Reprint of Efficient fungal UV-screening provides a remarkably high UV-B tolerance of photosystem II in lichen photobionts

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ABSTRACT

Lichen photobionts in situ have an extremely UV-B tolerant photosystem II efficiency (Fv/Fm). We have quantified the UV-B screening offered by the mycobiont and the photobiont separately. The foliose lichens Nephroma arcticum and Umbilicaria spodochroa with 1: intact or 2: removed cortices were exposed to 0.7 Wm⁻² UV-B for 4 h. Intact thalli experienced no reduction in Fv/Fm, whereas cortex removal lowered Fv/Fm in exposed photobiont layers by 22% for U. spodochroa and by 14% for N. arcticum. We also gave this UV-B dose to algal cultures of Coccomyxa and Trebouxia, the photobiont genera of N. arcticum and U. spodochroa, respectively. UV-B caused a 56% reduction in Fv/Fm for Coccomyxa, and as much as 98% in Trebouxia. The fluorescence excitation ratio (FER) technique comparing the fluorescence from UV-B – or UV-A-excitation light with blue green excitation light using a Xe-PAM fluorometer showed that these photobiont genera did not screen any UV-B or UV-A The FER technique with a Multiplex fluorometer estimated the UV-A screening of isolated algae to be 13–16%, whereas intact lichens screened 92–95% of the UV-A. In conclusion, the cortex of N. arcticum and U. spodochroa transmitted no UV-B and little UV-A to the photobiont layer beneath. Thereby, the upper lichen cortex forms an efficient fungal solar radiation screen providing a high UV-B tolerance for studied photobionts in situ. By contrast, isolated photobionts have no UV-B screening and thus depend on their fungal partners in nature.

1. Introduction

Lichens are long-lived associations between a mycobiont and one or more photobionts (a green alga and/or a cyanobacterium). The mycobiont provides water, minerals, and protection against high solar radiation and herbivores, whereas a main role of the photobiont is to fix CO₂. Lichens are poikilohydric organisms often inhabiting extreme environments with high solar radiation (Bjerke et al., 2002; Baniya et al., 2010). For example, the high-light adapted lichens Rhizocarpon geographicum and Xanthoria elegans survived several days exposure in the outer space (de la Torre et al., 2010). Even shade-adapted specimens of the old forest lichen Lobaria pulmonaria tolerates high UV-B doses due to efficient cortical UV-B screening: its cortical UV-B transmission is close to zero (Gauslaa et al., 2017). By contrast, epidermis of higher plants typically transmits 10% UV-B (Bilger et al., 2007). Exposure of an intact, but freely exposed photobiont layer in L. pulmonaria to UV-B increased the susceptibility of photosystem II (PSII) to UV, showing that the fungal cortex screens UV-radiation (Gauslaa et al., 2017). However, we do not know whether algal photobionts screen UV-radiation themselves.

Lichens often synthesize high amounts of secondary compounds (Molnár and Farkas, 2010; Huneck, 1999) that may screen photosynthetic active radiation (PAR) and UV radiation (Solhaug and Gauslaa, 2012). These secondary fungal compounds are often located as small crystals on the surface of the fungal hypha. Some compounds mainly occur in the upper cortex whereas others are located in the medulla (Fahsel, 1994). It is possible to non-destructively remove most of these compounds with acetone-rinsing (Solhaug and Gauslaa, 2001). However, acetone rinsing removing the majority of secondary compounds just slightly raised the UV-susceptibility of L. pulmonaria, probably because even a compound-deficient lichen cortex is an efficient UV-screen (Gauslaa et al., 2017).

