence parameters were used to assess minimum sub-zero temperature assuring functioning of photosynthetic photochemical processes in photosystem II (PS II) of Antarctic lichens. *Umbilicaria Antarctica* and *Xanthoria elegans* were measured within the temperature range from  $-20$  to  $+10^{\circ}\text{C}$  by a fluorometric imaging system. For potential ( $F_v/F_M$ ) and actual ( $\Phi_{II}$ ) quantum yields of photochemical processes the minimum temperature was found to be between  $-10$  and  $-20^{\circ}\text{C}$ . Non-photochemical quenching (NPQ) of absorbed excitation energy increased with temperature drop reaching maximum NPQ at  $-15^{\circ}\text{C}$ . Image analysis revealed intrathalline heterogeneity of chlorophyll *a* fluorescence parameters with temperature drop. Temperature response of  $\Phi_{II}$  exhibited an S-curve with pronounced intrathalline differences in *X. elegans*. The same relation was linear with only limited intrathalline difference in *U. antarctica*. The results showed that Antarctic lichen species were well adapted to sub-zero temperatures and capable of performing primary photosynthesis at  $-15^{\circ}\text{C}$ .

**Keywords** Chlorophyll fluorescence imaging · Extreme temperature · Freezing · Non-photochemical quenching

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## Introduction

Lichens are symbiotic organisms capable of surviving in extreme environments, including sub-zero temperatures. Lichens' high tolerance to freezing temperature is reported e.g. for *Xanthoria candelaria* and *Rhizoplaca melanophthalma* (Lange and Kappen 1972; Kappen 1989, 1993). Those species tolerated gradual or rapid freezing to  $-196^{\circ}\text{C}$ , even after being stored up for several years, almost immediately resumed normal photosynthetic rates when warmed and wetted. After 5–7 months of cold and continuous darkness, they remain green with intact photosynthetic pigments. In spite of the fact that optimum temperature for lichen photosynthesis lies above zero temperature (Uchida et al. 2006), it is well established that the majority of poikilohydric autotrophs abundant in alpine and polar environments photosynthesize at temperatures below  $0^{\circ}\text{C}$  (Reiter and Turk 2000; Kappen 2000). However, it is still under debate regarding the minimum freezing temperature for photochemical and biochemical processes of photosynthesis for individual lichen species. Schroeter et al. (1994) and Kappen and Schroeter (1997) measured photosynthetic rates gasometrically in Antarctic lichens and reported net  $\text{CO}_2$  uptake in *Umbilicaria aprina* at  $-17^{\circ}\text{C}$ . Another study (Reiter and Turk 2000) that used gasometric measurements of  $\text{CO}_2$  exchange brought the evidence that *Flavocetraria nivalis* exhibited positive net photosynthesis at  $-8^{\circ}\text{C}$ . It is therefore believed that for majority of lichens well adapted to low temperature, net photosynthesis has temperature minimum within the range of  $-5$  to  $-20^{\circ}\text{C}$ . For ecophysiological studies in lichens, it is essential to understand photosynthetic performance of lichens at freezing temperatures so that the mechanisms of lichen productivity can be analyzed in long-term studies performed in alpine (e.g. Reiter and Turk 2000), tundra

(Uchida et al. 2006), and polar regions (Schroeter et al. 2000; Pannowitz et al. 2003).

In lichens, primary photochemical processes of photosynthesis in photosystem II (PS II) and thylakoid membrane of a chloroplast are effective at low temperatures. For lichens, measured both in the field (Schlensog and Schroeter 2001) and in a laboratory (Hájek et al. 2001), either constant or slightly decreasing  $F_V/F_M$  is reported for the range of 0 to  $-5^\circ\text{C}$ . Effective yield of photochemical processes in PS II ( $\Phi_{II}$ ) is another characteristic investigated in low-temperature studies of lichen photosynthesis. Our last studies of central European lichen species measured under optimum hydration indicated that the minimum temperature for primary photochemical processes of photosynthesis, quantum yield of PS II ( $\Phi_{II}$ ) in particular, is about  $-10^\circ\text{C}$  for control (Hájek et al. 2001) and certainly below  $-10^\circ\text{C}$  for thalli treated with the addition of osmotically active sugar alcohol, ribitol (Hájek et al. 2006). The aim of the present study was to quantify temperature response curves for selected chlorophyll fluorescence (Chl fluorescence) parameters ( $F_V/F_M$ ,  $\Phi_{II}$ , NPQ) and find minimum temperatures for lichens collected in Antarctica using Chl fluorescence technique.

