Sensitivity of Antarctic freshwater algae to salt stress assessed by fast chlorophyll fluorescence transient

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Abstract
In this study, we investigated the effects of salt stress (2 mM NaCl) on excitation energy transfer from light harvesting complexes to photosystem II (PS II) in two Antarctic algal species: *Klebsormidium* sp. and *Zygnema* sp. Short-term salt stress led to a significant changes in the shape of chlorophyll fluorescence transient (OJIP). Analyses of the polyphasic fluorescence transients (OJIP) showed that the fluorescence yield at the phases J, I and P declined considerably with the time of exposition to salt stress. In both experimental species, OJIP transients reached lowest values of chlorophyll fluorescence signal after 30/60 min. of NaCl exposition. Then, OJIP shape and chlorophyll fluorescence showed species-specific recovery and rised towards original values (about 2/3 of untreated control). Analyses of chlorophyll fluorescence parameters derived from OJIPs showed that salt stress led to a decrease in the maximal efficiency of PS II photo-chemistry ($F_{V}/F_{M}$) in *Zygnema* sp. but not *Klebsormidium* sp. The results indicated that the probability of excitation energy transfer before and beyond $Q_{A}$, and the yield of electron transport beyond $Q_{A}$ is limited by salt-induced stress in *Zygnema* sp. In addition, salt stress resulted in a decrease in the photosynthetic electron transport per PS II reaction center, but both increase and decrease in the trapping per PS II reaction center was found. Performace index (PI$_{abs}$) was affected negatively in *Zygnema* sp. but possitively *Klebsormidium* sp. indicating that the latter species was more resistant to salt stress than *Zygnema* sp.

Key words: *Klebsormidium* sp., *Zygnema* sp., OJIP, photosynthesis, James Ross Island, Monolith Lake

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Introduction
Deglaciated northern part of the James Ross Island, Antarctica is rich in terrestrial lakes, streams and seepages (Nedbalová et al. 2013). Since 2004, freshwater auto-trophs have been studied in these ecosystems in order to evaluate their biodiversity and physiological processes. Komárek et al. (2008) and Komárek et Elster (2008)
reported several cyanobacterial species for the James Ross Island. Recently, a great number of studies has focused diatoms appearing mainly in seepages (e.g. Kopalová et al. 2009, Kopalová et al. 2012). Green freshwater algae of the James Ross Island are studied less frequently, however, their appearance in various lakes (Vácz et al. 2011, Skácelová et al. 2013) is reported. Physiological processes, photosynthesis in particular, of freshwater algae are studied mainly in laboratory experiments under controlled conditions. Kaplan et al. (2013) evaluated the effects of osmotic stress on photosynthesis and osmotically-induced changes in cell ultrastructure in Zygnema sp. from the James Ross Island. They reported a decrease in photosynthetic oxygen evolution rate as well as effective quantum yield measured by chlorophyll fluorescence technique. Salt stress and its effect on photosynthesis of Antarctic freshwater algae have been studied only recently. In these studies, both salt stress resistance (Kan et al. 2012) and sensitivity (Kaplan et al. 2013) are reported. To support the idea of dynamic character of Antarctic freshwater algae responses to salt stress, we designed an experiment using a short-term NaCl addition to cultivation medium and measurements of chlorophyll fluorescence parameters as markers of limitation of photosynthetic processes in photosystem II in Antarctic algae.

