Production of UV-B screens and changes in photosynthetic efficiency in Antarctic Nostoc commune colonies and a lichen Xanthoria elegans depend on a dose and duration of UV-B stress

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Abstract
The survival of non-vascular autotrophs in the extreme polar conditions and the principles of their high tolerance to extreme physical factors have been intriguing scientists in last decades. Therefore, this study focuses on the capacity of production of UV-B screening pigments in two model Antarctic species, one algal lichen, and colony of a cyanobacterium. Dose-dependent activation of protective mechanisms of Antarctic cyanobacterium (Nostoc commune) and algal lichen (Xanthoria elegans), synthesis of UV-B screening compounds in particular, were studied together with the changes in photosynthetic efficiency induced by a background photosynthetically active radiation (PAR) supplemented with UV-B radiation. The samples were exposed to different doses of UV-B (280–320 nm), low (0.7 W m\(^{-2}\)), medium (1.5 W m\(^{-2}\)) and high (3.0 W m\(^{-2}\)) for 5 days. Untreated samples (control) were shielded from UV-B radiation during experiment. Chlorophyll fluorescence parameters and secondary UV-B protective metabolites were analysed in the intervals of 24 h, 48 h and 120 h. Amount of UV-B screening pigments was measured spectrophotometrically using several specific wavelengths in UV-B absorption range. Results showed that if exposed to a low dose of UV-B radiation or a short-term treatment, both species exhibited an increase in UV-B screening pigments to protect the lichen photobiont against UV-B damage. However, if exposed to a high dose of UV-B radiation or a long-term treatment, a decrease of UV-B screening compounds occurred. This implies that Antarctic lichen and cyanobacterium can protect themselves against an increase of stress factors ranging within physiological limits, like e.g. increased synthesis of UV-B screening compounds thanks to a thinning of the ozone layer and consequent increase in UV radiation doses incident on Antarctic terrestrial ecosystems. Nevertheless, the likely increased UV-B radiation due to more intense depletion of stratospheric ozone layer may lead to alterations in UV-B tolerance in Antarctic lichens in future.

Key words: Chlorophyll fluorescence transient, effective quantum yield, ultraviolet radiation, UV absorbing compounds

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Introduction

Antarctic terrestrial non-vascular autotrophs are capable to survive extremes in physical factors of polar environment. Their resistance to individual stress factors, however, is species-specific and dependent on capacity of protective mechanisms of particular species. Antarctic lichens, thanks to synthesis and presence of photoprotective compounds (see e.g. Vrábliková et al. 2005), fast thallus dehydration and inactivation of photosynthetic apparatus of symbiotic algae and/or cyanobacteria on sunny days exhibit a high degree of resistance to high doses of photosynthetically active radiation (PAR).

Resistance against photoinhibition is reported for several Antarctic species, e.g. Umbilicaria aprina (Kappen et al. 1998), U. decussata (Barták et al. 2003), U. antarctica (Barták et al. 2004), and Usnea antarctica (Balariinová et al. 2014). Sensitivity of Antarctic lichens to UV-B radiation is, however, much less studied. Several experiments, however, have been done on lichen responses, their UV-B screening compounds in particular, to limited UV-B radiation using natural light and cut-off filters (e.g. alpine (Bachereau et Asta 1997) and Antarctic ecosystems (Huiskes et al. 2001, Edwards et al. 2004, Gautam et al. 2011). Only fragmentary knowledge exists on physiological processes in such organisms under UV-B stress, primary photochemical reactions of photosynthesis in particular. UV-B absorbing pigments are widespread across lichens from open sunny habitats, due to their ability to absorb biologically damaging UV-B radiation (Solhaug et al. 2003). Generally, lichens from sunny habitats have an effective absorption of UV-B thanks to numerous secondary metabolites, such as e.g. phenolics (Singh et al. 2012) located in the upper cortex layer. Amount of UV-B absorbing compounds typically increases with exposition to supplemental UV-B radiation accompanied with changes in chlorophyll fluorescence parameters (Czintalan et al. 2001, Solhaug et al. 2010). Due to stratospheric ozone depletion in Antarctica, UV-B increases in September-November and has numerous effects of functioning of higher plants (Caldwell et Flint 1994), mosses (e.g. Newsham 2003) and lichens (e.g. Gautam et al. 2011). Interspecific differences in the capability of Antarctic lichens to synthetize UV-B absorbing compounds, however, have been studied in much detail. Therefore, this study focuses on the responses of an Antarctic cyanobacterium (Nostoc commune) and algal lichen (Xanthoria elegans) to supplemental UV-B to evaluate an extent of photoprotective mechanisms and capacity to synthesize UV-B screens.

