Life strategies on physiology and metabolome of photobionts of different lichen species from Antarctic habitats compared to moderate habitats

I. Desiccation tolerance of photobionts of three Antarctic lichens in comparison to a lichen species from a moderate European habitat

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Acknowledgement

We thank Eva Posthoff for her invaluable help with the photobiont cultures. Thanks are also due to the organization committee of the XIIth SCAR Biology Symposium 2017, Leuven, Belgium. The last author is grateful to the German Research Foundation (DFG) for financing the research project Ot 96/15–1 as part of the Antarctic Priority Program (SPP 1158). Special thanks are due to the BGR (Bundesanstalt für Geologie und Rohstoffe, Andreas Läufer, Detlef Damaske) as well as to the British Antarctic Survey for the opportunity to collect the lichen samples used in this study. Thanks are also due to the staff at Rothera Station and Gondwana Station for logistic support. PC is supported by NERC core funding to the BAS Ecosystems programme. This paper also forms an output of the SCAR AntEco and AnT-ERA scientific programmes. Thanks to the anonymous reviewers for their helpful comments.

Abstract

The vegetation at terrestrial habitats across Antarctica is dominated by the poikilohydric symbiotic lichens. Terrestrial habitats generally are characterized by long durations of desiccation. Adaptation mechanisms on the physiological level of the algal partner (photobiont) are relevant key factors for successful colonization of lichens by severe environmental conditions. Isolated photobionts of the genus *Trebouxia* of the continental Antarctic lichens *Buellia frigida, Pleopsidium chlorophanum* and of the maritime Antarctic lichen *Umbilicaria antarctica* have been studied compared to the isolated photobiont of the Swedish lichen *Fulgensia bracteata* which originates from a moderate ecosystem. Both photosystems PS II and I and the ratio of linear and cyclic electron transport have been studied to get substantial knowledge to adaptation mechanisms on the physiology of photobionts with regard to desiccation but also to light stress. In relation to their Antarctic and European origin the results clearly show that the photobionts differ in response to their physiological potential under stress conditions. The photobionts of the Antarctic lichens demonstrate higher tolerance to the studied stress conditions. Although the photobionts of *U. antarctica* and *P. chlorophanum* are genetically identical the response pattern on the physiological level is clearly different. Results achieved on the photobiont of *F. bracteata* demonstrate obvious differences considering the stress tolerance to severe environmental conditions. The study exhibits considerable life strategies of the photobionts investigated and point to habitat-specific adaptations on the photosynthesizing partner with regard to physiology.

Keywords: Antarctica, lichens, desiccation, adaptation, isolated photobionts, physiology

Introduction

Antarctica is the driest continent due to low precipitation combined with the limited availability of liquid water caused by prevailing temperatures below the freezing point (Huiskes et al. 2006). Terrestrial, ice-free habitats across Antarctica are limited to less than 0.5 % of the entire continental area (British Antarctic Survey 2004). The rocky summits of buried mountain ranges (nunataks) standing above the surrounding ice-sheet form together with coastal landscapes, the contemporary ice-free terrestrial habitats of the Antarctic (Hughes et al. 2006). Lichens dominate the terrestrial vegetation across Antarctica. These symbiotic associations in general consist of a fungus (the mycobiont) and a green alga or cyanobacterium (the photobiont) responsible for carbon nutrition of the organisms (Henssen & Jahns 1974).

The most relevant limiting factor for lichens primarily is the water availability in both maritime and continental Antarctica (Kennedy 1993, Block 1996). Lichens are not able to actively regulate their hydrological balance and are dependent on water availability from their environment (Larson 1979; Green and Lange 1994). They are characterized by a poikilohydric life style. Poikilohydry causes a state of latent life called anabiosis (Jahns 1988) or anhydrobiosis (Kranner et al. 2005). Lichens are able to tolerate severe abiotic conditions such as extreme temperatures and strong radiation (Kappen 2000; de Vera 2003, 2004a; Backhaus et al. 2014; Sánchez et al. 2014).

A repetitive and long-lasting desiccation of the thallus can be described as a fundamental part of the poikilohydric life style of lichens. Reactivation occurs within a few minutes by moistening (Lange et al. 1998). In the Antarctic summer, the availability of melting water leads to irregularly distributed times of physiological activity of the lichens (Schroeter et al. 2011, 2017). The ability to tolerate desiccation processes constantly can be described as a crucial life strategy of the lichen symbiosis.

Photobionts of the genus *Trebouxia* Puymaly form the most frequent photosynthesizing partner in lichens (Ahmadjian 1960) and especially those belonging to *Trebouxia* clade S (phylogeny after Helms et al. 2001) are preferably represented in macro lichens across Antarctica (Romeike et al. 2002, Engelen et al. 2016). It is still a matter of discussion how they are able to survive aposymbiotically under natural conditions (Wornik and Grube 2010). Isolation and cultivation of lichen photobionts under artificial conditions has been experienced successfully (Stocker-Wörgötter 2001; Scharper and Ott 2003; Ruprecht et al. 2012).

The lichen symbiotic organism is characterized by a structure formed by the mycobiont which serves as protection for the photosynthesizing biont against excessive loss of water, severe temperatures as well as high irradiation (Jahns 1995; Nash 1996; Meeßen et al. 2013). The thallus structure creates a micro-environment for the photobiont, which makes it possible to be physiologically active even at hostile external environmental conditions (Honegger 2009) as they occur e.g. in Antarctica.