Recently, several chlorophyll fluorescence methods evaluating negative effects of stress to photosystem II exist. Among them, fast transient of chlorophyll fluorescence (OJIP) represents useful method because of easy use both in the field and laboratory conditions. Main advantage is a short time required for a single measurement. Thus, studies exploiting repetitive measurements taken in short intervals use this method. In principle, OJIP represents a polyphasic rise of chlorophyll fluorescence induced by high light intensity in dark-adapted plant sample. Chlorophyll fluorescence rises from minimal (F₀) to maximal (Fₘ or Fₚ). Within the transient, four important levels of chlorophyll fluorescence – O, J, I, P, can be distinguished (Strasser et al. 1995). It is well established that each level denotes particular phase of energy transport within and beyond PS II. The O, J, I, P levels are indirectly linked to the redox state of all the electron carriers of the photosynthetic electron transport chain from the water splitting complex side to photosystem I. In this method, mainly redox state of the primary quinone acceptor (QA) affects the absolute value of the chlorophyll fluorescence levels and the shape of OJIPs. Most recently, mathematical modeling is used to attribute chlorophyll fluorescence rise to individual processes of energy flow through PS II and electron transport chain in thylakoid membrane of a chloroplast (e.g. Lazár 2006, 2009).

OJIPs have been used for evaluation of stress effects in photosynthetic apparatus of higher plants (for review see Roháček et al. 2008). The shape of OJIPs and the parameters derived from them sensitively reflect negative changes caused in photosystem II and components of linear photosynthetic electron transport. Within last decade, OJIPs has been routinely used to evaluate salt stress mainly in crops (e.g. Mehta et al. 2010, Kalaji et al. 2011, Jafarinia et Shariati 2012). OJIPs are also frequently used for monitoring of water stress (Živčák et al. 2008) and negative effects heavy metals to photosynthetic apparatus (e.g. Haldimann et Tsimilli-Michael 2002). In aquatic higher plant Wolffia arrhiza (Wang et al. 2011), salinity stress was evaluated by OJIPs. Salt stress and leaf age effects on OJIPs were assessed by Touchette et al. (2012) in a marsh Juncus roemmerianus.

In unicellular green freshwater algae, OJIPs has been used to evaluate negative effects to photosystem II of heavy metals (Andosch et al. 2012, Zemri et al. 2012, Perreault et al. 2011), silver nanoparticles
(Okarroum et al. 2012), performance index $P_{\text{abs}}$ during cultivation in culture ($Dunaliella primalecta$, Kruskopf et Flynn 2006), dose-dependent inhibitory pattern caused by dried powdered macroalga Gracilaria temaneiformis on salt-resistant $Dunaliella salina$ (Ye et al. 2012). For Antarctic algal species, only limited number of photosynthetic studies exploiting OJIP approach has yet been done. Petrou et al. (2011) investigated sea-ice microalgal communities dominated by diatoms, their sensitivity to short-term photoinhibition in particular. The authors found dose-dependent decrease in variable chlorophyll fluorescence forming OJIP.

Within last decade, OJIP has been used for evaluation of salt stress induced negative changes in photosynthetic apparatus mainly in cyanobacteria (e.g. Lu et Von-Shak 2002). In our study, we focused on algae from Antarctica, because algal species from polar regions are considered highly tolerant to salt stress (Hoham et Duval 2001). Some strains of Klebsormidium sp. have shown aso broad tolerance to freezing and desiccation (Elster et al. 2008). However, there is only very limited information on salt tolerance of Klebsormidium sp. (Central European strain) - Karsten et Ridi (2010). Therefore, we focused on short-term salt stress in photosynthetic apparatus of Klebsormidium sp. and Zygnema sp. from Antarctica using the approach of NaCl addition into cell culture. We hypothesized that, in spite of the fact that both species occupy similar ecotopes at the James Ross Island, there are interspecific differences in sensitivity to salt stress. Moreover, we expected that, after initial salt-induced inhibition of photosynthesis, partial recovery towards pre-experimental values of photosynthetic parameters exist after hundreds of minutes of salt stress.

Material and Methods

Experimental species

For experiments, two Antarctic freshwater algae were used: Klebsormidium sp. and Zygnema sp. (see Fig. 1). Zygnema sp. and Klebsormidium sp. are quite abundant at the James Ross Island. They occur in shallow lakes such as Big Lachman Lake, Phormidium Lake and many other lakes and ponds (Komárek et al. 2008). Experimental strains were obtained from Culture Collection of Autotrophic Organisms (CCALA), Institute of Botany, Academy of Sciences of the Czech Republic, Třeboň. Individual strains used in our experiments were Klebsormidium sp. (CCALA, strain 859, Šnokhausová et Elster 2008/8) collected on seepages reaching Monolith Lake and Zygnema sp. (strain 880, Šnokhausová et Elster 2009/8).

