Temperature optima for growth and photosynthetic processes in *Trebouxia erici* isolated from an Antarctic lichen and cultivated in a temperature gradient

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Abstract
The temperature optimum for photosynthesis and growth of natural populations of *Trebouxia erici* isolated from an Antarctic lichen (*Usnea antarctica*) was determined using a long-term cultivation (26 days) at different temperatures. Several chlorophyll fluorescence parameters were used in *T. erici* cultivated in a liquid medium to assess the effect of cultivation temperature (0, 10, 20 and 30°C). Analysis of time courses of the capacity of photosynthetic processes in PS II (F_V/F_M), effective quantum yield of photosystem II (Φ_{PSII}), relative fluorescence decline ratio (RFd), and quenching of background chlorophyll fluorescence (qF_0) revealed that optimum temperature is between 10 to 20°C. Biomass production evaluated as a total chlorophyll production after 26 days of cultivation was maximal at 20°C. The results are discussed in relation to the data reported by other literature sources for *Trebouxia* sp. and other algae isolated from chlorolichens

Key words: alga, Antarctica, chlorolichens, chlorophyll fluorescence, stress

DOI: 10.5817/CPR2017-1-4

Introduction

Temperature optima for photosynthesis and growth of Antarctic lichens, their algal symbionts in particular, have been in the centre of interest of ecophysiologist since the majority of Antarctic lichens have a net photosynthetic temperature optimum below the temperature optima of their constituent algae and fungi (Friedman et Sun 2005). Similarly, lichen thalli sensitivity to dehydration stress differ from that of their photosynthesizing photobionts (Kosugi et al. 2009). Although the stress physiology of Antarctic lichen photosynthesis and related physiological processes have been studied extensively within the last few decades both in the field (*e.g.* Cao et al. 2015) and the laboratory, only fragmentary knowledge exists about the photobionts' physiological

Received May, 28th 2017, *accepted* July, 4th 2017.
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Acknowledgements: The authors would like to express their thanks to the laboratory infrastructure provided by the CzechPolar2 project (No. LM2015078) and the EEL laboratory staff. The authors also thank the anonymous reviewer for valuable comments that helped to improve the manuscript.

potential. Photosynthetic performance of green unicellular alga *Trebouxia* sp., a dominant lichen association-forming genus in chlorolichens has been studied mainly in relation to dehydration (Sadowsky et al. 2016), osmotic (Váczi et Barták 2006), artificially-induced oxidative stress (del Hoyo et al. 2011), and heavy metal stress (Álvarez et al. 2012).

The dependence of growth rate of terrestrial microalgae from polar regions on cultivation temperature has been investigated by many methods. Seaburg et al. (1981) reported optimum growth temperature for 35 species of Antarctic microalgae within the range of 7.7 to 18°C. Fogliano et al. (2010) reported that *Koliella antarctica*, an unicellular extremophilic green alga from the Ross Sea, Antarctica, exhibited higher growth rates and biomass production at 15°C of cultivation temperature than at 10°C.

Cultivation of algae in crossed gradient cultivator is a common method in experimental algology used typically for the testing of morphological variability, growth rates, adjustment of physiological processes, photosynthetic pigments content (for review *see* e.g. Kvíderová et Lukavský 2003). Recently, the cross gradients cultivation approach is used for determination of temperature optima of algae and cyanobacteria from polar region. In the last two decades, the approach was applied in filamentous algae of *Raphidonema* (Stibal et Elster 2005).

In this study, we hypothesized that *Trebouxia* strain EEL201 has a temperature optimum for growth at about 12 - 14°C. To support this hypothesis by experimental data, we applied chlorophyll fluorescence imaging method to evaluate photosynthetic parameters in *Trebouxia* culture cultivated at different temperatures.

Material and Methods

Cultivation

Trebouxia sp. was originally isolated from an Antarctic lichen *Usnea antarctica* collected at the James Ross Island (63.81 S, 57.83 W) and grown in a stock culture. The isolation was done by a gradient centrifugation method according to Gasulla et al. (2010) using a Percoll®. After isolation, the alga was cultivated on agar medium at 10°C. When algal culture was sufficiently developed, it was collected from the surface of agar medium and suspended in a liquid medium (nitrogen-enriched anorganic Bold's Basal Medium, 3N-BBM, Ahmadjian 1993). This medium is generally used for unicellular algae cultivation.