Nostoc commune is a semi-terrestrial autotroph that forms 1- to 5-mm thick, sheet-like colonies on alternating dry and wet bare soils in a variety of ecosystems ranging from polar to tropical regions (Novis et al. 2007). It is recognised for its unprecedented ability to survive extended periods of frost, drought and extreme temperatures as inactive dry crusts, while the colony rapidly swells and reactivates ion exchange, photosynthesis and respiration at moderate temperatures (0–35°C) when water becomes available (Sand-Jensen et Jespersen 2012). In polar regions, N. commune is reported as highly freeze tolerant (-60°C, Davey 1989) and maintaining photosynthetic processes within a wide range of relative water content (20–80% of full hydration, Kviderová et al. 2011) and temperature. Optimum temperature for N. commune photosynthesis, however, is reported as high as 25°C (Møller et al. 2014). Resistance of N. commune to UV-B is high because of a high capacity of the species to synthesize UV-B absorbing compounds (see Nguyen et al. 2013 for a review).

Xanthoria elegans is a lichen with a foliose thallus morphotype and has a circumpolar distribution. It is found in alpine
and polar regions frequently. The species forms small, typically 5 cm in diameter circles attached to stones or ground with small foliose lobes at the margins. Typically, the central part of the thallus has a crustose character. In Antarctica, *X. elegans* was studied in the field with the main emphasis given to the lichen-mediated weathering processes (Ascaso et al. 1990), pigment and UV-B absorbing compounds content (Edwards et al. 1998), dehydration tolerance of photosynthesis (Barták et al. 2005). Laboratory-based studies revealed a wide range of low and freezing temperature at which primary photosynthetic processes showed either no or minimal inhibition (Barták et al. 2007). *X. elegans* is reported to be UV-B tolerant (Nybakken et al. 2004) because of having the orange pigment parietin in upper cortex that works as a screen for the underlying pseudotissues containing intrathalline photobiont. Since *X. elegans* is a model species for exobiology, its resistance to UV radiation has been tested many times as a part of Earth-based (de Vera et al. 2003) and open space tests (Brandt et al. 2014). Preliminary studies showed, that amount of UV-B absorbing compounds increased in *X. elegans* after supplemental UV-B treatment (0.7 W m⁻²). The increase reached, however, only 107.5% of untreated control (Barták et al. 2014) suggesting that constitutive (natural) amount of the UV-B screens is high enough to cope with natural variation of UV-B incident on the lichen in Antarctica. In this study, the experiments were aimed to answer the question whether or not the changes in UV-B induced amount of UV-B screens are accompanied by changes in primary photosynthesis in the species. The hypothesis was tested that chlorophyll fluorescence parameters linked to primary photosynthetic processes in lichen symbiotic alga (Fᵥ/Fₘ and Φₚₛᵢ) are not negatively affected by physiological amounts of supplemental UV-B light thanks to involvement of photoprotective mechanisms.