Cultivation and handling of algae

Before experiments, cultivation was carried out in a liquid 3N BBM solution under constant temperature of 10°C and irradiance of 40 µmol m⁻² s⁻¹ of photosynthetically active radiation (PAR) with periodic dark phase (16/8 hours) in a Fytoscope FS130 (Photon Systems Instruments, Czech Republic) cultivator.

Salt stress

Stock solution of salt was prepared from 238.52 g NaCl per one liter destilled water. Osmotic potential (π) of the solution was 10 MPa. Experiment was performed using the following dilution procedure: 4 ml 3N BBM with 100 µl of a stock solution NaCl and 4 ml 3N BBM with 200 µl of stock solution NaCl. The
above-specified volume of sample of *Klebsormidium* sp. was pipetted into cultivation plate holes (Tissue culture test plates 12, TPP Switzerland) and exposed to salt stress. During exposition, chlorophyll fluorescence was measured on dark-adapted samples (10 min.) - see Chlorophyll fluorescence measurement. *Zygnema* sp. samples were exposed to salt stress and measured in a smaller sample volume (100 ml plastic caps fitting to predarkening clips) in order to optimize culture density and chlorophyll fluorescence signal.

**Chlorophyll fluorescence measurements**

Chlorophyll fluorescence transients (OJIPs) were measured by a FL-100 FluorPen (Photon Systems Instruments, Czech Republic) fluorometer. On samples, OJIPs measurements were repeated at time 0 (control) and then after 5, 30, 60, 120, 180, 240 and 300 min. of NaCl treatment. Recorded data were transferred to a PC and then analyzed using a FluorPen 1.0.4.1 software (Photon Systems Instruments, Czech Republic). OJIP-derived photosynthetic parameters such as $F_{V}/F_{M}$, $F_{M}/F_{0}$, and performance index ($Pl_{abs}$) were evaluated for both experimental species as exposed to salt stress (see Eqn.1). $Pl_{abs}$ is considered general indicator of vitality. It covers estimation of the amount of photosynthetic reaction centers (RC/ABS), the maximal energy flux which reaches the PS II reaction centers and the electron transport at a certain illumination (for details see Strasser et al. 2000). Additionally, maximum trapping rate per reaction centre (TRo/RC) was evaluated.

$$\text{TRo} / \text{RC} = \frac{M_{0}}{V_{J}} \times (1 / V_{J})$$

where $F_{0}$ means fluorescence intensity at 50 μs, $F_{J}$ is fluorescence intensity at the J step (at 2 ms), $F_{M}$ represents maximal fluorescence intensity, $V_{J}$ is relative variable fluorescence at 2 ms calculated as $V_{J} = (F_{J} - F_{0})/(F_{M} - F_{0})$, $M_{0}$ represents initial slope of fluorescence kinetics, which can be derived from the equation: $M_{0} = 4 \times (F_{300} \mu s - F_{0})/(F_{M} - F_{0})$. 

**Fig. 1.** Photo of cultivated strains of *Klebsormidium* sp., CCALA, strain 859 Šnokhausová et Elster 2008/8 (left) and *Zygnema* sp., strain 880, Šnokhausová et Elster 2009/8 (right). Photo by K. Skácelová.