Both desiccation and high radiation could lead to oxidative stress in lichen photobionts (Kranner et al. 2005). By conditions of high radiation, the excess light energy of the photosystem (PS) II in chloroplasts can be dissipated into heat by non-photochemical energy quenching (NPQ), which prevents the formation of reactive oxygen species (ROS) (Fernández Marín et al. 2010). Caused by desiccation, the loss of water can effect changes in intracellular pH. Additionally, changes in ion concentrations could influence the activity of enzymes (Kranner et al. 2008). For example, the core protein D1 of the PS II is degraded by the desiccation process (Richter et al. 1990, McKersie and Lesheim 2013), a mechanism evolved in photoinhibition (Krause and Jahns 2004). Proteins might be denatured and disruption of membrane functions may occur by water loss. This may also lead to the formation of ROS by desiccation stress (Konsugi et al. 2009). Increasing formation of ROS may cause damage to the photosystems I and II (Krause and Jahns 2004) resulting in a decrease of photosynthetic performance (deterioration of D1 core protein of the PS II). Damage to PS II also leads to a reduction of linear electron transport (LET), which reduces the downstream carbon assimilation. A possible pathway for repairing the damage and to resynthesize the impaired protein of PS II can be the stimulation of the cyclic electron transport (CET) concerning PS I. The promotion of the ATP synthesis and the recovery of PS II in a very short time can be generated by CET (Heber and Walker 1992; Bendall and Manasse 1995; Allakhverdiev et al. 2005). For augmentation of the knowledge on regulation mechanisms in the recent study the activity of PS I has been tested additionally to studies on the activity of PS II and its protection mechanisms (Sadowsky and Ott 2015).

The knowledge on the physiological potential of species of the genus *Trebouxia* and their stress tolerance has been increased fundamentally (Hoyo et al. 2001; Gasulla et al. 2009; Sadowsky and Ott 2012, 2015; Meeßen and Ott 2013; Meeßen et al. 2013, 2014; Backhaus et al. 2014; Sadowsky et al. 2016). Experiments conducted by Sadowsky and Ott (2012; 2015) demonstrated that isolated photobionts of selected Antarctic lichen species have a special potential for rehydration upon dehydration as well as freezing.

To enlarge the knowledge on stress tolerance and the physiological potential of isolated photobionts of Antarctic lichens, the present study focuses on the extent to which the physiological potential of isolated *Trebouxia* photobionts differ in relation to their Antarctic and European origin under stress conditions such as desiccation and high light intensity. The isolated photobionts of the continental Antarctic lichens *Buellia frigida* and *Pleopsidium chlorophanum* as well as of the maritime Antarctic lichen *Umbilicaria antarctica* were investigated and compared with the isolated

photobiont of the Swedish lichen *Fulgensia bracteata* originating from a rather moderate habitat. The photobionts of *P. chlorophanum* and *U. antarctica* are genetically identical and have been identified as *Trebouxia jamesii* clade S (Helms et al. 2001).

It can be postulated that adaptations of the lichen symbionts to the respective environment especially on the physiological potential of the isolated photobiont forms a crucial prerequisite to establish a successful symbiotic lifestyle. The study presented focuses on adaptation mechanisms with emphasis on the physiology of the photobiont comparing distinct environments.

Material and Methods

Material

The investigations were performed on isolated photobionts from lichen species colonizing Antarctic terrestrial habitats. *Buellia frigida* (Darb.), *Pleopsidium chlorophanum* (Wahlenb.) Zopf and *Umbilicaria antarctica* (Frey & I.M. Lamb) as well as from the lichen *Fulgensia bracteata* (Hoffm) Räs. growing on the Baltic island Gotland, southern Sweden, a more moderate environment. The identity of the photobionts and the geographical origin of the respective lichens are shown in table 1. The photobionts were isolated from lichen thallus fragments according to Yoshimura et al. (2002) and cultivated in liquid *Trebouxia-organic-medium* (TOM) with 1% (w/v) glucose according to Ahmadjian (1967) at low light intensity (20 µmol photons m^{-2} s^{-1}; diurnal cycles with 10 h of darkness) and 12 °C in a growth chamber (Rubarth Apparate GmbH, Germany).

Method

For chlorophyll fluorescence quenching analysis, the photobiont cultures were dark acclimated for 30 min at 20 °C. One ml of each photobiont culture was harvested per replicate (n = 4) and transferred to PVDF filters (GVWP 0.22 µm, Millipore, Durapore® Membrane Filters Ireland) by vacuum filtration to remove the medium. Samples were measured either directly, after 24 h (1 d) or 168 h (7 d) drying in a darkened desiccator with silica gel (rel. humidity < 5%). Subsequently all dried samples were reactivated with water at the start of the measurement with the Dual-PAM 100 (Pulse Amplitude Modulation Fluorimeter, Heinz Walz GmbH, Germany). The measuring system of the Dual-PAM 100 allows parallel recording of photosystem II and I activities. Each measurement series started with a dark-acclimation phase of 4.5 minutes, followed by a 12 minutes light phase with a gradually increase of light intensity to 40, 100, 211, and 342 µmol m^{-2} s^{-1}. At the end, 8 minutes of dark relaxation were applied. According to Huang et al. (2010), the ratio of cyclic electron flow (CEF) as well as linear electron flow (LEF) was determined from the data obtained from the measurements of PS II.

Data analysis

Data analysis was performed by MS Excel 2010. By two-sided *t* tests (level of significance $\alpha = 0.05$), significant differences between data sets were detected. The measuring points were always tested at the beginning and at the end of an experimental phase, in the light phase at the beginning and the end of each light intensity applied, respectively. The figures were created in GraphPad Prism 6.

Results

Light stress

The photobionts investigated exhibited different responses in their physiological reaction without a desiccation treatment (Fig. 1 & 2) during the initial dark phase of the measurement. The maximum quantum yield of PS II Fv/Fm of photobionts of the Antarctic lichens *Buellia frigida* (0.67±0.01), *Pleopsidium chlorophanum* (0.67±0.02) and *Umbilicaria antarctica* (0.68±0.01) was significantly higher than that of the photobiont of *Fulgensia bracteata* (0.58±0.01), (unpaired two-sided t test, $\alpha = 0.05$). The photobionts of the continental Antarctic lichens *B. frigida* and *P. chlorophanum* differed significantly to the photobiont of the lichen *U. antarctica* from southern maritime Antarctic considering the Fv/Fm values (unpaired two-sided t test, $\alpha = 0.05$).

