Physiological performance of a foliose macrolichen *Umbilicaria antarctica* as affected by supplemental UV-B treatment

Mariana Gonzalez Medina*, Roxana Avalos-Chacon

Faculty of Chemistry, National Autonomous University of Mexico, Mexico City, 04510, Mexico

Abstract

To date, a limited knowledge is available about *Umbilicaria antarctica* responses when it is exposed high doses of UV-B radiation. It is well established that resistance of Antarctic lichens to natural UV-B levels including increased doses during ozone hole period is high, thanks to numerous photoprotective mechanism. Capacity of the photoprotective processes, however, is not well known This study attempts to determine changes on the photosynthetic efficiency and on the synthesis of UV-B absorbing compounds of *U. antarctica* when exposed to low photosynthetically active radiation and extremely high intensity of UV-B light: 3.0 W m^{-2}, of UV-B for 3 hours, 6 hours and 7 days. During the experiment, chlorophyll fluorescence was measured to evaluate changes in photosynthetic apparatus of intrathalline alga. After 7 d exposition, amount of UV-B absorbing compounds was evaluated in *U. antarctica*. Heavy UV-B stress let to an increase in chlorophyll fluorescence kinetics (OJIPs), however, majority of parameters related to functioning of PS II remained unchanged indicating high resistance of *U. antarctica* to UV-B stress. Potential (F_V/F_M) and actual (Φ_{PSII}) yields of PS II were not affected by the UV-B treatment as well. In majority of cases, heavy UV-B treatment led to a decrease in the amount of UV-B absorbing compounds extracted from treated thalli.

Key words: Galindez Island, UV-B, spectral absorption, chlorophyll fluorescence

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*Corresponding author: M. G. Medina <mgm_14392@hotmail.com>
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Introduction

In Antarctica, the exposure to UV-B radiation varies during the year, being the months between September and November when most of the lichen's exposure to UV-B radiation occurs (Caldwell et Flint 1994). Various types of seasonal acclimation in lichens have been reported, but in general lichen acclimation is not well studied. Recent studies have shown that the mycobiont can acclimate with a UV-B induced fungal synthesis of sun-screening cortical pigments (Nybakken et al. 2004). In many lichen species, seasonality of involvement of particular photoprotective mechanisms in response to actual radiation regimen is expected, as shown for *e.g.* *Xanthoria parietina* (Vráblíková et al. 2005). Nowadays, many scientists are interested in studying lichens from polar regions due to their high tolerance to extreme physical factors. Lichen ability to synthetize several photoprotective compounds is in focus. Some studies (*e.g.* Buffoni-Hall et al. 2002, McAvoy 2006, Estêvão 2015) applied supplemental UV-B to alter synthesis of UV-B screens.

Solar UV radiation travels virtually unaltered from the sun to the earth's atmosphere. Once in the atmosphere, absorption and scattering by various gases and particles modifies the radiation profoundly, removing radiation that could be harmful to organisms (Bachereau et Asta 1997). In polar ecosystems, where the largest decrease in ozone concentration is occurring, UV-B radiation might reach up to 3 W m^{-2} in maritime Antarctica during ozone hole period (Montiel et al. 1999). Antarctic poikilohydric autotrophs have developed several photoprotective mechanism to cope with such high doses, such as *e.g.* UV-B screening pigments as demonstrated *e.g.* for mosses (Newsham 2003). Lichens that may dominate some Antarctic areas covered by vegetation are studied much less frequently (*see* e.g. Lud et al. 2001). Many macromolecules are potential targets for direct UV-B absorption, in particular DNA, membranes, lipids and proteins. UV-B radiation induces a wide range of direct and indirect effects on plants at a physiological and morphological level; it can produce a decrease on the efficiency of photosynthesis, alter the synthesis of phenolic compounds (Vráblíková et al. 2005). In Umbilicariaceae family, UV-B screeening compounds are reported (Fahselt 1993). Resistance against photoinhibition has been studied 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* (Balarinová et al. 2014); however, sensitivity of Antarctic lichens to UV-B radiation is less studied. Studies done with *U. antarctica* have demonstrated that it is well adapted to sub-zero temperatures and is capable of performing photosynthesis at -15ºC (Barták et al. 2007). *U. antarctica* can resist high light stress synthesizing antioxidants for photoprotection (Barták et al. 2004).