## Materials and methods

### Lichen thalli collection and handling

*Umbilicaria antarctica* and *Xanthoria elegans* were collected in the Maritime Antarctic at Galindez Island in a close vicinity of Ukrainian station Vernadsky ( $65^\circ14'43''\text{S}$ ,  $64^\circ15'24''\text{W}$ ). The thalli in a naturally dry state were transferred to the laboratory in Brno, Czech Republic, stored under a dim light ( $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at  $5^\circ\text{C}$ . Before measurements, the thalli were gradually hydrated by regular spraying with demineralized water for 48 h. Then, their vitality and physiological status were tested fluorometrically (OS1-FL fluorometer, OptiScience, USA) using  $F_V/F_M$  as a marker. When maximum  $F_V/F_M$  ratio was reached (typically 0.55–0.60), the thalli were considered optimally hydrated. For both species, it represented water potential of  $-3$  to  $-1$  MPa (WP-4T water potential meter, Decagon, USA, data not shown) checked before and after measurements. For measurements at manipulated temperature, we selected the specimens that exhibited highest  $F_V/F_M$  and  $\Phi_{II}$ . To study the effect of freezing temperatures on primary photosynthetic processes in lichens, *U. antarctica* and *X. elegans* were chosen as experimental species. The two species are quite abundant along the Antarctic Peninsula (Krzewicka and Smykla 2004; Olech 2001, 2004; Øvstedal and Lewis Smith 2001) and typical for vegetation oases in the maritime and continental Antarctic.

### Chlorophyll fluorescence measurements

Response of primary photosynthetic processes to the temperature range from  $-20$  to  $+10^\circ\text{C}$  was monitored by Chl fluorescence parameters measured by a PC-linked fluorometer FluorCam 700 MF (Photon Systems Instruments, Czech Republic) equipped with a CCD camera and image analysis software (FluorCam v. 6.0). During measurements, temperature within the measuring chamber was maintained constant by a thermo-regulator controlled cooling unit (ConBrio, Czech Republic) consisting of two Peltier coolers, one adjacent to the metal plate on which an experimental lichen thallus was placed, the other maintaining air temperature inside the measuring chamber. The Peltier coolers were connected to a water loop with circulating water (thermostat Labio, Czech Republic) ensuring effective cooling. During the low-temperature experiments, both chamber and lichen thallus temperatures were monitored with a set of Cu–Co thermocouples linked to a data-logger (VV/VX Minicube, Environmental Monitoring Systems, Czech Republic). Optimally hydrated lichen thalli were placed in the measuring chamber and exposed consequently to a temperature decreasing in a  $5^\circ\text{C}$  step within the range of 10 to  $-25^\circ\text{C}$ . At each temperature, thalli equilibrated for 0.5 h at moderate irradiance of  $30 \mu\text{mol m}^{-2} \text{s}^{-1}$ . After the equilibration, a set of Chl fluorescence parameters was measured using Chl fluorescence imaging. For detailed description of the method and its application in lichen photosynthetic studies see, e.g. Barták et al. (2000, 2004).

