

Desiccation and rehydration in the lichen *Cladonia convoluta* monitored by laser scanning microscopy

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ABSTRACT Changes in chlorophyll fluorescence emission from the lichen *Cladonia convoluta* were followed by imaging techniques. We compared fluorescence images of both the green (cortex) and the gray (fungal) side of the lichen at various stages of desiccation and re-watering. Confocal laser scanning microscopy made the imaging of fluorescence from individual algal colonies possible inside the lichen, under the cortex in a non-invasive way.
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KEY WORDS

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chlorophyll fluorescence

Lichens acquired special importance during evolution, by enabling photosynthetic algae to become established in previously barren terrestrial environments. Lichens are symbionts, an association beneficial for both fungi and algal cells. It is well established, that the photosynthetic activity of the photobiont (the algae) decreases when the mycobiont compartment (the fungi) loses water. A large group of lichens are tolerant to desiccation, *i.e.* their photosynthesis and other physiological parameters fully recover after rehydration (Bewley 1979; Proctor 1990). We have shown earlier, by measuring variable chlorophyll fluorescence, that the decline of photosynthetic activity was much slower during desiccation than its recovery upon rehydration (Sass et al. 1995). These experiments were carried out collecting and averaging fluorescence data from a relatively large area of lichens. In the frame of the present work, we studied the spacial distribution of steady state chlorophyll fluorescence from lichens using imaging techniques.

Materials and Methods

Cladonia convoluta lichens were collected from dry grassland on sand (*Festucetum vaginatae danubiale* Soó) near Fülöpháza, Hungary (19° 14' E; 47° 30' N) at 130 m above

sea level. *Cladonia convoluta* (Lam.) P. Cout. is a terricolous foliose type desiccation tolerant lichen with *Trebouxia* as green algal photobiont (Tuba et al. 1994). All measurements were made on the entire basal squamules of *Cladonia convoluta*. Before the desiccation experiments, cleaned thalli were rehydrated and kept moist resulting in full photosynthetic activity. As physiological responses of desiccation tolerant plants are greatly affected by the rates of water loss (Schonbeck and Bewley 1981; Tuba et al. 1995), thalli with full photosynthetic activity were allowed to desiccate slowly as in their natural habitat. Rehydration was achieved by oversaturating thalli with distilled water under room light.

Red ($\lambda > 675$ nm) fluorescence images were taken by a FLA2000 Bioimager (Fujifilm Ltd. Japan), using 633 nm excitation from a He-Ne laser. For microscopy, lichen thalli were put between two layers of microscope cover glass and measured using a confocal laser scanning system (LSM 510, Karl Zeiss, Germany) in combination with an inverted microscope. In these experiments, the cortex faced the 633 nm He-Ne laser excitation and fluorescence emission was observed as $\lambda > 650$ nm. LSM images were scanned at 0.8 s per frame, averaging 4 images.

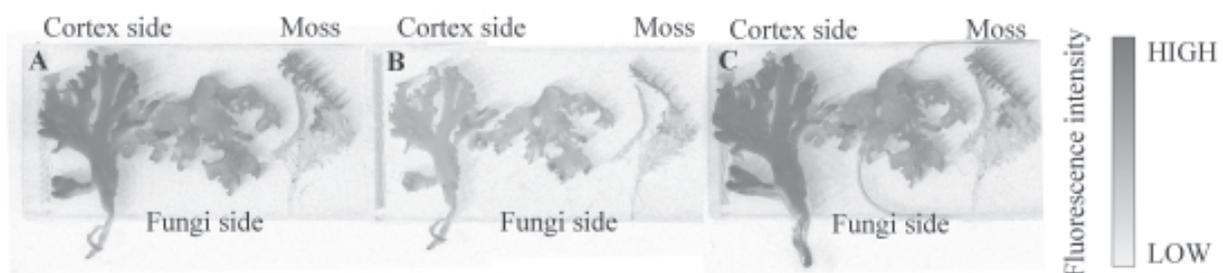


Figure 1. Images of 633 nm excited red ($\lambda > 675$ nm) fluorescence from the cortex and fungal side of the lichen *Cladonia convoluta*, and from the moss *Tortula ruralis*. (A) wet samples, (B) after 21 hours of desiccation and (C) after 1 hour subsequent re-watering.

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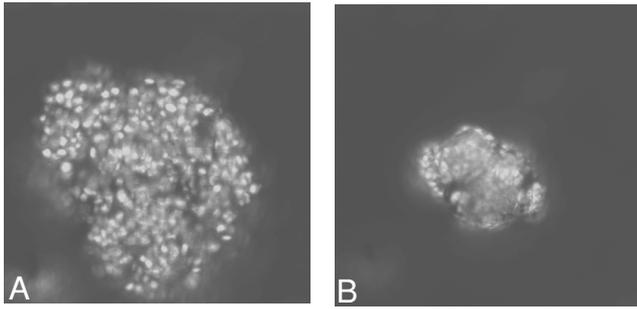


Figure 2. LSM images of 633 nm excited red ($\lambda > 650$ nm) fluorescence detected in lichen (A) wetting state, (B) after 1 hour 50 minutes of desiccation at 70mm from the cortex. Image size 230 x 230 mm.

Results and Discussion

Figure 1 illustrates changes in chlorophyll fluorescence measured at the upper green (cortex) and at the lower gray (fungal) side of *Cladonia convoluta* thalli at various stages of desiccation and re-hydration. For comparison, images of fluorescence from a desiccation tolerant moss, *Tortula ruralis*. In *Cladonia convoluta* red fluorescence was more intense from the cortex side than from the fungal one (Fig. 1A). Steady state fluorescence gradually decreased during desiccation until it reached ca. 40% of the original value (Fig. 1B), but was completely restored after 60 min subsequent rewetting (Fig. 1C).

In lichens, photobionts are located under the cortex layer and initiate association with fungi making their mechanical separation very difficult. Confocal laser scanning microscopy (LSM) enabled us to measure from this area without damaging the integrity of the thallus. Figure 2 shows images of

chlorophyll fluorescence acquired at 70 mm depth from the surface of the cortex. This picture also indicates that the fluorescence is lower in dry state and the colony size of algal cells decreased during the desiccation.

Acknowledgments

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