Following the dark phase, with gradual increase of light intensity, the different photobionts displayed a continuing decrease of actual PS II quantum yield Y(II) depending on the respective light intensity (Fig. 1). The Y(II) values of the photobionts of *P. chlorophanum* and *U. antarctica* were slightly but not significantly higher than those obtained by the photobionts of *B. frigida* and *F. bracteata*. Considering the Y(II) values of the photobionts of *B. frigida* and *F. bracteata* no significant difference could be detected (unpaired two-sided t test, $\alpha = 0.05$).

By switching on the light independent of its intensity, an activation of the quantum yield of regulated energy dissipation (Y(NPQ)) and non-regulated energy dissipation (Y(NO)) could be recognized in the photobionts tested (Fig. 1). All investigated photobionts showed increasing Y(NPQ) and decreasing Y(NO) upon gradually increasing light intensity. Among the photobionts differences in the intensity of the increase of Y(NPQ) and the decrease of the Y(NO) could be detected (Fig. 1). The photobiont of *F. bracteata* exhibited the lowest values of Y(NPQ) and the highest of Y(NO) at the end of the light phase (342 µmol photons m^{-2} s^{-1}) compared to the photobionts of the Antarctic lichens. During the gradual increase of light intensity, no obvious changes in Y(NO) of the photobiont of *F. bracteata* occurred. The photobionts of the continental Antarctic lichens *B. frigida* and *P. chlorophanum* demonstrated a conspicuous increase of Y(NPQ). The Y(NPQ) values exceeded Y(NO) values during the gradual increase of light intensity. During the whole light phase, no significant differences has been detected for Y(NPQ) and Y(NO) between these two photobionts (unpaired two-sided *t* test, $\alpha = 0.05$). It could also be observed, that the clade S photobionts of *P. chlorophanum* and *U. antarctica* (genetically identical) differed from each other in terms of their response of the protective mechanisms considering Y(NPQ) as well as Y(NO) with respect to the induced light stress.

Considering the activity of PS I in the photobionts studied, the quantum yield of PS I (Y(I)) decreased with increasing light intensity, similarly to Y(II) as described above (Fig. 2). Only the Y(I) values of the clade S photobionts *P. chlorophanum* and *U. antarctica* differed significantly by the light intensity from 100 to 342 µmol photons m^{-2} s^{-1} (unpaired two-sided *t* test, $\alpha = 0.05$). For the photobionts studied, by increasing light intensity an increase of the donor side limitation of PS I (Y(ND)) and a decrease of the acceptor side limitation of PS I (Y(NA)) has been detected (Fig. 2). Comparing the photobionts of the Antarctic lichens and the photobiont of the Swedish lichen the largest increase of Y(ND) and decrease of Y(NA) has been detected in *F. bracteata*. A significant difference occurred on the Y(ND) values of the photobionts of *P. chlorophanum* and *U. antarctica* concerning the light intensity of 100 µmol photons m^{-2} s^{-1} (unpaired two-sided *t* test, $\alpha = 0.05$). The photobionts of *B. frigida* and *F. bracteata* showed no significant difference of Y(NA) as observed between *P. chlorophanum* and *U. antarctica* (unpaired two-sided t test, $\alpha = 0.05$).

The ratio of linear (LET) to cyclic electron transport (CET) of all photobionts showed a decrease of the linear electron transport as well as of the cyclic electron transport by increasing light intensity (Fig. 3.) and differed among the various photobionts. The photobionts of the Antarctic lichens exhibited a ratio of LET to CET comparatively much lower than in the photobiont of *F. bracteata*. In the photobiont of the maritime Antarctic lichen *U. antarctica* the LET decreased during the gradual increase of the light intensity but no increase of CET has been recognized.

Y(II) regenerated in all photobionts investigated during the dark relaxation phase following the light phase (Fig. 1). The highest Y(II) values have been detected by the photobiont of *U. antarctica* and the lowest by the photobiont of *B. frigida*. No significant differences considering Y(II) appeared between the photobiont of *F. bracteata* and *B. frigida* as well as *P. chlorophanum* (unpaired two-sided *t* test, $\alpha = 0.05$). By switching off the light, Y(NPQ) and Y(NO) immediately decreased in all photobionts studied and did not fully regenerate within the dark relaxation phase (Fig. 1). Y(I) increased in all investigated photobionts but in different extents (Fig. 2). The photobiont of the Swedish lichen *F. bracteata* showed a steep increase of Y(I) compared to the photobionts of the Antarctic lichens. A significant difference considering Y(I) has been recognized between the photobionts of *F. bracteata* and *P. chlorophanum* as well as of *U. antarctica* (unpaired two-sided *t* test, $\alpha = 0.05$). By turning off the light, all photobionts investigated displayed no values of Y(ND) but differences in Y(NA) among the photobionts could be observed. The values of Y(NA) were clearly higher in the photobionts of Antarctic lichens compared to the photobiont of the Swedish lichen *F. bracteata*. But no significant difference has been detected among the photobionts of the Antarctic lichens. Due to the dark relaxation phase the photobionts studied showed an increase of LET except the photobiont of *U. antarctica*.

Effect of desiccation

All isolated photobionts studied have been reactivated by water after 24 h (1d) and 168 h (7d) of desiccation. Clear differences in the reactivation kinetics have been recognized (Fig. 1 & 2). Considering the photobionts of the Antarctic lichens the effect of 1d desiccation can be described as a minor effect compared to the control while the photobiont of the Swedish lichen *F. bracteata* displayed a distinct effect already after 1d desiccation that appeared almost in the same range by 7d desiccation (Fig. 1 & 2).