In our experiments, Chl fluorescence imaging technique provided high resolution ( $512 \times 512$  pixels) false-colour images of Chl fluorescence distribution over a lichen thallus. For each pixel of the images taken, the kinetics of Chl fluorescence induced by the below-specified light treatment was recorded and basic Chl fluorescence parameters determined. In dark-adapted state, lichen thalli were exposed to experimental protocol using actinic light and saturation light pulses that allowed calculating and visualizing  $F_V/F_M$  and  $\Phi_{II}$  distribution over a thallus. The measurements started with  $F_0$  determination at low light ( $5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), followed by a maximum Chl fluorescence ( $F_M$ ) determination at ( $1,500 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Then, after 5 min exposure to a constant moderate irradiance ( $100 \mu\text{mol m}^{-2} \text{s}^{-1}$ , sufficient for lichens, tested before), the lichen thallus exhibited steady-state Chl fluorescence ( $F_S$ ). In this state, maximum Chl fluorescence ( $F_M'$ ) in light-adapted state was determined and quantum yield of photochemical processes in photosystem II ( $\Phi_{II}$ ) calculated as  $(F_M' - F_S)/F_M'$ . Non-photochemical quenching was calculated as  $\text{NPQ} = (F_{\text{Minit}} - F_M')/F_M'$  (see e.g. Vráblíková et al. 2005). After switching off the actinic light,  $F_M''$  Chl fluorescence level was determined and

used for qE determination. The images were taken for typically five lichen thalli at each experimental temperature. The images were then analyzed using a FluorWin software and the above-specified Chl fluorescence parameters were evaluated either for the whole thallus or selected tiny spots (typically eight, each of which 5 mm in diameter) representing thallus area exhibiting high/low photosynthetic activity.

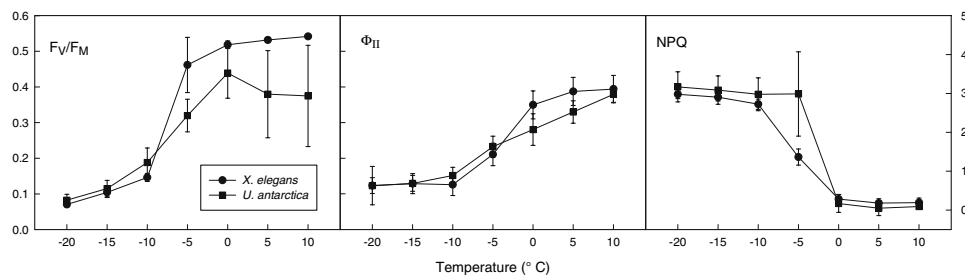
## Results

When calculated for whole thallus, mean Chl fluorescence parameters of *X. elegans* and *U. antarctica* showed a similar response to decreasing temperature (Fig. 1), however, some species-specific differences were found. It was particularly apparent for  $F_V/F_M$  which was significantly higher in *X. elegans* than *U. antarctica* within the temperature range of 0–10°C. For both species, critical low temperature at which  $F_V/F_M$  started to decline was –5°C.  $F_V/F_M$  further decreased with temperature reaching their minimum values at about –15°C. Temperature-induced change in  $\Phi_{II}$  was similar in both species; however, the  $\Phi_{II}$  decline with

temperature decrease was steeper in *X. elegans* than *U. antarctica*.

Within a single thallus, significant differences in  $F_V/F_M$ ,  $\Phi_{II}$ , and NPQ found between different thallus parts proved heterogeneity in distribution of the parameters. Maxima of the two parameters showed species-specific patchiness. In *X. elegans*, maximum  $F_V/F_M$  was predominantly found in close-to-central parts of the thalli where fruiting bodies, if formed, were located. In *U. antarctica*, areas of maximum  $F_V/F_M$  were located in irregularly distributed patches within the thalli.