The BBM composition was follows: $CaCl_2$ (25 mg l^{-1}), NaCl (25 mg l^{-1}), $NaNO_3$ (250 mg l^{-1}), $MgSO_4$ (75 mg l^{-1}), KH_2PO_4 (105 mg l^{-1}), K_2HPO_4 (75 mg l^{-1}), and 3 ml of trace metal solution with the following concentration was added to the 1000 ml of the above solution: $FeCl_3$ (0.194 g l^{-1}), $MnCl_2$ (0.082 g l^{-1}), $CoCl_2$ (0.16 g l^{-1}), $Na_2MoO_4 * 2H_2O$ (0.008 g l^{-1}), and $ZnCl_2$ (0.005 g l^{-1}).

In order to find temperature demands for *T. erici* photosynthesis and growth, the method of crossed gradients of temperature and light (Labio cross gradients cultivator, Labio, Prague, Czech Republic) was used. The system construction was described earlier by Kvíderová et Lukavský (2003). Therefore, here we report only the basic information about the system. The device consists of 2 independent systems. One controls temperature (the temperature system), and the second controls irradiation (illumination system). In our study, however, a constant cultivation irradiance was used.

The basis of the temperature system is an aluminium plate with dimensions 80×60×4 cm having a hot and cooled side. Cooling and heating took place at the opposite sides of the plate. Cooling took place continuously allowing to achieve a stable temperature gradient. *T. erici* cultivation took place on this aluminum plate. We placed cultivation Petri dishes with innoculated algae to a certain place over the aluminium plate to cultivate at 0, 10, 20 and 30°C. In the text, the temperature treatments are abbreviated A = 0°C, B = 10°C, C = 20°C and D = 30°C. The Petri dishes were covered with a plastic foil to prevent the medium (BBM, *see* above) to evaporate. During the cultivation, the Petri dishes with *T. erici* were exposed to 120 μmol m^{-2} s^{-1} of photosynthetically active radiation.

Chlorophyll fluorescence

During cultivation of *T. erici*, the acclimation of primary photosynthetic processes was measured by chlorophyll fluorescence repeatedly, typically in 3 days interval. The chlorophyll fluorescence was measured by a HFC-010 FluorCam (Photon Systems Instruments, Drásov, Czech Republic) using the approach of slow Kautsky kinetics supplemented with saturation pulses. The measurements started on pre-dark-adapted samples. The microbiological plate with 4 holes filled with the *T. erici* culture were pre-darkened for 5 min. (*i.e.* sufficient time to allow full opening of reaction centres of PS II – tested before). Then, the algal culture was exposed to a weak light to induce background chlorophyll fluorescence to determine F_0. Then, a saturation pulse was given in order to induce and record maximum chlorophyll fluorescence (F_M). Following 10 s of dark adaptation, the algae were exposed to actinic light for 300 s and the kinetics data, particular chlorophyll fluorescence levels F_P (peak chlorophyll fluorescence recorded after 2 s of actinic light on) and F_S (steady state chlorophyll fluorescence) recorded. Then, a saturation pulse was given to induce maximum of chlorophyll fluorescence in light-adapted state (F_M'). Then, actinic light was switched off and background chlorophyll fluorescence (F_0') recorded. Finally, after 20 s, another saturation pulse was given and peak chlorophyll fluorescence level (F_M'') recorded.

From the recorded data, the following chlorophyll fluorescence parameters were calculated using the below equations:

$$F_V/F_M = (F_M - F_0)/F_M \qquad \textit{Eqn.}\ 1$$

$$\Phi_{PSII} = (F_M' - F_S)/F_M' \qquad \textit{Eqn.}\ 2$$

$$RFd = (F_P - F_S)/F_S \qquad \textit{Eqn.}\ 3$$

$$qF_0 = (F_0 - F_0')/F_0 \qquad \textit{Eqn.}\ 4$$

Mean values (means of 4 replicates for each temperature) of the above-specified parameters were calculated and plotted against the time of cultivation.

Pigment content

At the end of cultivation period, the algal cultures of particular temperature treatments were filtered on disks, re-diluted in DMSO and pigment content (chlorophyll *a*, chlorophyll *b*, total carotenoids) evaluated spectrophotometrically using the absorbances at 480, 665 and 649 nm using the formulae by Welburn (1994).

Statictical analysis

Treatment-dependent differences in the chlorophyll fluorescence parameters were evaluated by ANOVA, if not stated otherwise.