In our experiment, we applied several chlorophyll fluorescence methods in order to determine the resistance of *U. antarctica* to extremely high UV-B radiation. Since intrathalline heterogeneity of chlorophyll fluorescence was reported earlier in foliose macrolichens (Barták et al. 2000), chlorophyll fluorescence imaging of several parameters was applied in our study. In similar studies, key parameters F_0, F_M, F_0', and F_M' are measured and basic parameters are calculated in order to evaluate stress effects in PS II. F_0 acceptor fluorescence emission is measured when the primary quinone acceptor Q_A is oxidized and non-photochemical quenching is inactive, F_0' - fluorescence emission measured when Q_A is oxidized and non-photochemical quenching is active, F_M - maximum fluorescence emission measured when Q_A and the plastoquinone pool are reduced and non-photochemical quenching is inactive,

F_M' - fluorescence emission measured when Q_A and plastoquinone pool are reduced and non-photochemical quenching is active,

F_S - steady-state fluorescence emission in light (Nedbal et Whitmarsh 2004).

Material and Methods

Sample collection and handling

Thalli of *U. antarctica*, a foliose macrolichen, were collected from rock walls at Galindez Island, close to Vernadsky station (65° 14′ 44′′ S, 64° 15′ 20′′ W), Antarctica by a Czech participants of Ukrainian expedition. After collection, thalli were dried under natural conditions and transfered to a laboratory where stored in a refrigirator. Before experiments, thalli of *Umbilicaria antarctica* were hydrated by demineralized water for 24 h at 0°C until full hydration was reached. Vigorous thalli showing the highest values of chlorophyll fluorescence parameters (F_V/F_M, Φ_{PSII} - *see* below for definitions) were chosen for the experiments.

UV-B exposition

Hydrated thalli were placed in Petri dishes with ice to maintain the temperature and covered with a UV-B transmitting transparent foil to prevent dehydration during the exposition time. Afterwards, the lichen thalli were exposed to a high light dose of UV-B radiation (3.0 W m^{-2}), for 3 hours, 6 hours and 7 days at 0°C. 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). After 3 and 6 hours and on the day 7, chlorophyll (Chl) fluorescence parameters were measured and samples for spectroscopic measurements were taken.

Chlorophyll fluorescence measurements

To evaluate the UV-B treatment-related effects on photosynthetic performance. Chlorophyll fluorescence was measured with a HFC-10 fluorometer (PSI, Czech Republic) after 3 and 6 hours of exposure and after 1 week of exposure. Thalli of *Umbilicaria antarctica* were predarkened for 5 min at 0°C to produce a dark-adapted thalli on which background (minimum) chlorophyll fluorescence F_0 was measured. Then, the thalli were exposed to a saturating pulse of light to induce maximum chlorophyll fluorescence (F_M). Then, the thalli were light-adapted for 5 min until the steady state chlorophyll fluorescence (F_s) was reached. At this time, thalli were exposed to another saturating pulse of light and a value for the maximum chlorophyll fluorescence on light-adapted sample (F_M') was obtained.

To determine whether or not *Umbilicaria antarctica* shows a decrease in primary photosynthetic processss when exposed to a high light dose of UV-B radiation (3.0 W m^{-2}), we calculated potential and effective quantum yields of photosynthetic processes in PS II: F_V/F_M and Φ_{PSII} (Genty et Meyer 1994).

$F_V/F_M = (F_M - F_0)/F_M$ *Eqn.1*

$\Phi_{PSII} = (F_M' - F_S)/F_M'$ *Eqn.2*

Additionally, measurements of fast chlorophyll fluorescence transients (OJIPs) were done on *U. antarctica* thalli using a FluorPen (Photon Systems Instruments, Czech Republic). Untreated control thalli and those exposed to supplemental UV-B (3.0 W m^{-2}) for 3, and 6 h, respectively were used. From standard 17 chlorophyll fluorescence parameters (for more details *see* Strasser et al. 1998), the most sensitive one was selected and analyzed (Table 1).

Spectrophotometric measurements

The thalli of *Umbilicaria antarctica* were desiccated for 24 hours in a lyophilizator, then homogenized for UV-B screening compounds evaluation. Equal amounts were then weighed (0.1 g) and secondary products were extracted with ethanol. Using a UV-VIS spectrophotometer (Specord 205, Analytik Jena, Germany) the absorbances of the extracts were measured within the wavelength range of 190-700 nm.