$Pl_{abs}$ = \frac{1 - (F_{0}/F_{M})}{M_{0}/V_{J}} \times \frac{F_{M} - F_{0}}{F_{0}} \times \frac{1 - V_{J}}{V_{J}}$

Eqn. 1
Results and Discussion

Shape of OJIPs was affected by salt stress (see Fig. 2). Generally, chlorophyll fluorescence signal decreased with duration of salt stress showing maximum decrease after 30 min. of exposition to NaCl. Such response was found for *Klebsormidium* sp., however in *Zygnema* sp. a minimum chlorophyll fluorescence signal was found later, after 60 min., then followed by a slight rise. This indicated dynamic character of PS II response to NaCl-induced stress during the first few hours of exposition. However, dose-related response, *i.e.* the higher NaCl concentration, the more pronounced decrease in chlorophyll fluorescence signal and more flattened OJIP shape might be expected. This response could be supported by data by Zhang et al. (2010) who reported that NaCl exposition led to gradually decreased chlorophyll fluorescence signal forming an OJIP in *Spirulina platensis* exposed to different NaCl (0, 0.2, 0.4, 0.6 and 0.8 M NaCl) concentrations for 12 h. An apparent interspecific difference was found in OJIP shape. While in *Klebsormidium* sp., the ratio of chlorophyll fluorescence at level I to level P reached values about 0.7, it was found much higher in *Zygnema* sp. (above 0.9). Moreover, the ratio increased with the duration of salt stress indicating strong limitation of reoxidation rate of quinones and thus decreased ability of PS II to transfer absorbed light energy to photosynthetic linear electron transport chain. This might be interpreted as less effective energy transport through PS II in *Zygnema* sp. than in *Klebsormidium* sp. both in untreated control and salt-stressed samples.

![Fig. 2. OJIPs recorded during salt treatment (2mM NaCl for 300 min.) in Klebsormidium sp. (left) and Zygnema sp. (right).](image)

Also chlorophyll fluorescence parameters derived from OJIPs supported the idea of more pronounced negative effect of salt stress on PS II functioning in *Zygnema* sp. than *Klebsormidium* sp. While \((F_P-F_0)/F_P\) parameter (here it is an equivalent for potential quantum yield of photochemical processes in PS II \(- F_V/F_M\)) showed a marked decrease of about 46% during initial period of salt treatment (120 min.) in *Zygnema* sp., no response was found for the same parameter in *Klebsormidium* sp. (see Fig. 3). It is well established for microalgae that a slight decrease in \(F_V/F_M\) values during salt and osmotic stress does not bring substantial limitation to photosynthetic processes within the first few hours of salinity stress (Affenzeller et al. 2009). Prolonged salt stress, however, may decrease photosynthetic efficiency as shown...
for salt-stressed green alga *Scenedesmus* (Demetriou et al. 2007).

Similarly to $F_{V}/F_{M}$, $F_{M}/F_{0}$ ratio showed a decline within the first 120 min. of salt treatment in *Zygnema* sp., contrastingly to *Klebsormidium* sp. that exhibited no change. Trapping rate per reaction centre (TRo/RC) that is considered a specific flux of trapped energy leading to reduction of $Q_{A}$ per RC showed different responses in the two experimental species. It showed an increase (*Zygnema* sp.) and decrease (*Klebsormidium* sp.) after 30 min. exposition followed by a gradual return to pre-experimental values. This indicated that salt stress affected energy transformation before $Q_{A}$ to only limited extent while photosynthetic electron transport beyond $Q_{A}$ was strongly suppressed at least in *Zygnema* sp. - see low PI_{abs} in Fig. 4. This can be supported also by generally low values of ETo/RC (not shown here). This indicates that in spite of the fact that a shape of OJIPs shows a sort of recovery after 5 h of salt treatment, PS II of *Zygnema* sp. is able to transfer energy to photosynthetic linear electron transport chain to only very limited extend. It can be interpreted as a strong inhibition of primary photosynthetic processes in salt-treated *Zygnema* sp. Decline in functioning of PS II in *Zygnema* sp. during first hours of exposition to salt stress may be attributed to direct osmotic effect on PS II and electron carries which is typical for a rapid phase of salt stress-induced PS II inhibition (Al-lakhverdiev et al. 2000).