Results

Time courses of F_V/F_M reflected the effect of cultivation temperature. While a decrease from 0.60 to 0.40 was observed from day 0 to day 3 at the A treatment, followed by a gradual increase of more or less constant rate with the time of cultivation, the D treatment led to a very different time curve. F_V/F_M remained as high as the pre-experimental value (about 0.6) for the first 5 days of exposition. Then, a dramatic decrease in F_V/F_M was apparent to a minimum found on day 12 (about 0.2). With the further time of cultivation, F_V/F_M slightly increased to 0.4 (day 19), however, a consequent decrease to 0.32 (day 26) was seen. The B, and C temperature treatments had a similar effect to a time course of F_V/F_M. In the C treatment, however the F_V/F_M decrease was less steep than in the D treatment within the first 10 days of cultivation. At the end of cultivation, the final F_V/F_M values were quite comparable for B and C treatment (about 0.47). At the low temperature (treatment A), final F_V/F_M reached a much higher value (0.5) then at the highest temperature (treatment D, 0.35).

Except of the treatment D, effective quantum yield showed an increase within the period from day 0 to day 12, followed by a more or less constant value of about 0.22 (A treatment), or slight decrease to about 0.20 in B, and C treatments. The highest temperature treatment led to an increase from 0.1 to the maximum value of 0.32 (day 5) followed by a decrease to a minimum (0.19 – day 12) and consequent increase to a final value of 0.28 (day 26). Relative fluorescence decline ratio (RFd) had initial value within the range of 1.5-2.0. Low-temperature treatment (A) led to a dramatic decrease to 0.5 on day 3 followed to a gradual increase with the time cultivation. In spite of the fact the final RFd value did not reach the initial value, it was the highest when compared to the B, C, and D treatments. In the highest temperature treatment (D), RFd showed a biphasic decline with the time of exposition, fast decline within the days 0-5, followed by much slower decline within the days 5-26. Quenching of background chlorophyll fluorescence (qF_0) exhibited slightly increasing trend with cultivation time in A treatment, however, no clear trend was apparent in the other treatments. Both qF_0 increase followed by a decrease (B, C treatments), and more or less constant qF_0 value was found (D treatments).

Biomass production evaluated at the end of the cultivation revealed that maximum Chl *a*, Chl *b* and total carotenoids were produced at 20°C followed by 10°C. This might be interpreted as optimal temperatures for growth and photosynthetic pigments synthesis. A remarkable decrease in Chl *a,* Chl *b* and total carotenoids content was found in the D treatment. This is indicative for the high temperature effects on functioning of PS II and chloroplastic photosynthetic apparatus. Temperature effect, however was apparent in some ratios. Chl *a* / Chl *b* decreased with cultivation temperature: 4.63 (A), 3.82 (B), 6.64 (C), and 2.81 (D). Total chlorophyll to carotenoids ratio, however, did not show temperature effects: 3.00 (A), 3.30 (B), 3.37 (C), 3.19 (D).

The effect of temperature on the four tested chlorophyll fluorescence parameters was statistically significant ($P < 0.001$).