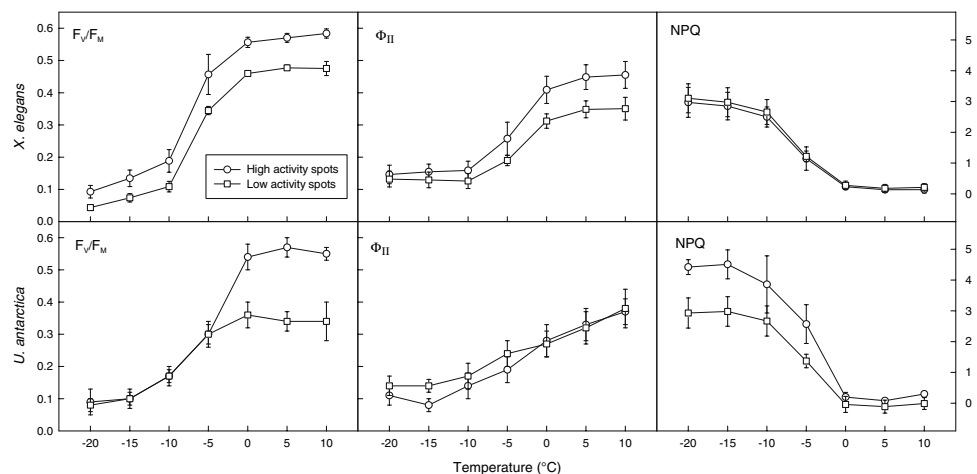
In the images of  $F_V/F_M$  and  $\Phi_{II}$  distribution over lichen thalli (data not shown), some apparently demarked local maxima were apparent. In thalli of *U. antarctica*, few areas exhibiting  $F_V/F_M$  higher than mean were located between thallus centre and margin, apparently distant from umbilicus. Areas with low photochemical activity were located to marginal parts. The difference between thallus parts exhibiting high and low photosynthetic photochemical activity was apparent mainly at the temperature range of 0–10°C (See Fig. 2). Gradual decrease in thallus temperature led to inhibition of photosynthetic activity both in the areas exhibiting high and low  $F_V/F_M$ . First signs of the inhibition were



**Fig. 1** Temperature response curves of chlorophyll fluorescence parameters: potential yield of PS II ( $F_V/F_M$ ), actual quantum yield of photochemical processes of PS II ( $\Phi_{II}$ ) and non-photochemical

quenching (NPQ) recorded for whole lichen thalli of *Xanthoria elegans* (closed circles) and *Umbilicaria antarctica* (closed squares). The bars indicate  $\pm$ SD. Data points are means of at least five replicates

**Fig. 2** Potential yield of PS II ( $F_V/F_M$ ), actual quantum yield of photochemical processes of PS II ( $\Phi_{II}$ ) and non-photochemical quenching (NPQ) in response to temperature. Data were recorded for thalli parts exhibiting either high (open circles) and low (open squares) photosynthetic activity in *Xanthoria elegans* (upper row) and *Umbilicaria antarctica* (lower row). The bars indicate  $\pm$ SD. Data points are means of at least eight replicates



manifested, similarly to the whole thalli, at  $-5^{\circ}\text{C}$ . In *X. elegans*, maxima of  $F_v/F_M$  were located to the youngest growing parts of individual thalli (see also Discussion). Minima were found either in the oldest central parts where thallus was dropped off (sometimes with missing lichen biomass) or close to the margin. Distribution of areas exhibiting high/low  $\Phi_{II}$  was, irrespective of experimental temperature, similar to  $F_v/F_M$  (data not shown). However, contrastingly to  $F_v/F_M$ , the decrease in  $\Phi_{II}$  was found within the whole temperature range, more apparently in *U. antarctica*. Another difference was that  $\Phi_{II}$  did not differ in the thallus parts exhibiting high and low capacity of photosynthetic photochemical processes ( $F_v/F_M$ ). Non-photochemical quenching exhibited typical response with temperature decrease. In both species, it increased at freezing temperatures showing differences between thalli parts typical by different  $F_v/F_M$ .