**Fig. 3.** Timecourses of $F_{M}/F_{0}$ (left) and $F_{V}/F_{M}$ (right) in *Klebsormidium* sp. (□) and *Zygnema* sp. (●) exposed to salt stress.

**Fig. 4.** Timecourses of Performance index (PI_{abs} - left) and trapping rate per reaction centre (TRo/RC - right) in *Klebsormidium* sp. (□) and *Zygnema* sp. (●) exposed to salt stress.
Performance index ($\text{PI}_{\text{abs}}$) is a chlorophyll fluorescence parameter derived from OJIPs that is accepted as overall indicator of vitality and proper functioning of PS II. It has been used in many studies of salt stress effects in higher plants (e.g. Kalaji et al. 2011, Bacarin et al. 2011), however only limited number of studies used $\text{PI}_{\text{abs}}$ as indicator in stress physiology of algae (e.g. Einali et Shariati 2012, Zhou et al. 2013) and cyanobacteria (e.g. Bueno et al. 2004, Zhang et al. 2010). In our study, *Klebsormidium* sp. showed higher $\text{PI}_{\text{abs}}$ values in untreated control than *Zygnema* sp. When exposed to salt stress, $\text{PI}_{\text{abs}}$ value decreased slightly within the first 30 min of exposition in *Klebsormidium* sp. However, $\text{PI}_{\text{abs}}$ recovered to pre-exposition values after 120 min. of exposition and even increased with duration of salt stress. In *Zygnema* sp., salt stress-induced decrease in $\text{PI}_{\text{abs}}$ reached 0 after 120 min. and did not recover (see Fig. 4). It might be interpreted as either destruction or functional inability of reaction centres caused by salt stress.

Interspecific differences in OJIPs and several chlorophyll fluorescence parameters might be interpreted that *Klebsormidium* sp. exhibited more pronounced resistance to salt stress than *Zygnema* sp. There are many mechanisms that may cause such differences, some of them might be related to cultivation conditions that were probably more favourable for *Klebsormidium* sp. than *Zygnema* sp. Involvement of physiological mechanisms that improve salt resistance of algae, such as e.g. ABA content (Yoshida et al. 2004), sugar degradation and glycerol production (Miyasaka et Ikeda 1997), regulation of lipid biosynthesis (Lu et al. 2012, Meijer et al. 2001), and specific proteins expression (Yokthongwattana et al. 2012, Mastrobouni et al. 2012) might be also considered.

### Concluding remarks

Analyses of the OJIPs showed that salt stress led to a decrease in the maximal efficiency PS II photochemistry ($F_V/F_M$), the probability of electron transfer beyond $Q_A$, and the yield of electron transport beyond $Q_A$. In addition, salt stress resulted in a decrease in the electron transport (not shown here) per PS II reaction center, but either an increase or no change in the trapping rate per PS II reaction center. However, almost no change in ($F_V/F_M$) in *Klebsormidium* sp. and substantial decrease of this parameter in *Zygnema* sp. indicated a high tolerance of PS II to salt stress only in *Klebsormidium* sp. Our finding that *Klebsormidium* sp. exhibits a high degree of tolerance to salt stress may be supported by the theory of cross adaptation to cold and drought in green algae from polar regions Li et Li (2011). These authors reported that cold or drought tolerant plants may also show salinity tolerance. The above authors showed that a *Chlorella* strain NJ-18 from the Antarctica, exhibited increased salinity tolerance relative to a temperate strain *C. vulgaris* UTEX 259 of the same genus. In terms of photosynthetic activity, the Antarctic strain was more tolerant to salinity stress than the temperate one. Therefore, our results suggest that salinity tolerance found for *Klebsormidium* sp. and to a certain extent in *Zygnema* sp. may have been developed in parallel to cold tolerance typical for algal species from Antarctica. Such adaptive mechanism may provide an advantage for survival and physiological performance of the two studied species at harsh Antarctic environments.
References


SALT STRESS IN ALGAE