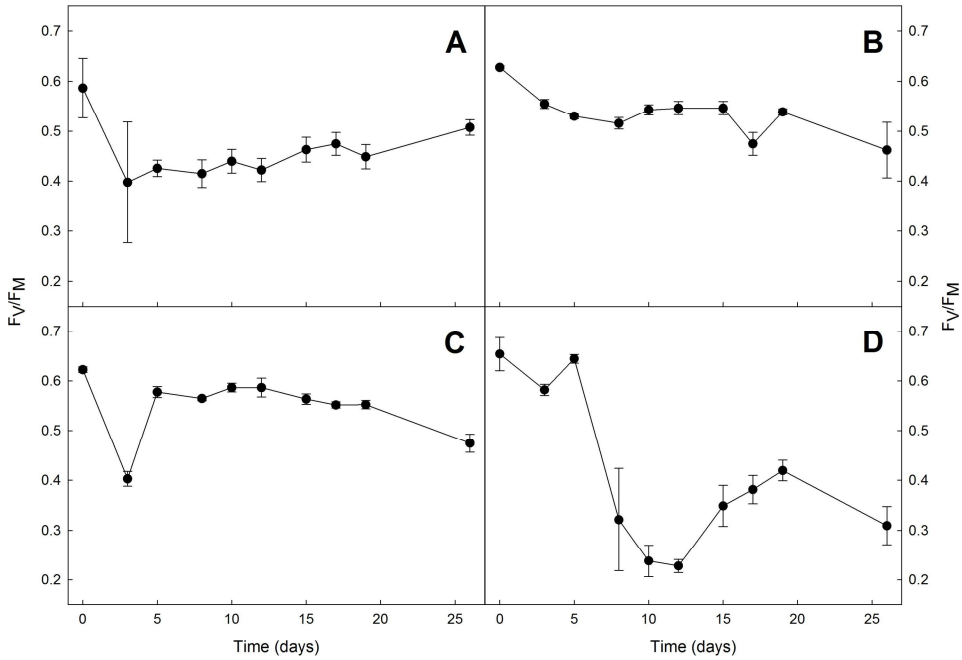

Fig. 1. Time courses of capacity of photosynthetic processes in PS II (F_V/F_M) for low (A = 0°C), semi-low (B = 10°C), high (C = 20°C) and extremely high (D = 30°C) temperature.

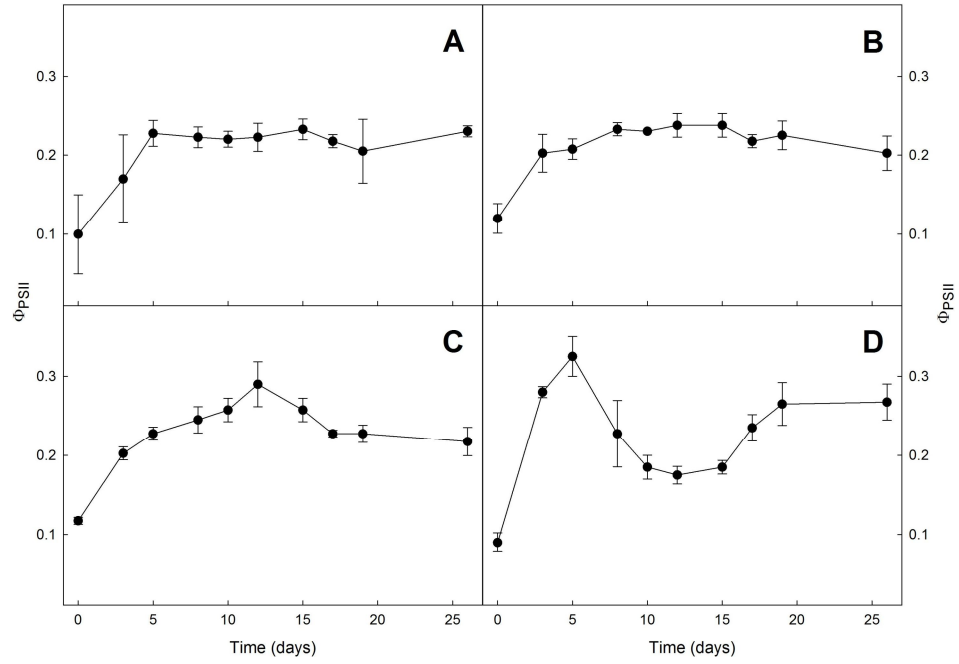

Fig. 2. Time courses of effective quantum yield of PS II (Φ_{PSII}) measured as a long-term effect of low (A = 0°C), semi-low (B = 10°C), high (C = 20°C) and extremely high (D = 30°C) temperature.

