Does *Parmelina tiliacea* lichen survive at low temperature of liquid nitrogen?

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Summary

Dry and wet states of the lichen *Parmelina tiliacea* were carried out to investigate recovery of the photosynthetic apparatus to liquid nitrogen storage for 1 week. In the present study, the chlorophyll *a* fluorescence rise and the maximum quantum yield of primary photochemistry ϕ_{Po} (F_V/F_M) were analysed. When wet thalli were stored for 1 week in to liquid nitrogen; the photosystem II reaction centers were inactivated. However, recovery of dry state thalli differed remarkably, suggesting that photosystem II reaction centers had remained intact. These results indicate that the state of the thallus in is the factor determining the recovery and survive of lichen following storage in liquid nitrogen.

Key words *Parmelina tiliacea*; liquid nitrogen; dry and wet state; chlorophyll *a* fluorescence rise

Lichens are a symbiotic association between a heterotrophic mycobiont (fungus) and an autotrophic photobiont (photosynthetic partner; an eukaryotic green alga or a cyanobacterium). Lichens in their dehydrated state (dry state) are characterised by a total inactivation of photosynthetic activity and gas exchange (Kappen and Breuer 1991; Oukarroum et al. 2012). Like other poikilohydric organisms, down-regulation of their metabolism at dehydrated state permits lichens to maintain the functional integrity of their membranes by immobilisation of the cytoplasm in a stable multicomponent glassy matrix (Wolfe et al. 1999). However, addition of water to dehydrated lichens can restore their photosynthetic activity within a few minutes (Coxson 1988; Soni and Strasser 2008; Oukarroum et al. 2012, Sánchez et al. 2014). Many studies showed that lichen species are remarkably resistant to extreme environment conditions by developing adaptation strategies to cope with harsh ecological conditions (Sánchez et al. 2014 and see references therein).

Parmelina tiliacea (Hoffm.) Ach. (*P. Tiliacea*) is a symbiotic association between *Parmelina* (a genus of lichenised fungi) and *Trebouxia jamesii* algae a photosynthetic partner. Hydrated *P. tiliacea* thalli present functional photosynthetic PSII units and exhibit a polyphasic OJIP Chlorophyll *a* fluorescence rise (ChlF) during the first second of illumination following a dark-to-light transition (Oukarroum et al. 2012). Indeed, ChlF is widely used to assist the viability of lichen for example after simulated space exposure (Backhaus et al. 2014). The analysis of changes in OJIP transients provides detailed

information of the photosynthetic performance mainly structure and function of the photosystem II (Strasser et al. 2004). In this brief communication, thalli of *P. tiliacea* were stored for 1 week in liquid nitrogen (-196°C) and our objective is to investigate their recovery.

Thalli of *P. tiliacea* were collected from the south sides of oak trunks, around the laboratory in Jussy, Geneva (Switzerland). All lichens were brought to the laboratory the same day and air-dried for 48 h at room temperature under low light conditions ($\sim 12 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$). For this experiment, fragments of a single thallus were used. To assess the tolerance of dehydrated (dry state) and hydrated *P. tiliacea* thalli (wet state) to liquid nitrogen storage, they were stored in liquid nitrogen for 1 week. Here, the hydrated state was initiated by adding a drop of water on dry thalli (Oukarroum et al. 2012). All ChlF measurements were conducted on thalli of *P. tiliacea* kept in dark with a fluorometer (Plant Efficiency Analyser, Hansatech Instruments Ltd, King's Lynn, UK) with high time resolution (10 μs) and with a maximum pulse intensity of $\sim 3000 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$. The measurement consisted of a single strong 1 s light pulse (600 W m^{-2} , an excitation intensity) provided by an array of six light-emitting diodes (peak 650 nm) in the PEA instrument. We noted here that ChlF was first measured in the two states of *P. Tiliacea* before the samples were stored in liquid nitrogen, this to verify the state of their photosynthetic activity. After 1 week storage, the thalli were transferred to room temperature conditions and ChlF measurements were made immediately, after 5 min, 1 week and two months. The maximum quantum yield of primary photochemistry ϕ_{Po} (= 1-

$(F_0/F_M) = F_V/F_M$) was also calculated, it is one of the ChlF parameters that give useful information about the state of plants and their vitality. ϕ_{P_0} corresponds to the efficiency by which an absorbed photon will be trapped by PSII reaction centers (Strasser et al. 2004).