**Material and Methods**

**Sample collection**

*N. commune* was collected from wet sites at a 1500 m² vegetation oasis located on a seashore (6 m a.s.l.) in a close proximity to the Czech Antarctic station Johann Gregor Mendel (φ = 63° 48´ 2.3´´ S, λ = 57° 52´ 56.7´ W). The site is well supplied by melt water from neighboring snow patches. Therefore, rich algal and cyanobacterial mats, moss and lichen flora is found there. The site of collection is located on a long-term experimental plot that serves for several long-term projects, such as e.g. simulation of atmospheric warming and its effects on vegetation using an open top chambers (OTC) approach (Barták et al. 2009), yearlong in situ measurements of photosynthetic processes in *Bryum* sp. using chlorophyll fluorescence (Barták et Váczi 2014). The site of collection is located in between two streams (Bohemian and Algal stream). The mean annual temperature recorded 200 m from the study site is -4.6°C (Mendel station weather station, 2 m above ground). The warmest month is January with monthly temperature of 2.5°C (Mendel station) while the coldest one is July with monthly mean temperature below -11.1°C (Láska et al. 2011).
X. elegans was collected from several sites on the northern slopes of the Berry Hill at the distance ranging from 2–4 km E from Mendel station. The lichen was found on small stones in narrow lines along temporary streams and seepages. The collecting sites altitude ranged from 50–100. Climate of the sites of collections is similar to the above-mentioned Mendel station characteristics, however, some minor differences might be found. Climate of the sites is characterized by data from neighbouring field weather station located at Komarek’s slopes (63° 48’ 12” S, 57° 50’ 20” W). Detailed characteristics are given in Skácelová et al. (2015). After collection, samples of both experimental lichen species were dried out under natural conditions, then packed and transferred from Antarctica to a laboratory, where stored in dry state in dark at 5°C.

Fig. 1. Photo of experimental species: Nostoc commune (upper panels), Xanthoria elegans (lower panels).

Handling the samples

Before experiments, thalli of X. elegans and N. commune colonies were hydrated for 48 h at 5°C until full hydration was reached (checked by effective quantum yield, data not shown). Hydrated thalli were then exposed to low light (10 µmol m⁻² s⁻¹, PAR) supplemented with a low (0.7 W m⁻²), medium (1.5 W m⁻²), and high (3.0 W m⁻²) dose of UV-B radiation for 5 days at 24°C. The lichen thalli were placed in petri dishes covered by a UV-B transmitting transparent foil to prevent dehydration during the exposition time. UV-B was provided by a UVB Broadband TL lamp (Phillips, the Netherlands, TL 20W/12 RS SLV) which emits radiation in the ‘B’ bandwidth of the UV spectrum (290 to 315 nm). On days 0, 1, 2, and 5, chlorophyll (Chl) fluorescence parameters (see below) were measured and samples for pigment analyses taken.
**Chlorophyll fluorescence**

To evaluate UV-B treatment-related effects on primary photosynthetic processes, Kautsky kinetics supplemented with quenching analysis were measured by a HFC-10 fluorometer (PSI, Czech Republic) on day (d) 0, 1, 2, 5. Segments of *X. elegans* and *N. commune* thalli were predarkened for 5 min at 24°C and then subjected to a saturation pulse of light to induce maximum chlorophyll fluorescence (F_M) on dark-adapted sample. Then actinic light was switched off for 5 min to induce variable chlorophyll fluorescence in light-adapted sample. After the steady state chlorophyll fluorescence (F_S) was reached, a saturation pulse was applied and a maximum chlorophyll fluorescence on light-adapted sample recorded (F_M'). From Kautsky kinetics records and particular levels of chlorophyll fluorescence (F_0, F_M, F_S, F_M' – for definitions see e.g. Roháček et al. 2008), the following chlorophyll fluorescence parameters were calculated using the formulae:

\[
\frac{F_V}{F_M} = \frac{F_M - F_0}{F_M} \quad \text{Eqn. 1}
\]

\[
\Phi_{PSII} = \frac{(F_M' - F_S)}{F_M'} \quad \text{Eqn. 2}
\]

Particular chlorophyll fluorescence parameters were plotted against time of UV-B treatment and related to the supplemental UV-B dose.