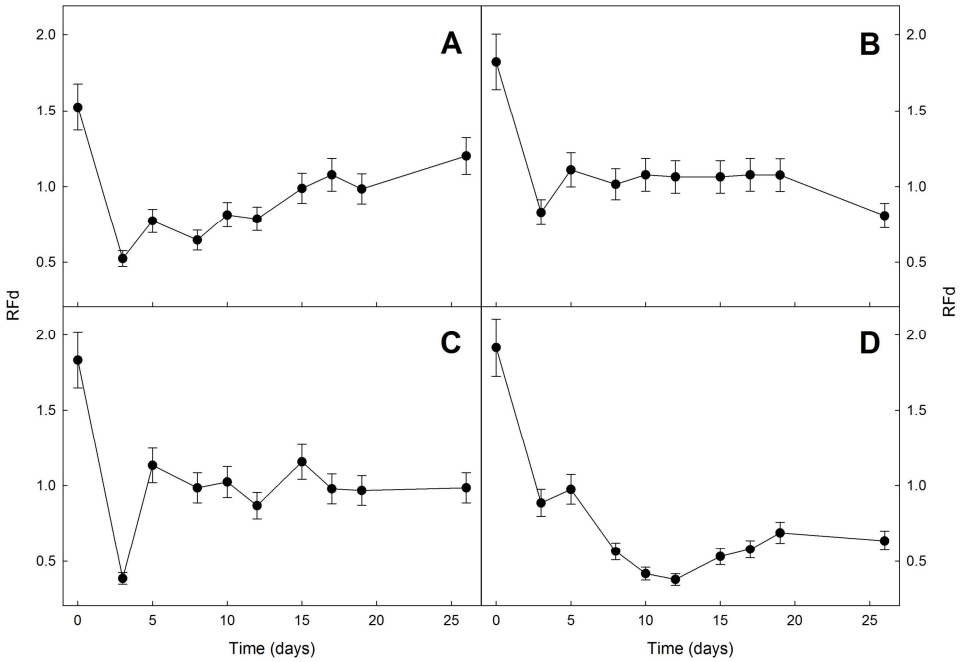

Fig. 3. Time courses of relative fluorescence decline ratio (RFd) measured as a long-term effect of low (A = 0°C), semi-low (B = 10°C), high (C = 20°C) and extremely high (D = 30°C) temperature.

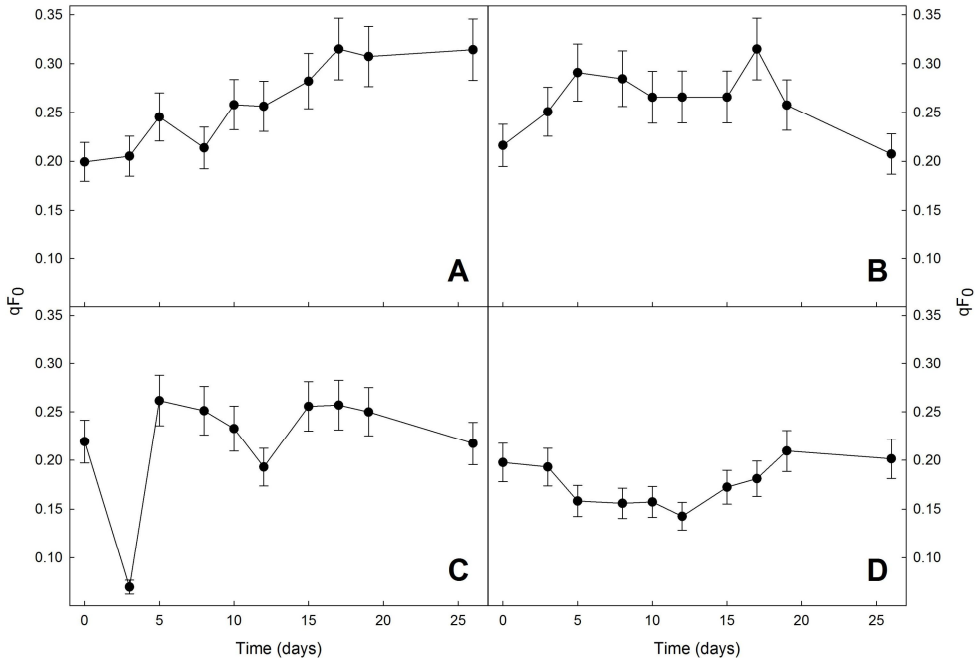

Fig. 4. Time courses of quenching of F_0 (qF_0) measured as a long-term effect of low (A = 0°C), semi-low (B = 10°C), high (C = 20°C) and extremely high (D = 30°C) temperature.

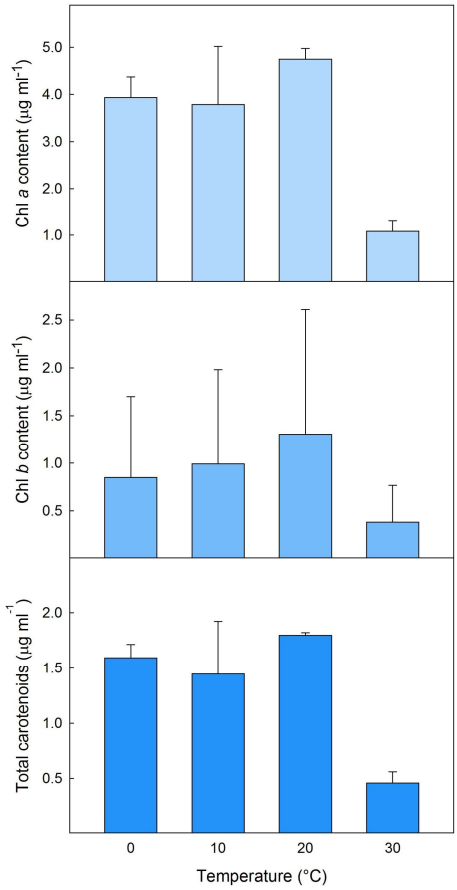

Fig. 5. Content of photosynthetic pigments evaluated at the end of cultivation (day 26) as dependent on cultivation temperature (A = 0°C, B = 10°C, C = 20°C, and D= 30°C).