## Discussion

The response of  $F_v/F_M$ ,  $\Phi_{II}$  and NPQ showed for both species that photochemical processes in PS II of both studied lichen species still have detectable activity at least at the temperature of  $-10^{\circ}\text{C}$ . Below that point, freezing temperature brings a substantial inhibition of photochemical processes of photosynthesis. Incident light might be an interacting factor limiting primary photosynthetic processes. The phenomenon of low-temperature photoinhibition was reported, e.g. for poikilohydric mosses (Lovelock et al. 1995). For Antarctic lichens, either no (Kappen et al. 1998) or moderate photoinhibition (Barták et al. 2003) was found in field and laboratory studies, respectively. On chloroplast level, net photosynthesis decreases due to low supply of ATP and NADPH from temperature-dependent limitation of photochemical processes related to electron transport through thylakoid membrane. However, in spite of extreme low values of  $\Phi_{II}$ , found at sub-zero temperature, positive net photosynthesis need not be necessarily diminished. Lichens possess several mechanisms assuring cell functioning at low temperature. At freezing temperatures from 0 to  $-10^{\circ}\text{C}$ , due to the presence of osmotically active compounds, cells of mycobiont are increasingly cavitated and their protoplast closely attached to cell wall while photobiont cells are still in function (Schroeter and Scheidegger 1995). Another protective mechanism is e.g. ice nucleation activity (Worland et al. 1996) that reduces intracellular formation of ice crystals with sharp edges. At extremely low freezing temperature, however, negative effects of intra- and extracellular ice formation in lichens cannot be excluded. At the temperatures below critical, ice formation may cause decline of functioning of cells of symbiotic algae and thus affect primary photochemical

processes. Our samples were exposed to decreasing temperature under optimum hydration. Therefore, negative effects of extra- and/or intracellular freezing of water on photosynthesis might be higher than expected in partly or fully desiccated thalli. Such alleviation of the negative effect by a progressive thalli desiccation during freezing was documented for Antarctic lichens (Schroeter et al. 1997) and moss (Kennedy 1993). Moreover, the most apparent decrease in  $F_v/F_M$  and  $\Phi_{II}$  was found at about  $-5^{\circ}\text{C}$  which corresponds to the temperature reported as critical for ice-nucleation activity in fully hydrated lichen thalli (*Umbilicaria aprina*, Schroeter and Scheidegger 1995) during gradual freezing. Haranczyk et al. (2003) showed for several lichens with contrasting anatomy that  $-5^{\circ}\text{C}$  is an edge temperature for freezing of water in their thalli.

Non-photochemical quenching increased at sub-zero temperature similarly as found in an earlier study (Hájek et al. 2001). This indicated low temperature-induced involvement of quenching mechanism scavenging excitation energy absorbed by light harvesting complexes of PS II. The NPQ increase might be attributed both to zeaxanthin-dependent component manifested as an increase in DEPS (Lovelock et al. 1995) and qE (our data, not shown), and PS II quenchers-dependent thermal energy dissipation (Heber et al. 2006).

At each experimental temperature, primary photosynthetic processes exhibited spatial heterogeneity over examined thalli, similarly to previous studies on lichens under diverse environmental conditions such as, e.g. high light (Barták et al. 2000, 2004), osmotic (Hájek et al. 2006), and chemical stress (Jensen and Siebke 1997). In the present study, the heterogeneity of  $F_v/F_M$ ,  $\Phi_{II}$  and NPQ might be attributed to thallus anatomy, thallus thickness, distribution of growing pseudomeristems, and age of photobiont cells present in particular thallus zones. It is clear that the investigated *X. elegans* has been formed by several formerly independent thalli growing for decades before they reach each other. Finally, they formed a semi-uniform cover over a stone. The heterogeneity in  $F_v/F_M$  and  $\Phi_{II}$  of investigated thallus, therefore, showed a certain regularity corresponding to the distribution of round-shaped objects forming the respective independent thalli. The highest  $F_v/F_M$  and  $\Phi_{II}$  were found close to the object edges. This is the same type of  $F_v/F_M$  heterogeneity observed earlier in *X. elegans* in the field (Barták et al. 2005) during gradual desiccation.

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