**Pigments and secondary metabolites analyses**

To evaluate pigment content (Chl a, Chl b, carotenoids) and UV-B screening compounds according to (Buffoni-Hall et al. 2002), ethanol extracts from the samples collected before and during treatment(s) were analysed. Absorbances of the extracts were measured by UV-VIS spectrophotometer (Specord 205, Analytik Jena, Germany) within the wavelength range of 190–700 nm.

**Results and Discussion**

**Slow Kautsky kinetics**

Shapes of the chlorophyll fluorescence (Kautsky) kinetics were species specific (see Fig. 2) and dependent on dose of UV-B treatment. Generally, they reflect redox state of electron carriers in chloroplastic thylakoid membrane and involve also numerous processes associated with primary photochemistry of photosynthesis such as e.g. non-photochemical quenching of absorbed light energy, ATP synthesis, as well as Calvin-Benson cycle (Stirbet et Govindjee 2011, Stirbet et al. 2014). Within the slow Kautsky kinetics, several phases can be distinguished (P, S, M and T), where P is peak chlorophyll fluorescence level reached shortly after exposure to light, S stands for semi-steady state, M for a local maximum, and T for a terminal steady state level of chlorophyll fluorescence. In some plant species, M peak is missing, or several steady states (S1, S2, etc.) and maxima (M1, M2, etc.) might be observed. Slow Kautsky kinetic shape is difficult to interpret, however, it may sensitively indicate negative effects of variety of stresses in photosynthetic apparatus. In our study, slow Kautsky kinetics differed between the two experimental species both in control and UV-B-affected samples. In *X. elegans*, the curve exhibited
$P$ peak some 0.8-1.0 s after actinic light was switched on followed by a polyphasic curve ending in a plateau (equivalent to $T$) found after 5 min exposition to actinic light. However, a consequent increase from $P$ to $M$ peak was apparent. The $M$ peak was reached typically after 33 s (in untreated control). Absolute value of $M$ peak was always higher than that of $P$ peak. From $M$ chlorophyll fluorescence level, an exponential decrease to was seen. Steady-state chlorophyll fluorescence level ($T$) was then reached after 240 s of actinic light. The shape of slow Kautsky kinetics recorded in *X. elegans* was similar to those recorded earlier in several lichens possessing green alga as major photobiont. The curves typically show $P$ level, a value of which is lower than $M$ level which is achieved at about 40 s of actinic light on (e.g. Conti et al. 2014, *Stereocaulon vesuvianum*). $P$ and $M$ values are, however, species-specific. In some Antarctic lichens, $P$ might be higher than $M$ as shown e.g. for *Usnea antarctica* (Barták et al. 2012).

**Fig. 2.** Slow chlorophyll fluorescence induction (transient, Kautsky kinetics) with indication of important chlorophyll fluorescence levels $O$, $P$, $S$, $M$, and $T$. Abbreviations have the following meanings: $O$ (origin) is the first measured minimum fluorescence level, $P$ is the peak found within first few seconds when actinic light is switched on, $S$ stands for semi-steady state chlorophyll fluorescence, $M$ for a maximum chlorophyll fluorescence and $T$ for a terminal steady state level. $F_M$, $F_M'$ and $F_M''$ denotes to maximum chlorophyll fluorescence reached after saturation pulse of strong light is given on dark-adapted samples, light-adapted samples, and the samples in a short-term dark, *i.e.* few tens of seconds after actinic light is switched off.
Those Kautsky kinetics recorded for *N. commune* were rather flat (Fig. 2) exhibiting typical “cyanobacterial” features. The shape of the curve was found very similar to earlier studies done on several cyanobacteria (*e.g.* *Synechococcus* sp. – Cambell et al. 1998, Tsimilli-Michael et al. 2009). Typically, variable chlorophyll fluorescence ($F_V$) was almost constant during light adaptation when actinic light was switched on (10–250 s in Fig. 2) showing, however, slightly decreasing trend. Therefore, $M$, $S$, and $T$ chlorophyll fluorescence levels were not easily distinguishable due to small differences in chlorophyll fluorescence signal. However, $M$ chlorophyll fluorescence level ($M > P$) was reached typically at about 170 s of actinic light. This was well comparable to data presented by Kaňa et al. (2009), who reported $M$ higher than $P$ and $T$ chlorophyll fluorescence levels. Such special features of chlorophyll fluorescence kinetics in *N. commune* are associated with a general pathway of absorbed light energy transfer in antennae which involves phyco-cyanin, allophycocyanin and chlorophyll molecules in cyanobacteria (Stirbet et Govindjee 2011).