Discussion

F_V/F_M is a sensitive indicator of the temperature effect on the primary photosynthetic processes. From our data follows that the highest temperature (D treatment) had the most negative effects on the capacity of photosynthetic processes in PS II. Therefore, we may suggest that a wide temperature span 10 - 20°C does not bring negative changes of F_V/F_M in *T. erici* when cultivation time is long enough to allow acclimatory changes (*see* F_V/F_M value, day 26 in Fig. 1). However, temperature responses of F_V/F_M for different *Trebouxia* strains may differ significantly as reported by del Hoyo et al. (2011).

Considering F_V/F_M and Φ_{PSII} time courses at different cultivation temperature, we may suggest that optimum temperature for the primary photochemical processes is within the range of 12-16°C for the studied *T. erici.* Such a conclusion, however, does not consider the whole photosynthetic pathway because no biochemical processes were studied in this experiment. Some other aspects of cultivation procedure, such as *e.g.* the likely mixotrophy and unknown propor-

tion of carbohydrates taken by the cultivated *T. erici* from medium may play a role. Therefore, combination of fluorometric and oxymetric methods estimating photosynthesis in lichen algae is a necessity for future studies. Existing data from previous studies using an oxymetric approach (*see* e.g. Domaschke et al. 2013) suggest that the photosynthetic and growth optima for *Trebouxia* sp. isolated from lichens from polar regions is about 11°C. Similarly, Marečková et Barták (2016) reported 10 and 15°C as the optimum temperature of maximal F_V/F_M values in two *Trebouxia*-possessing lichens from the Antarctica.

Increase in qF_0 is typically associated with pronounced involvement of non-photochemical quenching mechanisms and well documented to day/night changes in available radiation. It is reported for higher plants, that qF_0 increases during night (dark period) - Roháček (2002). On the other hand, high light stress leading to photoinhibition of photosynthetic processes brings a decrease of qF_0 towards negative values (Rosenqvist et van Kooten 2003) since F_0' is higher than F_0 in such situations (*c.f.* Eqn. 4). Since temperature effects on qF_0 have been studied only marginally in algae and cyanobacteria, we may hypothesize that the response of *T. erici* followed the general rules found in higher plants. Our data suggest a low temperature-induced increase in non-photochemical quenching in LHC II. Such a qF_0 increase localized in antennae contributed to overal non-photochemical quenching which rised as well in A-treated *T. erici* (from 0.28 to 0.92, data not shown).

The chlorophyll fluorescence decrease ratio (vitality index) provides an useful information on the physiological state of photosynthesis (Lichtenthaler et Rinderle 1998). RFd has been applied with great success as stress detector in photosystem II. In algae, RFd was shown to be sensitive to nutrient supply by Fodorpataki et al. (2013) who reported decrease in RFd due to limitation of nitrogen. Similarly, RFd was used to assess stress effects of heavy metals (Fodorpataki et al. 2010) and toxic compounds in cyanobacterium (CeO_2, Rodea-Palomares et al. 2012). Biological stressors may also cause RFd decline in algae as demonstrated for a parasite effects on brown alga (Gachon et al. 2006). For lichen-associated *Trebouxia* sp., Tuba et al. (1996) showed a dramatic RFd decline in *Cladonia* exposed to severe drought stress. For indication of low-temperature stress in lichen symbiotic alga *Trebouxia* sp., however, RFd is used only scarcely (*see* e.g. Sehnal et al. 2014). Our data suggest that the lowest temperature (treatment A) brings gradual RFd increase from 0.2 to about 0.3 which might be interpreted as a gradual acclimation of photosynthetic processes preformed at PS II towards optimal functioning at this temperature. At the D temperature, no such phenomen is seen since RFd remained constant throughout the cultivation time. In our experiment, *Trebouxia* showed about ten times lower RFd values than reported for a wide range of higher plants exposed to low temperature (*e.g.* Georgieva et Lichtenthaler 2006 for pea plants, Pererra-Castro et al. 2017 for alpine plants). Low RFd found in our study were, however, well comparable to the earlier *Trebouxia sp.* data reported by Sehnal et al. (2014) for cultivations on BBM agars.