After 7d of desiccation, all photobionts studied showed a significant decrease of Y(II) compared to the hydrated photobionts (control) during the dark acclimation phase. The genetically identical (clade S) photobionts of *P. chlorophanum* and *U. antarctica* exhibited a significant decrease of Y(II) compared to the 1d desiccation (unpaired two-sided *t* test, $\alpha = 0.05$) along the gradual light intensities from 40 – 342 µmol photons m^{-2} s^{-1}. Significant differences related to Y(II) could be detected in all photobionts studied after 7d of desiccation except of the photobiont of *B. frigida* in comparison to the control (Fig. 1). Only at the light intensity of 40 µmol photons m^{-2} s^{-1} the photobiont of *B. frigida* showed a significant difference of Y(II) correlating to the control (unpaired two-sided t test, $\alpha = 0.05$). Considering photosystem I the photobionts of the Antarctic lichens tested showed an increase of Y(I) after 1d of desiccation and a reduction after 7d of desiccation (Fig. 2). The Y(I) values measured after 7d of desiccation largely corresponded to those of the control conditions. Generally, no significant differences have been recognized (unpaired two-sided t test, $\alpha = 0.05$).

The photobiont of *F. bracteata* displayed a reduction of Y(I) after 1d of desiccation. An effect not changing after 7d of desiccation. A significant increase of Y(ND) has been recognized after 7d of desiccation in both the photobionts of *P.*

chlorophanum and *U. antarctica*. The Y(ND) of the photobiont of *F. bracteata* displayed clearly lower values after 1d as well as after 7d of desiccation related to the control (unpaired two-sided *t* test, $\alpha = 0.05$).

A clear increase of the CET of both the photobionts of *B. frigida* as well as of *F. bracteata* could be recognized after 1d desiccation but the photobiont of *B. frigida* displayed a minor increase in comparison with the high increase of the photobiont of *F. bracteata* followed by an additional minimal increase after 7d of desiccation. The highest increase of CET has been detected in the photobiont of *P. chlorophanum* after 7d of desiccation. Only after 7d of desiccation at the light intensity of 211 µmol photons m^{-2} s^{-1} the photobiont of *U. antarctica* showed a clear increase of the CET.

Considering the following dark relaxation phase, the photobionts of *P. chlorophanum* and *U. antarctica* showed a significant decrease in Y(II) after 1d of desiccation as well as after 7d desiccation (unpaired two-sided *t* test, $\alpha = 0.05$). Related to the Y(NPQ) a complete regeneration after desiccation has not been recognized in the photobionts studied. The Y(NO) differed significantly among all photobionts studied but was significantly higher of the photobiont of *P. chlorophanum* after 7d of desiccation (unpaired two-sided t test, $\alpha = 0.05$). Considering 1d as well as 7d desiccation a significant decrease in Y(I) has been recognized after 7d of desiccation by the photobionts of *P. chlorophanum* and *U. antarctica* while Y(NA) increased (unpaired two-sided t test, $\alpha = 0.05$). In the dark relaxation phase after 7d of desiccation the clade S photobionts of *P. chlorophanum* and *U. antarctica* exhibited a difference concerning the ratio of LET to CET. The photobiont of *P. chlorophanum* as well as the the photobiont of *F. bracteata* show a similar ratio of LET to CET while the photobiont of *U. antarctica* displayed an obvious increased LET after 7d of desiccation.

Discussion

Water availability is a key factor for metabolic activity of poikilohydric organisms as lichens. By metabolic activity the photobiont of the lichen symbiosis nourishes the mycobiont (Henssen & Jahns 1974). In this context adaptation mechanisms on the physiological level of the photobiont are of high importance for successful colonization of habitats characterized especially by environmental conditions. The photobionts studied clearly differ in their physiological reaction to light stress and prolonged desiccation. The strongest effect on desiccation considering the activity of both photosystems has been recognized in photobionts of the Antarctic lichens after 7d of desiccation. The photobiont of *F. bracteata* only showed a strong effect after 1d of desiccation.

Photobionts of Buellia frigida, Pleopsidium chlorophanum and Umbilicaria antarctica

As described above, oxidative stress in lichen photobionts can be triggered by desiccation and high radiation (Kranner et al. 2005, Konsugi et al. 2009), that could lead to formation of reactive oxygen species (ROS) (Fernández Marín et al. 2010) which in turn can influence the activity of enzymes (Kranner et al. 2008) related to the photosystems. The Antarctic photobionts studied showed a reduced PS II activity after 7d of desiccation. It indicates to insufficient D1 core proteins of the PS II reaction center, which may have been degraded by the desiccation process (Richter et al. 1990, McKersie and Lesheim 2013). This represents an increased risk to formation of ROS which can cause additional damage to the photosystems (Krause and Jahns 2004). Despite the reduced activity of PS II a constant response of the protective mechanisms (NPQ and NO) has been recognized in the photobiont of *B. frigida* by dissipation of light energy. Independent of the experimental treatment by desiccation and light no considerable changes of the PS I activity in donor as well as acceptor side limitation occurred. The activity of the PS I of the photobiont of *B. frigida* did not get

affected by desiccation after 1d as well as 7d of treatment, also recognized by Gasulla et al. 2009 for the algae *Trebouxia erici*. Huang et al. 2010 describe, that in the higher plant *Dalbergia odorifera* the PS I remained very stable whereas the PS II will be inhibited due to stress situation and a stable PS I complex favours a fast recovery of PS II activity. Based on these results the photobiont of *B. frigida* demonstrated the highest tolerance with regard to light stress and desiccation. Previous investigations on the physiological response of Antarctic lichen photobionts revealed that the photobiont of *B. frigida* showed a higher potential on desiccation resistance regarding the slower reduction in the quantum efficiency of PS II and a cold resistance potential concerning reduced quantum efficiency of PS II only after 48 h of freezing at -25 °C (Sadowsky and Ott 2012).