Results and Discussion

Fast chlorophyll fluorescence transients showed an increase in absolute values with UV-B stress (Fig. 1A). Shape of OJIPs, however, remained unchanged (*see* F_P -normalized curves Fig. 1B). Therefore, OJIP-derived characteristics related to PSII functioning were more or less unchanged in UV-B-affected *U. antarctica* and comparable to untreated control (Table 1). Thus, short-term UV-B treatment did not lead to any change in photosynthetic processes in PS II. Such conclusion is supported by the chlorophyll fluorescence parameters derived from slow Kautsky kinetics supplemented with quenching analysis. Majority of parameters were insensitive and showed either no or very small change with UV-B treatment. NPQ increased slightly which could be attributed to early phase of activation of photoprotective processes in photosynthetic apparatus of symbiotic alga. Such a high resistance of *U. antarctica* to strong, but short-term UV-B treatment is not suprising since Antarctic lichens possess UV-B absorbing compounds that protect chlorophyll molecules from UV-B induced stress (Gautam et al. 2011). The resistance might be related to a high, constitutive amount of UV-B absorbing compounds, umbilicaric acid and phenolic compounds in particular (Swanson et al. 1996). Intrathalline amount of UV-B absorbing compound could be increased in short-term UV-B treated *U. antarctica* (Krábková 2013).

Spectral curves of ethanol extracts showed several differences between control and UV-B treated thalli. Generally, absorption curves of UV-B treated thalli showed either no change or a decrease in particular peaks (*see* Fig. 2). Therefore, two subgroups of ´UV-B resistant´ and ´UV-B sensitive´ thalli could be distinguished in thalli treated by high UV-B dose (3 W m^{-2}). In sensitive thalli, a decrease in absorption peaks at 215, 270, and 310 nm was apparent while the 208 nm peak remained unchanged. It could be attributed to a treatment-induced degradation of the compound having maximum absorption in the above specified particular waveleghts – umbilicaric acid (Huneck et Yoshimura 1996). It seems that sensitivity of *U. antarctica* to UV-B induced degradation is specific for particular thalli since some thalli did not show any change compared to control.

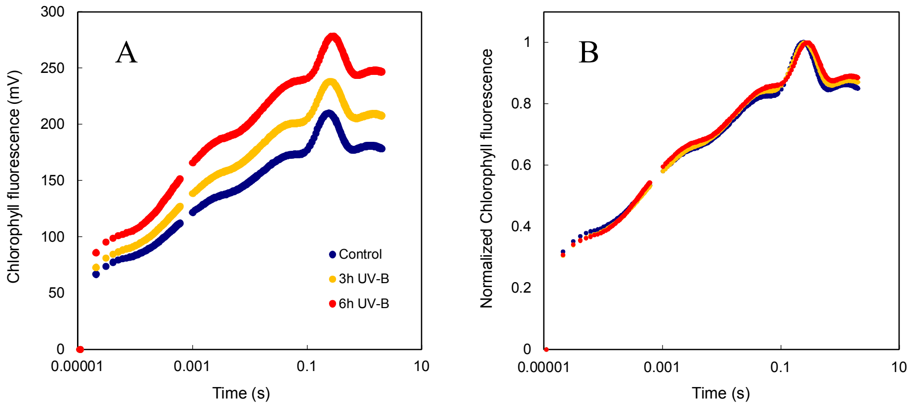

Fig. 1. A – Fast chlorophyll fluorescence transients (OJIPs) of *Umbilicaria antarctica* measured before (control), and after 3, 6 h exposition to additional UV-B (3 W m^{-2}). Absolute values of chlorophyll fluorescence are presented in left panel. B – F_P-normalized data are presented in right panel.

	Control		**After 3h**		**After 6h**	
	Mean	± Std	Mean	± Std	Mean	± Std
OJIP						
F_V/F_0	**1.906**	± 0.345	**2.172**	± 0.432	**2.235**	± 0.280
F_V/F_P	**0.714**	± 0.033	**0.679**	± 0.045	**0.688**	± 0.030
Phi_Pav	**772.6**	± 11.04	**882.7**	± 12.85	**891.1**	± 8.11
Pi_Abs	**0.787**	± 0.342	**1.014**	± 0.346	**0.934**	± 0.314
ABS/RC	**2.577**	± 0.278	**2.780**	± 0.352	**2.701**	± 0.299
TRo/RC	**1.743**	± 0.147	**1.875**	± 0.153	**1.852**	± 0.139
ETo/RC	**0.915**	± 0.026	**1.032**	± 0.087	**0.956**	± 0.087
KK_QMA						
F_V/F_M	**0.549**	± 0.020	**0.545**	± 0.015	**0.547**	± 0.023
Φ_{PSII}	**0.475**	± 0.021	**0.483**	± 0.028	**0.484**	± 0.025
NPQ	**0.524**	± 0.034	**0.542**	± 0.043	**0.558**	± 0.040

Table 1. Parameters (means and standard deviations - Std) derived from OJIPs and slow Kautsky kinetics supplemented with quenching mechanisms analysis (KK_QA) measured before and after exposition to supplemental UV-B. Symbols and abbreviations: Time to reach F_M given in ms (Phi_Pav), performance index (PI_{ABS}), absorption flux (for PS II antenna chlorophylls) per reaction center (ABS/RC), trapped (maximum) energy flux per PS II (TRo/ABS), electron transport flux per RC of PS II (ET_0/RC), non-photochemical quenching (NPQ).