Supplemental UV-B treatment did not bring much change to the shape of Kautsky kinetics both in *X. elegans* and *N. commune* (Fig. 2). However, time at which $M$ was reached was shorter in UV-B treated (24.2 ± 1.2 s) than in control samples of *X. elegans* (32.8 ± 3.4 s). UV-B treatment had also effects on chlorophyll fluorescence parameters calculated from particular signals derived from Kautsky kinetics supplemented with saturation pulses, such as *e.g.* $\Phi_{PSII}$, exhibited dose- and treatment duration-effect (see Fig. 3).

**Chlorophyll fluorescence parameters**

Both potential ($F_V/F_M$) and effective quantum yield of PS II photochemical processes ($\Phi_{PSII}$) showed only a slight change in absolute values with dose and time of exposition to supplemental UV-B light. There were, however, interspecific differences in time courses of the two chlorophyll fluorescence parameters. While either no change (or even slight increase) in $F_V/F_M$ and $\Phi_{PSII}$ was apparent in *X. elegans* treated by 0.7 W m$^{-2}$ and/or 1.5 W m$^{-2}$, the same doses brought a decrease of these parameters in *N. commune*. The highest UV-B dose (3.0 W m$^{-2}$) led to a decrease in $F_V/F_M$ and $\Phi_{PSII}$ found in *X. elegans* after 24 and 48 h of the treatment. Further treatment (120 h) led to a consequent increase in $F_V/F_M$ and $\Phi_{PSII}$ documenting a high capacity of chloroplastic photosynthetic apparatus to cope with high UV-B doses. Similar response was found in *N. commune*, however, recovery was incompleated in these species after 120 h of supplemental UV-B treatment (see Fig. 3).

Fluorometric parameters measured in this study, however, revealed interspecific differences. Negative changes in primary photochemical processes of photosynthesis ($F_V/F_M$, $\Phi_{PSII}$) in response to treatments were more pronounced in *N. commune* than *X. elegans*. Interspecific differences were demonstrated in the shape of Kautsky curves. Generally, variable Chl fluorescence ($F_V$) recorded during actinic light period showed polyphasic course in *X. elegans*, while it was found close to steady-state fluorescence ($F_S$) in *N. commune* colonies (see Fig. 2).
Fig. 3. The effect of supplemental UV-B treatments on time courses of chlorophyll fluorescence parameters ($F_{v}/F_{m}$, $\Phi_{PSII}$) for *X. elegans* and *N. commune*. Data points represent means of at least 5 measurements. Error bars are standard deviations.

Time courses of $F_{v}/F_{m}$ and $\Phi_{PSII}$ showed that primary photosynthetic processes are quite resistant to UV-B radiation in both species. Such resistance might be documented by earlier studies of Nybakken et al. (2004) - *X. elegans*. Resistance of *N. commune* against supplemental UV-B is attributed to effective energy quenching of absorbed light in antennae and involvement of hundreds of proteins into acclimation responses to UV-B stress (Ehling-Schulz et al. 2002).