Concluding remarks

Photosynthetic processes in lichenized *Trebouxia* may sustain even at subzero temperature in lichen thalli (Barták et al. 2007), however thermoresistance of isolated algae may differ from that measured in lichens. Typically, it is lower than in lichens. The optimum temperature for F_V/F_M and Φ_{PSII} found in our study is consistent

with earlier studies done on cryptoendolithic lichen communities from Ross Desert, Antarctica. Ocampo-Friedman et al. (1988) found that two *Trebouxia* strains isolated from cryptoendolithic lichens had growth optima at around 17°C and maxima at around 20°C. Similar temperatures (15°C in the majority of cases) are reported by Schoefield et Ahmajian, (1972) for five of six *Trebouxia* strains isolated from thallose Antarctic lichens. For *Hemichloris antarctica* strain from Antarctic cryptoendolithic community the range of -4 to 22°C is reported with the optimum ranging between 12 to 18°C (Tschermak-Woess et Friedmann 1984).

Our data on optimum cultivation temperature are well comparable to the previous studies made in *Trebouxia* sp. For batch cultures of *Trebouxia* sp., Hájek et al. (2009, 2012, 2016) found fastest growth at 10°C. Similarly, Teoh et al. (2004) reported the highest specific growth rates of several Antarctic microalgae for temperature ranging from 6°C to 14°C. Recent study on *Trebouxia* sp. (Balarinová et al. 2013) reported a faster growth in 10°C than 15°C.

References

AHMADJIAN, V. (1993): The Lichen Symbiosis. John Wiley & Sons, New York. 250 p.

ÁLVAREZ, R., DEL HOYO, A., GARCÍA-BREIJO, F., REIG-ARMIÑANA, J., DEL CAMPO, E.M., GUÉRA, A., BARRENO, E. and CASANO, L.M. (2012): Different strategies to achieve Pb-tolerance by the two *Trebouxia* algae coexisting in the lichen *Ramalina farinacea*. *Journal of Plant Physiology*, 169: 1797-1806.

BALARINOVÁ, VÁCZI, P., BARTÁK, M., HAZDROVÁ, J. and FORBELSKÁ, M. (2013): Temperature-dependent growth rate and photosynthetic performance of Antarctic symbiotic alga *Trebouxia* sp. cultivated in a bioreactor. *Czech Polar Reports*, 3: 19-27.

BARTÁK, M., VÁCZI, P., HÁJEK, J. and SMYKLA, J. (2007): Low-temperature limitation of primary photosynthetic processes in Antarctic lichens *Umbilicaria antarctica* and *Xanthoria elegans*. *Polar Biology*, 31: 47-51.

CAO, S., ZHANG, J., ZHENG, H., LIU, CH. and ZHOU, Q. (2015): Photosynthetic performance in Antarctic lichens with different growth forms reflect the diversity of lichenized algal adaptation to microhabitats. *Polish Polar Research*, 36: 175-188.

DEL HOYO, A., ALVAREZ, R., DEL CAMPO, E.M., GASULLA, F., BARRENO, E. and CASANO, L.M. (2011): Oxidative stress induces distinct physiological responses in the two *Trebouxia* phycobionts of the lichen *Ramalina farinacea*. *Annals of Botany,* 107: 109-18.

DOMASCHKE, S., VIVAS, M., SANCHO, L.G. and PRINTZEN, C. (2013): Ecophysiology and genetic structure of polar versus temperate populations of the lichen *Cetraria aculeata*. Oecologia, 173 (3): 699-709, doi:10.1007/s00442-013-2670-3.

FODORPATAKI, L., GERÁJ, J., DEÁK, H., BARNA, S. and KOVÁCS, B. (2013): Influence of inorganic nutrients on parameters of biomass production in a local strain of the microalga *Scenedesmus Acuminatus*. *Contributii Botanice*, XLVIII: 83-94.

FODORPATAKI, L., KERESZTES, Z.G., BARTHA, C. and BARNA, S. (2010): Bioindicator of water pollution in the somes river using biochemical and physiological parameters of the green alga *Scenedesmus opoliensis* P. Richter. *Egyptial Journal of Phycology*, 11: 49-68.

FOGLIANO, V., ANDREOLI, C, MARTELLO, A., CAIAZZO, M., LOBOSCO, O., FORMISANO, F., CARLINO, P. A., MECA, G., GRAZIANI, G., DI MARTINO RIGANO, V., VONA, V., CARFAGNA, S. and RIGANO, C. (2010): Functional ingredients produced by culture of *Koliella antarctica*. *Aquaculture*, 299: 115-120.