Although the photobionts of *P. chlorophanum* and *U. antarctica* are genetically identical (Helms et. al. 2001) associated with fungi of different genera as well as forming different phenotypes, they are characterized by different physiological response pattern. Already after 1d of desiccation of the photobiont of the maritime Antarctic lichen *U. antarctica* a considerable decrease of the regulated energy dissipation (Y(NPQ)) has been recognized although no effect in Y(II) appeared. Only the photobiont of *U. antarctica* showed higher Y(NPQ) values at different light intensities after 7d of desiccation while the Y(NPQ) values after 1d of desiccation were significantly lower. During the phase of gradual light intensity a strong increase in the non-regulated energy loss (Y(NO)) has been detected during the desiccation process. The results clearly demonstrate that the physiological resources for the regulation to protection against excess light energy are impaired. Additionally, the significant high values of Y(NO) indicate an increased sensitivity at high light intensities (Krause und Jahns 2004), that is expanded after 7d of desiccation. Considering the significant decrease in Y (I) no relevant changes in donor and acceptor side limitation of PS I have been recognized after 7d of desiccation. The functionality of the PS I was not affected by desiccation (Gasulla et al. 2009). The increased cyclic electron transport (CET) at higher light intensities (211 and 342 µmol photons $m^{-2} s^{-1}$) effected a clear reduced risk of photoinhibition after 7d of desiccation. In the linear electron transport (LET) electrons are transferred on $NADP^+$ used for the carbon cycle while the electrons of CET will get returned to the plastoquinone pool. In this cycle, H^+ ions are transported into the thylakoid lumen and only ATP is synthesized. Due to the increased CET, the electron flow can be ensured caused by stress situation as excess light. The increased ATP formation can be used for the synthesis of the D1 proteins and the repair of damaged PS II subunits (Allakhverdiev et al. 2005).

The photobiont of *P. chlorophanum* clearly differed to the photobiont of *U. antarctica* on the physiological level with respect to light and desiccation stress. By reduced PS II activity after reactivation of 7d of desiccation, the initial high values of Y(NO) decreased continuously with increasing light intensity, while the values of Y(NPQ) increased. The reduced Y(I) correlates with the decreased Y(II), i.e. the less electrons are mobilized by PS II, the less electrons are transported to the PS I, the less result in a limitation of PS I by the donor side that has been recognized in the photobiont of *U. antarctica*. Despite this reduction of Y(I), Y(ND) increased with increasing light intensity and down regulated Y(I), showing that the system is physiologically well-regulated because the excess energy has been derived (Schreiber and Klughammer 2008). The activity of PS I obviously is affected by desiccation in contrast to the results achieved by *Trebouxia erici* (Gasulla et al. 2009). These results are not congruent with the photobiont of *U. antarctica* as well as of *B. frigida*. The considerable increase of CET may support to resist the risk of photoinhibition, additionally.

Photobiont of Fulgensia bracteata

The photobiont of *Fulgensia bracteata* gets highly affected by desiccation compared to the photobionts of Antarctic lichens. After 1d of desiccation a strong effect on the activity of both photosystems gets obvious that does not change after 7d of desiccation. A strong decrease of the quantum yield of the Y(II) was detected indicating a reduced photosynthetic performance that might be an indication to insufficient D1 core proteins of the PS II reaction center, triggered by the desiccation process (Richter et al. 1990, McKersie and Lesheim 2013). The additionally reduced regulated energy dissipation of PS II (Y(NPQ)) has a diminished ability to regulate energy extinguishing of the incident radiation of the actinic light (Krause and Jahns 2004). The increased non-regulated energy dissipation of PS II (Y(NO)) exceeded Y(NPQ) indicating a higher photosensitivity similar to *U. antarctica*. This kind of similarity among photobionts from different clades of the genus *Trebouxia* has already been recognized and described by Sadowsky and Ott (2012).

The reduced ability to regulate energy dissipation of the photobiont of *F. bracteata* can result in an increased formation of reactive oxygen species (ROS), which can cause additional damage to the photosystems (Krause and Jahns 2004). Based on the desiccation process, a strong influence on the activity of the downstream PS I in the electron transport chain could be observed and a reduced limitation by the donor side of the PS I (Y(ND)) has been recognized. As described above for *U. antarctica*, the physiology of the photobiont of *F. bracteata* got well regulated because the excess energy has been derived (Schreiber and Klughammer 2008). A high and constant level on the acceptor side limitation of PS I (Y(NA)) in the photobiont of *F. bracteata* has been observed already at low light intensity. Y(NA) represents the over-reduction on the acceptor side of the PS I, which contributes to the photoinhibition of PS I (Huang et al. 2010). The high and constant level of Y(NA) suggest that obviously not enough PS I connected acceptors are present to cope with the delivered electrons (Pfündel et al. 2008). This increases the risk of damaging both the PS I as well as PS II, since the excess energy cannot be sufficiently dissipated in heat. The risk of photoinhibition of the PS I of the photobiont of *F. bracteata* might be minimized by the inhibition of the linear (LET) and the increased cyclic electronic transport (CET) as has been recognized in the experiments. To postulate, a damage of the PS II caused by desiccation could be avoided by an increase of CET at an initial state of desiccation of the photobiont of *F. bracteata*. Considering desiccation and light stress the physiology on PS II as well as PS I of the photobiont of *F. bracteata* from a moderate European habitat differ clearly to the photobionts of the lichens from Antarctic habitats.