Among green algal lichen photobionts, sporopollenin-like substances occur in the cell wall of Coccomyxa, but not in Trebouxia.
Trebouxia jamesii

2. Materials and methods

2.1. Lichen material

Umbilicaria spodochroa was collected from sun-exposed rocks at Hvaler, Østfold S Norway (59°08′36″N, 10°55′21″E); Nephroma arcticum from partly shaded N-facing rock in open spruce forests in Ski, Akershus, S Norway (59°44′22″N, 10°58′34″). We selected these two species because they were sufficiently large and robust to allow freely exposed photobiont layers of the respective photobiont genera to be prepared. Both were collected in typical habitats of the two respective species. Furthermore, we wanted to use one lichen with and one without a sporopollenin-producing photobiont. Many thalli of both species were collected in May, air-dried, and stored at −18°C. We completed all experiments within less than one month after collection. Before the UV exposure, lichens were moistened and placed at approx. 1 cm2 of the lower cortex including the medulla was removed from partly shaded N-facing rock in open spruce forests in Ski, Akershus, S Norway (59°44′22″N, 10°58′34″). We selected these two species because they were sufficiently large and robust to allow freely exposed photobiont layers of the respective photobiont genera to be prepared. Both were collected in typical habitats of the two respective species. Furthermore, we wanted to use one lichen with and one without a sporopollenin-producing photobiont. Many thalli of both species were collected in May, air-dried, and stored at −18°C. We completed all experiments within less than one month after collection. Before the UV exposure, lichens were moistened and placed at approx. 30 μmol m−2 s−1 light for one day to recover from possible photoinhibition prior to collection in the field (Solhaug et al., 2018). For direct exposure of the photobiont layer, a portion of approximately 1 cm2 upper cortex was carefully scraped off each specimen of U. spodochroa, whereas approx. 1 cm2 of the lower cortex including the medulla was removed from N. arcticum thalli. A highly fragile structure did not allow removal of the upper cortex in the latter species. We carefully scraped off the upper cortex from healthy uniform lichen thalli using a scalpel under a preparation microscope. The following categories, (1) intact thalli, (2) scraped thalli with exposed photobiont layers, as well as (3) cultured photobionts were exposed to UV irradiation before Fv/Fm were measured by chlorophyll fluorescence imaging tools.

2.2. Photobiont cultivation

Stock cultures of the lichen photobionts Trebouxia erici (UTEX 911), T. assimetrica (SAG 48.88), T. jamesii (SAG 2103) and Coccomyxa sp. (P. Váčzi lab. collection) were maintained in an axenic culture on Bold's Basal Medium (BBM-agar, prepared according to Ahmadjian, 1993) in Petri dishes. The photobionts were cultivated at 20°C using 16/8 h light/dark photoperiod with irradiance of 30 μmol m−2 s−1 from Philips TL 18W/827 fluorescent tubes. The spectral irradiance distributions were measured with an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, Florida, USA) (Fig. 1), and the biologically effective UVB radiation from UV-B broadband fluorescent tubes, Philips TL 20W/12 RS for 4 h. This UV-B treatment represents a common, high UV-B level during a sunny summer day in Norway (Solhaug et al., 2003). Cellulose diacetate film (Jürgen Rachow, Hamburg, Germany, 0.10 mm, 50% cut-off at 295 nm) screened the radiation below 295 nm. During both UV exposure and subsequent recovery, the samples were exposed to PAR of 40 μmol m−2 s−1 from Philips TL 18W/827 fluorescent tubes. The spectral irradiance distributions were measured with an Optronic model 756 spectroradiometer (Optronic Laboratories, Orlando, Florida, USA) (Fig. 1), and the biologically effective UVB was estimated with the green weighting function normalised to 1 at 300 nm (Green et al., 1974). Control samples had a cover of polycarbonate sheets that screened all UV radiation. Three 4 h UV-exposure experiments were done. In the first experiment, we compared the four different photobiont species. In the second experiment, we simultaneously exposed (1) intact thalli, (2) thalli with scraped cortex of U. spodochroa, and (3) its cultivated photobiont Trebouxia jamesii to UV. Likewise, the third experiment simultaneously used (1) intact thalli, (2) thalli with scraped cortex of N. arcticum, (3) and its cultivated photobiont genus Coccomyxa.

2.4. Photosystem II efficiency

Maximal photosystem II efficiency (Fv/Fm, Van Kooten and Snell, 1990) was measured after 15 min dark adaptation using Handy FluorCam imaging chlorophyll fluorometer (Photon System Instruments, Brno, Czech Republic; all exciting light λmax = 620 nm). During the measuring protocol, the samples were exposed to low measuring light (1 μmol m−2 s−1) to determine basal fluorescence signal Fo and subsequently to strong saturating pulse (3000 μmol m−2 s−1) to
determine maximal fluorescence Fm. Fv/Fm was calculated using the formula: (Fm-Fo)/Fm.