Before liquid nitrogen storage, we observed that dehydrated *P. Tiliacea* thalli did not show an increase in their fluorescence intensity (absence of variable ChlF) on a dark-to-light transition (Fig. 1A). However, in hydrated lichens (following addition of a drop of water) the typical OJIP transient was recorded indicating that their photosynthetic activity was restored after dry state (Fig. 1A, see also Oukarroum et al. 2012 for more details). The fluorescence intensity increases quickly from a minimum fluorescence intensity (O) to a maximum fluorescence intensity (P) and with two intermediate steps labelled as J and I. This OJIP transient was divided into three phases (O–J, J–I and I–P) and these phases reflect three different reduction processes of the electron transport chain (Strasser et al. 2004). Subsequently to liquid nitrogen storage (after 5 min), ChlF measurements of dehydrated state of *P. tiliacea* thalli did not showed any variable on OJIP transient as well as observed before exposure to liquid nitrogen, however, the hydrated state presents an increase in ChlF intensity (Fig. 1B). Rehydration of these thalli (and kept in dark for 5min) indicated that the dry state (stored in this state) showed the typical OJIP transient, on the other hand, the hydrated state showed also an OJIP transient but with high fluorescence intensity at J step (Fig. 1C). When these lichen were kept at room condition for 1 week and after following addition of water we observed a drastic decrease of ChlF

intensity in hydrated state, contrary in dry state when all OJIP steps were maintained (fig. 1D). Interestingly, after two months of recovery, the *P. tiliacea* thalli stored in their hydrated state did not show any variable ChlF compared to dry state (Fig. 1E). In addition, we observed that liquid nitrogen storage had a significant effect on ϕ_{Po} for both states (Fig. 2). While this parameter decreased in hydrated state compared to value calculated before treatment, it increased in dehydrated state.

Here, we found that PSII in hydrated state of *P. Tiliace* is sensitive to liquid nitrogen storage. This sensitivity can be described by the hazardous effect of liquid nitrogen storage on cell physiology, especially in this study on photosynthesis. Possible interpretation of this decrease of photosynthetic activity in hydrated state can be interpreted by impaired PS II photochemical efficiency. We supposed that when hydrated thalli (wet state) were stored, the formed water crystals would destroy all membranes and then defect all physiological reactions mainly photosynthesis activity. According to the findings of the present work, we assume that *P. Tiliace* tolerance is influenced first by the physiological state of the *P. Tiliace* thalus. In other words, the dry state of lichens is responsible for extreme tolerance of thalli towards low temperature. The results of this current study are in accordance with previous results which demonstrate that the dry state can resist to harsh environmental condition such as high temperature (Oukarroum et al. 2012) or UV (Meeßen et al. 2014; Nybakken et al. 2004). However, further investigations on physiological mechanism should be done in order to understand this tolerance and survivability to low temperature in dry state. Among this highly resistance, lichen

represent the best candidate for astrobiological research, this idea is also supported by Sancho et al. (2008).

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Figures captions

Figure 1: Chl *a* fluorescence OJIP rise in *Parmelina tiliacea* lichen stored 1 week in liquid nitrogen in hydrated state (wet state) and dehydrated state (dry state). O, J, I, and P indicate PSII rapid fluorescence transients. Chl *a* fluorescence rise was measured before liquid nitrogen treatment (A), immediately after liquid nitrogen treatment (B), after rehydration (C), after 1 week (D) and after two months (E).

Figure 2: Change of maximum quantum yield of primary photochemistry ($\phi_{P0} = F_V/F_M$), in *Parmelina tiliacea* lichen stored 1 week in liquid nitrogen in hydrated state (wet state) and dehydrated state (dry state).

Figure 1

Figure 2