Conclusion

The results of former studies (Sadowsky and Ott 2012, 2015) on the physiological potential of lichen photobionts from Antarctic habitats pointed to adaptations which substantially contribute to the strategy of stress tolerance and subsequently, to the colonization capacity of the lichen species. The results of the study presented are in accordance to the results of the former studies. The actual study focuses on the comparison of photobionts of lichen species colonizing harsh environmental conditions with a photobiont of a lichen species from a moderate habitat. All photobionts studied belong to the genus *Trebouxia*. The study emphasized on differences on the physiological level considering the potential of adaptation mechanisms with regard to stress conditions as desiccation and light depending on respective environmental conditions. The photobionts of the four lichen species investigated differed substantially on PS II and PS I as well as on the ratio of LET to CET. The PS I downstream in the electron transport chain provides the evidence of a reduced ability to separate the charge on PS II via an early occurring donor site limitation. The results demonstrate different responses to desiccation and light stress. The photobiont of *B. frigida* from a continental Antarctic habitat showed a considerable resistance to desiccation and light stress according to former initial results achieved on drought

and sub-zero temperature (Sadowsky and Ott 2012). Remarkably, the genetically identical photobionts of *P. chlorophanum* and the endemic *U. antarctica* (Helms et al. 2001) react different to the stress parameters applied and exhibited a lower potential stress resistance compared to the photobiont of *B. frigida.* Already after 1d of desiccation the photobiont of *U. antarctica* exhibited an impairment of PS II which results in a loss of protection mechanisms while an impairment of PS II of the photobiont of *P. chlorophanum* only occurs after 7d of desiccation. After 1d of desiccation of the photobiont of *F. bracteata* the activity of PS II strongly will be downregulated followed by a substantial decrease of NPQ. NO is increasing strikingly with the consequence of a very high photosensitivity. The photobiont of *F. bracteata* originating from a moderate habitat demonstrates obvious differences considering the stress tolerance to severe environmental conditions which indicates to distinct life strategies compared with the photobionts of Antarctic lichen species. With respect to desiccation and light stress the physiology on PS II as well as PS I of the photobiont of *F. bracteata* differ conspicuously to the photobionts of the lichens from Antarctic habitats. Although the photobionts investigated were cultivated under standardized conditions distinct responses to the applied stress parameters have been maintained which can be postulated to be genetically fixed. Although this study has been performed by isolated photobionts the results clearly indicate to the range of adaptation mechanisms on the level of physiology depending on environmental conditions at the habitat. The investigations on the physiology of the photobionts studied have been supplemented by research on their metabolome (Determeyer-Wiedmann, Mettler-Altmann, Sadowsky, Ott in prep.).

Abbreviations

ANOVA	analysis of variance
ATP	adenosintriphosphat
CET	cyclic electron transport
ETR	electron transport rate
Fv/Fm	maximum quantum yield of PS II
LET	linear electron transport
NPQ	non-photochemical quenching
PAM	pulse-amplitude modulation
PS I	photosystem I
PS II	photosystem II
ROS	reactive oxygen species
TOM	*Trebouxia* organic medium
Y(I)	photochemical quantum yield of PS I
Y(II)	effective quantum yield of PS II
Y(NA)	non-photochemical quantum yield of PS I - acceptor side limitation
Y(ND)	non-photochemical quantum yield of PS I - donor side limitation
Y(NO)	quantum yield of non-regulated energy dissipation in PS II
Y(NPQ)	quantum yield of regulated energy dissipation in PS II
1d	24 hours
7d	168 hours

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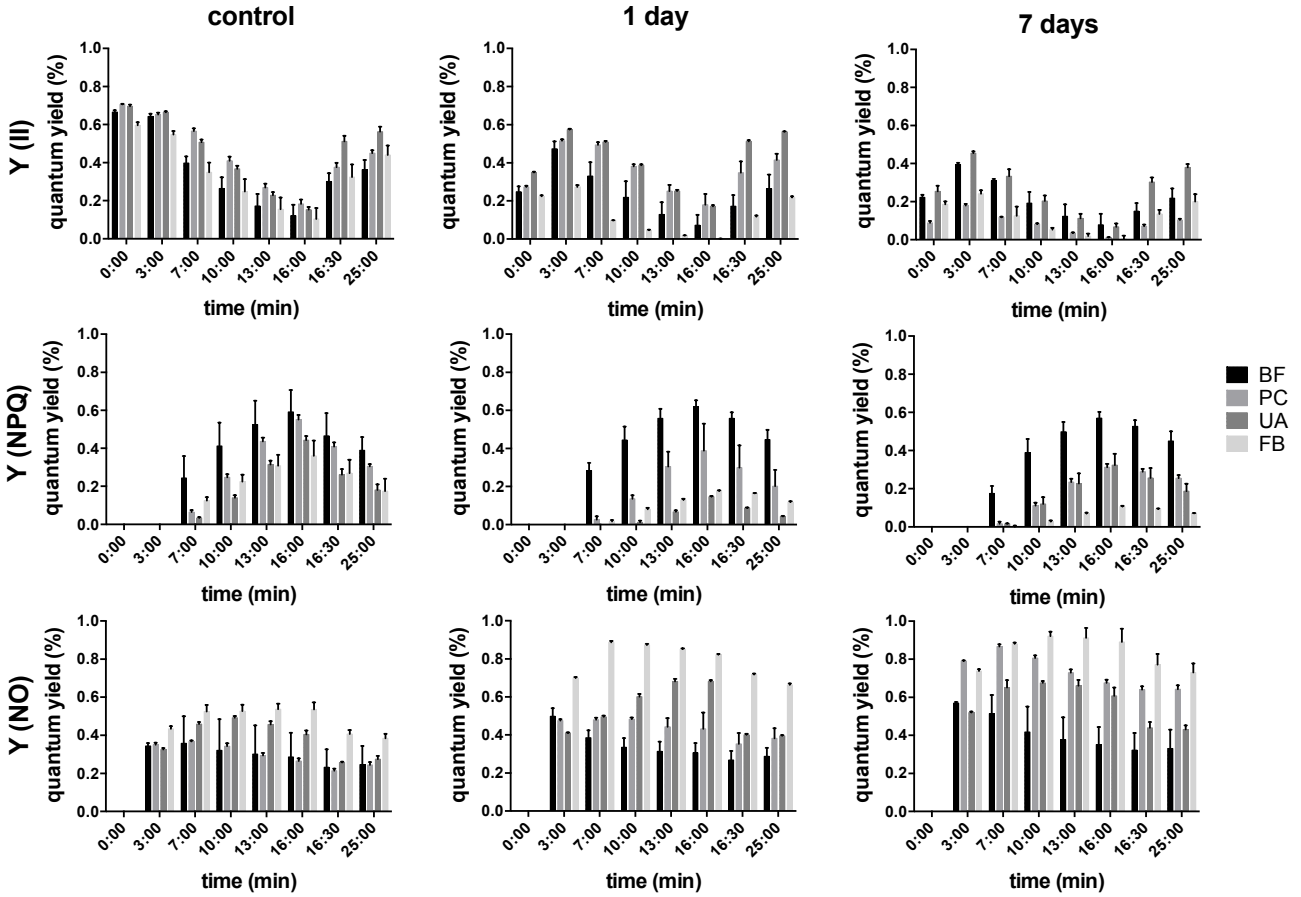
Tab. 1 Species of photobionts and origin *Clade*-classification according to Helms et al. 2001.