Measurements were done before the UV exposure, during the UV-B exposure (after 1, 2, 3, 4 h) and during the recovery period (after 1, 3, 6, 18, 30 h).

2.5. Fluorescence excitation ratio method (FER)

UV screening was measured with the fluorescence excitation ratio (FER) technique using the fluorometers Multiplex 3 (FORCE A, Orsay, France) and Xe-PAM fluorometer (Walz, Effeltrich, Germany). Both instruments excite chlorophyll fluorescence at two wavelengths: one UV-wavelength absorbed by cells or cell walls before the beam reaches
with the Xe-PAM fluorometer using the method of Bilger et al. (1997). Photobiont cells were concentrated on filter paper by dripping a photobiont suspension on the paper. The paper covered with photobionts was attached to the sample holder in the fluorometer with double-sided tape. The setup of the instrument with filters, sample holder and light source followed Burchard et al. (2000) and Pescheck et al. (2010). The fluorescence signals induced by UV-A (λ_{max} = 366 nm, 32 nm half bandwidth), UV-B (λ_{max} = 314 nm, 18 nm half bandwidth) and broadband blue-green (λ = 420–550 nm) were normalised with fluorescence signals from blue plastic foil (fluorescence standard, Walz); the signal from a non-fluorescing green foil was subtracted from all measurements. The signals from photobionts were compared with the signal from isolated chloroplasts assumed to have very low screening. Because the normalised F_{UV}/F_{BG} ratios were slightly higher for lichen photobionts than for isolated chloroplasts, we presented the results as F_{UV}/F_{BG} ratios for both photobionts and chloroplasts. Calculation of percent screening based on zero screening for chloroplasts would have resulted in negative values probably caused by slight screening in the chloroplasts.

### 2.6. Statistical analysis

We used factorial analysis of variance and Fisher’s LSD test (Dell Statistica ver.13, 2016) to determine the significant differences between (P > 0.05) measured parameters.

### 3. Results

#### 3.1. UV-B effects on photosystem II efficiency in algal cultures

Mean Fv/Fm at start (time = 0) differed among all (P < 0.001; one-way ANOVA) algal cultures. It was highest in *Coccomyxa* (0.738 ± 0.001), lower in *T. ericii* (0.651 ± 0.002), but lowest in *T. asymmetrica* (0.580 ± 0.001) and *T. jamesii* (0.590 ± 0.002). During the UV-B exposure to 0.7 W m^{-2} for 4 h, all algal cultures rapidly experienced strong reductions in maximum Fv/Fm. UV-B reduced Fv/Fm by 75% in *Coccomyxa* and by 88, 95 and 97% in *T. ericii*, *T. asymmetrica* and *T. jamesii*, respectively. *Coccomyxa*, *T. ericii*, and *T. asymmetrica* recovered almost 100% during 30 h under 40 μmol photons m^{-2} s^{-1}, whereas the recovery of *T. ericii* was 86% (Fig. 2A). Control algal cultures not exposed to UV-B had quite constant Fv/Fm during the experiment (Fig. 2B).

#### 3.2. UV-B effects on photobionts in intact lichens, exposed photobiont layers, and in asymbiotic photobiont cultures

When the three categories of the *N. arcticum* photobiont were exposed to 4 h UV-B, Fv/Fm was reduced by 56% in the *Coccomyxa* culture (start Fv/Fm = 0.709 ± 0.003), by 14% in scraped thalli (start Fv/Fm = 0.559 ± 0.025), whereas UV-B had no effect on photobionts in intact thalli (start Fv/Fm = 0.677 ± 0.010). Nevertheless, all samples recovered completely during 30 h under 40 μmol photons m^{-2} s^{-1}, whereas the recovery of *T. ericii* was 86% (Fig. 2A).