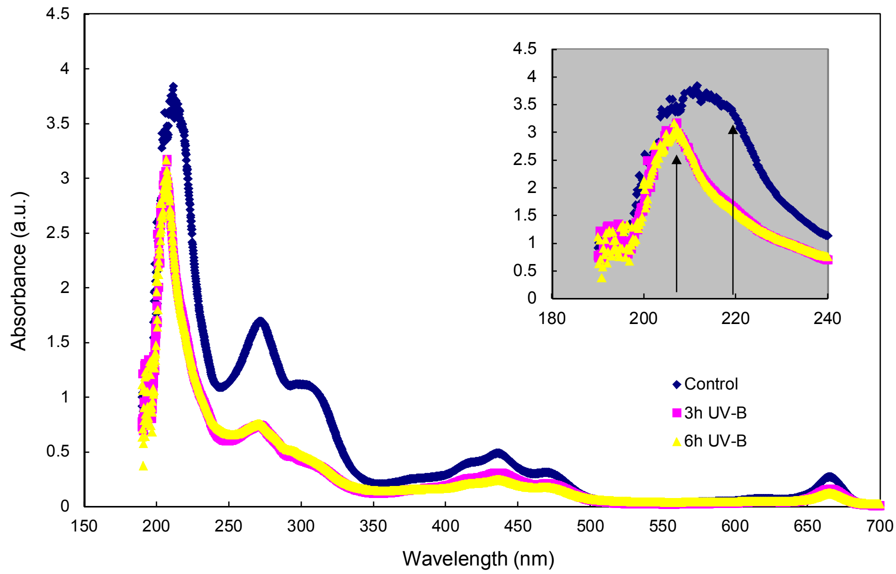

Fig. 2. Absorbance of ethanol extracts of *Umbilicaria antarctica* from control (untreated) thalli and those exposed to supplemental UV-B (3 W m^{-2}) for 3, and 6 h, respectively. Inset graph shows a decrease in absorbance in selected wavelengths (210 and 220 nm, respectively).

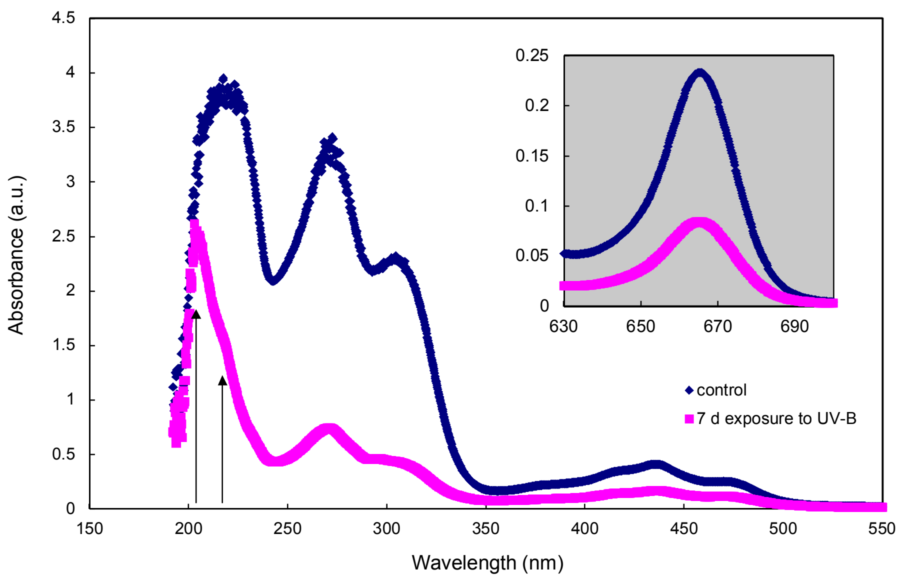

Fig. 3. Absorbance of ethanol extracts of *Umbilcaria antarctica* from control (untreated) thalli and long-term UV-B exposed (3 W m^{-2}) ones, respectively. Pronounced decrease in absorbance is apparent at 210 nm (*c.f.* Fig. 2). Inset graph shows absorption of chlorophyll in control and treated thalli. UV-B-induced decrease in the 664 nm peak is distinguished.

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