Mycobiont	**Photobiont**	**Origin**	**Reference**
Buellia frigida	*Trebouxia sp*., clade A identical to NCBI (AY667580.1)	Gondwana Station, North Victoria Land, continental Antarctica	Brandt 2011
Pleopsidium chlorophanum	*Trebouxia jamesii*, clade S	Gondwana Station, North Victoria Land, continental Antarctica	Brandt 2011
Umbilicaria antarctica	*Trebouxia jamesii*, clade S	Rothera Point, Adelaide Island, Antactic Peninsula	Romeike et al. 2002
Fulgensia bracteata	*Trebouxia sp*., clade I subgroup 1	Gotland Schweden	Schaper and Ott 2003



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591 **Fig. 1 Physiological activity of photosystem II of isolated photobionts** Values presented as mean and standard
592 deviation as well as specific time points to give an overview of effects of desiccation and increasing light intensity. 7 to
593 16 min display the illumination phase with increasing light intensity (40, 100, 211, 342 µmol photons m^{-2} s^{-1}).
594 Photobionts were examined by: BF *Trebouxia sp*., clade A of *Buellia frigida*; PC *Trebouxia jamesii*, clade S of
595 *Pleopsidium chlorophanum*; UA *Trebouxia jamesii*, clade S of *Umbilicaria antarctica*; FB *Trebouxia sp.*, clade I of
596 *Fulgensia bracteata*. To determine physiological activity of PS II, the quantum yield of PS II (Y(II)) and its quantum
597 yield of regulated energy dissipation in PS II (Y(NPQ)) as well as of non-regulated dissipation in PS II (Y(NO)) were
598 measured.