Likewise, UV-B exposure of the three photobiont categories of *U. spodochroa* resulted in 98% reduction of Fv/Fm in cultured *T. jamesii* (start Fv/Fm = 0.609 ± 0.004), 22% reduction in the scraped thalli (start Fv/Fm = 0.656 ± 0.011) and no reduction in the photobiont of the intact thalli (start Fv/Fm = 0.661 ± 0.011). Fv/Fm for both intact and scraped thalli completely recovered within 30 h at 40 μmol photons m^{-2} s^{-1}, whereas cultured *T. jamesii* recovered to 94% of the original Fv/Fm (Fig. 3C). By contrast, Fv/Fm did not change in any categories of *N. arcticum* and *U. spodochroa* photobionts during the experiment excluding UV-B (Fig. 3B and D).
3.3. Estimation of UV-A and UV-B screening using the Xe-PAM fluorometer

The ratio between UV induced fluorescence and blue-green induced fluorescence (FUV/FBG) was similar or slightly higher for algal cultures compared to isolated chloroplasts (Fig. 4). We assumed that chloroplasts had very low UV-A and UV-B screening. Similar or higher FUV/ FBG ratios in photobionts than in chloroplasts were consistent with low or no screening for all species both in the UV-A and UV-B spectral range.

3.4. Estimation of UV-A screening using the multiplex fluorometer

UV-A screening in pure T. jamestii and Coccomyxa cultures was 13.3 and 15.6%, respectively, whereas intact thalli of U. spodochroa and N. arcticum had UV-A screening of 95.0 and 92.7%, respectively (Fig. 5).

4. Discussion

Cortical screening by the mycobiont efficiently protects PSII against harmful UV-B radiation in photobionts in N. arcticum and U. spodochroa (Fig. 2), as previously shown for the old forest lichen Lobaria pulmonaria (Gauslaa et al., 2017). Yet, even exposed photobiont layer were substantially more UV-B-resistant than pure photobiont cultures (Fig. 3), presumably because algal cells in a photobiont layer are surrounded by medullary hyphe with UV-B screening compounds. Another explanation may be that algae within the lichen has acclimated to higher UV than algae cultured for a long time in a lab. Additional, but low UV screening in pure algal photobionts was confirmed by the two FER techniques.

With respect to the two FER techniques, the portable Multiplex instrument measures UV-A screening only, whereas the Xe-PAM fluorometer assesses UV-A and UV-B screening independently. However, UV-A screening may also indicate UV-B screening because many UV-A-absorbing compounds absorb even more in the UV-B range. For algal cultures, the Multiplex instrument showed less than 15% screening assuming no UV-screening in the reference chloroplasts, whereas Xe-PAM indicated that the algal cultures had similar or even less screening than chloroplasts. Together, these measurements show that pure cultures of studied lichen photobionts hardly screen any UV. By contrast, the Multiplex instrument showed 85% UV-A screening for intact lichens. This is likely an underestimation of the UV-B screening because the cortical secondary compound usnic acid in N. arcticum absorbs much more UV-B than UV-A (McEvoy et al., 2007). Furthermore, if cortical transmission of N. arcticum and U. spodochroa is similar to unpigmented shade-adapted thalli of the old forest lichen Lobaria pulmonaria (Gauslaa et al., 2017), their cortical UV-B screening should be close to 100%.

The FER data did not support the hypothesis that the sporopollenin present in Coccomyxa screens UV (Figs. 4 and 5). Nevertheless, Coccomyxa seems to be more resistant against UV-B than Trebouxia, but the explanation is likely rather efficient repair of UV damage than efficient screening. In the macroalgae Ulva clathrate, UV-B resistance with no screening is based on fast repair rate of PSII, whereas UV-B resistance in Rhi...omolipid reaction in the same sites efficiently screens UV. By contrast, their photo-bionts Coccomyxa and Trebouxia are highly susceptible to UV-B because they lack UV-screening. Therefore, Coccomyxa and Trebouxia totally depend on their fungal partner to survive UV-B under natural conditions.

Author contributions

PV and KAS designed the study. PV and KAS performed the experimental studies. All results and data were analyzed and interpreted by PV, YG and KAS. PV, YG and KAS wrote the manuscript.

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References