**Secondary metabolites in response to UV-B**

Enhanced synthesis of UV-B absorbing compounds (assessed according Buffoni-Hall et al. 2002) was apparent within the first 24 h of supplemental UV-B treatments in both experimental species (see Fig 4). Further treatment, however, led to different responses that were dose-dependent. In *X. elegans*, lowest dose (0.7 W m$^{-2}$) caused initial increase in UV-B absorbing compounds content (280 nm) followed by constant values found after 48 and 120 h of treatment. Higher supplemental UV-B dose (1.5 W m$^{-2}$) led to a slight increase from maximum values (100% = the value found after 24 h of treatment) to 104.8 and 106.1% found after 48 h, while a decrease to 91.2 and 85.7% (from maximum values) was apparent after 120 h. The decrease (after 120 h) was more pronounced in a strong (3.0 W m$^{-2}$) supplemental UV-B dose treatment (61.4 and 71.0%, respectively).
In *N. commune*, the response was generally similar, however, negative responses were achieved earlier (48 h) in 1.5 W m\(^{-2}\) treatments than in *X. elegans*. Moreover, the strong supplemental UV-B treatment (3.0 W m\(^{-2}\)) caused rather a decrease in UV-B absorbing compounds found after 24 h. The content remained more or less constant within the overall treatment time, however, a slight increase of UV-B absorbing compounds was found after 48 and 120 h, respectively. This indicates that *N. commune* responded more sensitively to high doses and/or duration of treatment than *X. elegans*.

Shape of absorbance spectra of ethanol extracts are shown in Fig. 6. The spectral curves in *N. commune* were similar to a preliminary study (Trnková et al. 2014) who reported several peaks caused by UV-absorbing compounds. In UV-B-treated *N. commune* colonies, absorbance rose mostly in a broad peak at 380 nm that can be attributed to scytonemin content. In UV-C region, two narrow peaks at 209 and 260 nm were observed. Moreover, an increase in color-less mycosporine, an extra-cellular, water-soluble UV-A/B-absorbing compounds associated with extracellular glycan synthesis is reported in some studies (e.g. Ehling-Schulz et al. 1997) exploiting UV-B treated *N. commune*. Recently, a wide number of mycosporine-like amino acids (MAA) has been characterised as an important part of UV-B resistance of *N. commune* (e.g. Nazifi et al. 2015). These compounds are water soluable with an absorption maximum at 334 nm.
Apart from the above-discussed peaks of absorbance, spectral curves showed several distinguished peaks in UV-B absorbing range (see Fig. 6). Therefore, the responses of *X. elegans* and *N. commune* to supplemental UV-B were assessed for several maxima found in particular wavelengths. In *X. elegans*, UV-B absorbing compounds exhibited increased values after 24 h treatment for three wavelengths (203-207, 310 and 354 nm) but not in 233 nm. In *N. commune*, data did not show single trend (Fig. 7), however, the UV-B absorbing compounds showing a peak in 310 nm were much less responsive to supplemental UV-B treatment than the others. However, relatively small increase in UV-B induced synthesis of UV-B absorbing compounds suggest that natural (constitutive) contents of these compounds is high enough to cope with natural variations of UV-B radiation incident on lichen- and/or *Nostoc*- dominated vegetation cover in Antarctic terrestrial ecosystems.
**Fig. 7.** Time courses of UV-B absorbing compounds determined for specific wavelengths as affected by dose of supplemental UV-B treatment for *X. elegans* and *N. commune*.

Data presented in this study support the conclusion of Singh et al. (2010, 2011) that majority of Antarctic higher plants and autotrophic organisms have a high capacity to synthesize photoprotective secondary compounds when exposed to increased levels of UV-B. Thanks to contents of such compounds, resistance of *N. commune* to UV-B is high both in colonies and heterospecific microbial mats (Quesada et al. 1999). For lichens from polar regions, a similar conclusion could be made. Lud et al. (2001) reported that an increase in UV-B dose led to enhanced synthesis of UV-B absorbing compounds in *Turgidosculum complicatum*. Comparably, UV-B induced synthesis of UV-screening secondary compounds, parietin in particular, was found by Nybakken et al. (2004) for *X. elegans*.

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UV-B ABSORBING COMPOUNDS