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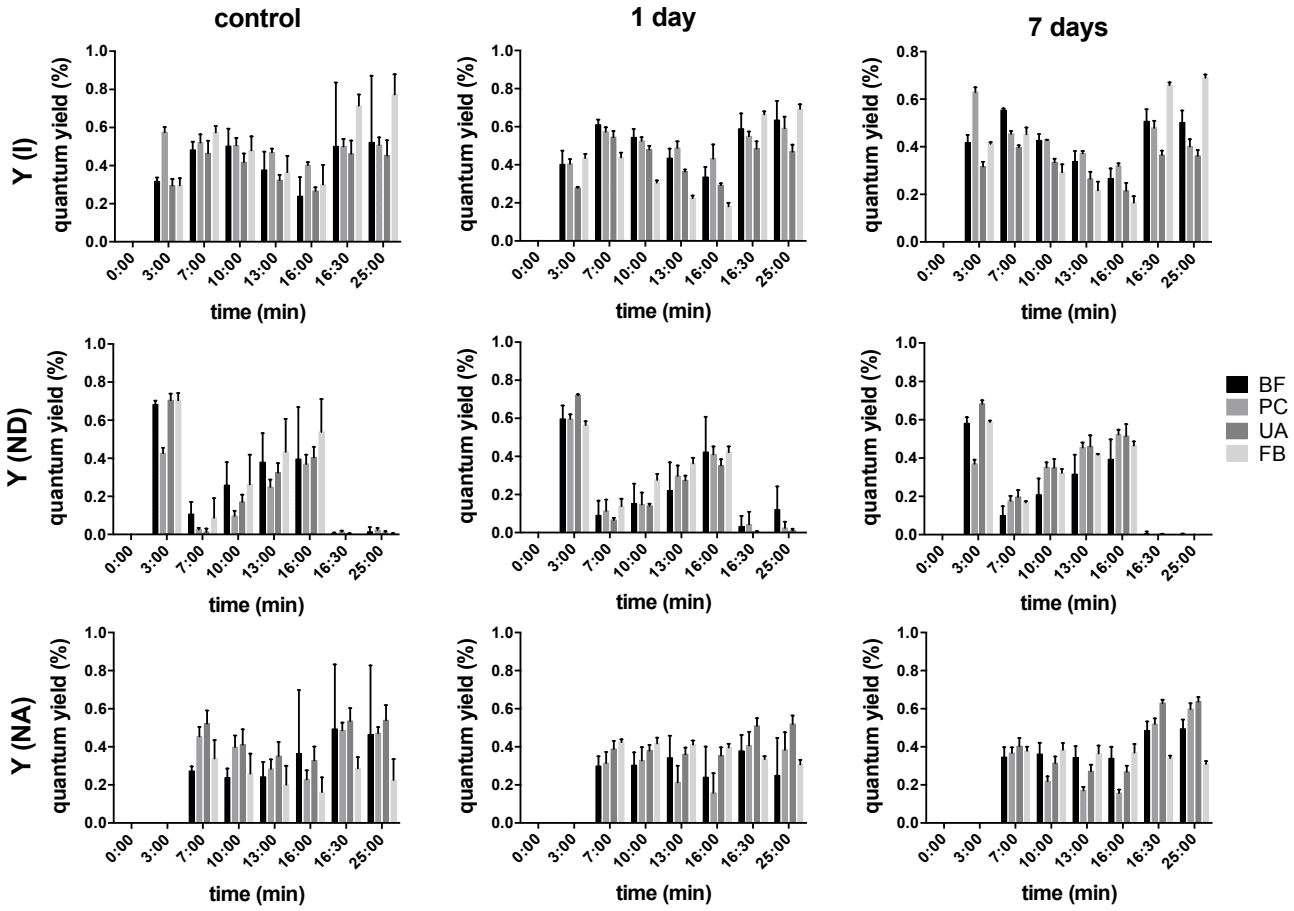
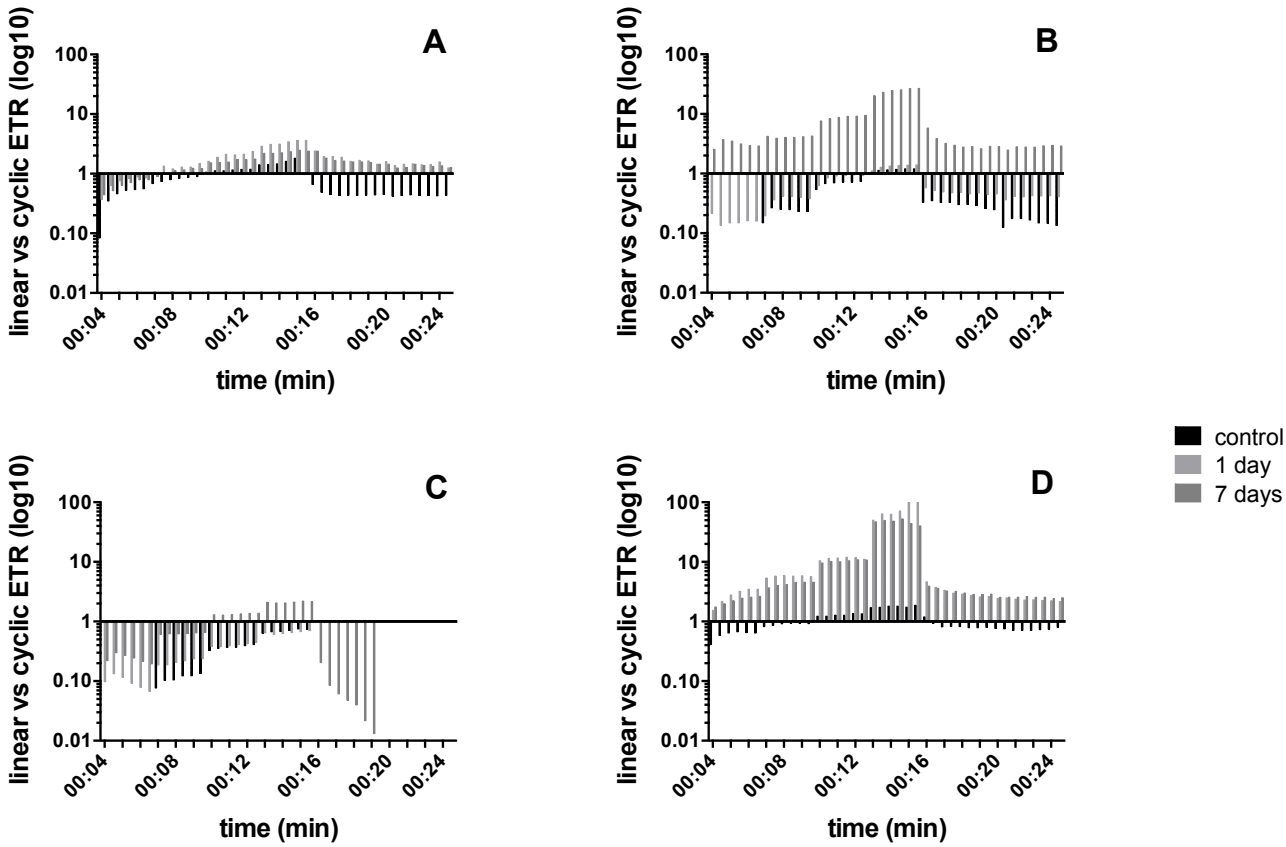


Fig. 2 Physiological activity of photosystem I of isolated photobionts Values presented as mean and standard deviation as well as represent specific time points to give an overview of effects of desiccation and increasing light intensity. 7 to 16 min display the illumination phase with increasing light intensity (40, 100, 211, 342 µmol photons m^{-2} s^{-1}). Photobionts were examined from: BF *Trebouxia sp*., clade A of *Buellia frigida*; PC *Trebouxia jamesii*, clade S of *Pleopsidium chlorophanum*; UA *Trebouxia jamesii*, clade S of *Umbilicaria antarctica*; FB *Trebouxia sp.*, clade I of *Fulgensia bracteata*. To determine physiological activity of PS I, the quantum yield of PS I (Y(I)) and its donor side limitation (Y(ND)) as well as acceptor side limitation (Y(NA)) were measured.



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610 Fig. 3 Linear versus cyclic electron transport rate of isolated photobionts after reactivation of different dehydration
611 treatments and upgraded light intensity: Bars represent the ratio of linear and cyclic electron transport rates calculated
612 by values obtained from the measurements of PS II of the isolated photobionts: (A) *Trebouxia* sp., clade A of *Buellia*
613 *frigida*; (B) *Trebouxia jamesii*, clade S of *Pleopsidium chlorophanum*; (C) *Trebouxia jamesii*, clade S of *Umbilicaria*
614 *antarctica*; (D) *Trebouxia* sp., clade I of *Fulgensia bracteata*. Recording starts at 4 min due to irradiation of the lowest
615 light intensity. The illumination phase starts at 4 min with 40 µmol photons m^{-2} s^{-1} and ends by 16 min with 342 µmol
616 photons m^{-2} s^{-1}. Values below 1 at the y-axis represent linear electron transport and over 1 represent cyclic electron
617 transport.