FRIEDMAN, E.I., SUN, H.J. (2005): Communities Adjust their Temperature Optima by Shifting Producer-to-Consumer Ratio, Shown in Lichens as Models: I. Hypothesis. *Microbial Ecology*, 49: 523-527.

GACHON, C.M.M., KUPPER, H., KUPPER, F.C. and ŠETLÍK, I. (2006): Single-cell chlorophyll fluorescence kinetic microscopy of *Pylaiella littoralis* (Phaeophyceae) infected by *Chytridium polysiphoniae* (Chytridiomycota). *European Journal of Phycology*, 41: 395-403.

GASULLA, F., HERRERO, J., ESTEBAN-CARRASCO, A., ZAPATA, J.M. and GUÉRA, A. (2010): Photosynthesis in Lichen:Light Reactions and Protective Mechanisms. *In*: M. Najafpour (ed.): Advances in Photosynthesis - Fundamental Aspects. pp. 149-174.

GEORGIEVA, K., LICHTENTHALER, H. K. (2006): Photosynthetic response of different pea cultivars to low and high temperature treatments. *Photosynthetica*, 44: 569-578.

HÁJEK, J., VÁCZI, P. and BARTÁK, M. (2009): Photosynthetic electron transport at low temperatures in the green algal foliose lichens *Lasallia pustulata* and *Umbilicaria hirsuta* affected by manipulated levels of ribitol. *Photosynthetica*, 47: 199-205.

HÁJEK, J., VÁCZI, P., BARTÁK, M. and JAHNOVÁ, L. (2012): Interspecific differences in cryoresistance of lichen symbiotic algae of genus *Trebouxia* assessed by cell viability and chlorophyll fluorescence. *Cryobiology*, 64: 215-222.

HÁJEK, J., BARTÁK, M., HAZDROVÁ, J. and FORBELSKÁ, M. (2016): Sensitivity of photosynthetic processes to freezing temperature in extremophilic lichens evaluated by linear cooling and chlorophyll fluorescence. *Cryobiology*, 73: 329-334.

KOSUGI, M., ARITA, M., SHIZUMA, R., MORIYAMA, Y., KASHINO, Y., KOIKE, H. and SATOH, K. (2009): Responses to desiccation stress in lichens are different from those in their photobionts. *Plant Cell Physiology*, 50: 879-88.

KVÍDEROVÁ, J., LUKAVSKÝ, J. (2003): The cultivation of *Pheodactylum tricornutum* in crossed gradients of temperature and light. *Algological Studies,* 110: 67-80.

LICHTENTHALER, H.K., RINDERLE, U. (1988): The role of chlorophyll fluorescence in the detection of stress conditions in plants. *Critical Reviews in Analytical Chemistry*, 19: 329-383.

MAREČKOVÁ, M., BARTÁK, M. (2016): Effects of short-term low temperature stress on chlorophyll fluorescence transients in Antarctic lichen species. *Czech Polar Reports*, 6: 54-65.

OCAMPO-FRIEDMANN, R., MEYER, M.A., CHEN, M. and FRIEDMANN, E.I. (1988) Temperature response of Antarctic cryptoendolithic photosynthetic microorganisms. *Polarforschung*, 58: 121-124.

PERERRA-CASTRO, A.V., BRITO, P. and GONZÁLEZ-RODRÍGUEZ, A.M. (2017): Changes in thermic limits and acclimation assessment for an alpine plant by chlorophyll fluorescence analysis: Fv/Fm vs. Rfd. *Photosynthetica*, 55: accepted for press.

RODEA-PALOMARES, I., GONZALO, S., SANTIAGO-MORALES, J., LEGANÉS, F., GARCÍA-CALVO, E., ROSAL, R., FERNÁNDEZ-PINAS, F. (2012): An insight into the mechanisms of nanoceria toxicity in aquatic photosynthetic organisms. *Aquatic Toxicology*, 122–123: 133-143.

ROHÁČEK, K. (2002): Chlorophyll Fluorescence Parameters: The Definitions, Photosynthetic Meaning, and Mutual Relationships. *Photosynthetica*, 40: 13-29.

ROSENQVIST, E., VAN KOOTEN, O. (2003): Chlorophyll fluorescence: A general description and nomenclature. *In*: J. R. de Ell, P. M. A. Toivonen (eds.): Practical Applications of Chlorophyll Fluorescence in Plant Biology, Springer/Kluwer, New York, pp. 31-77.

SADOWSKY, A., METTLER-ALTMANN, T. and OTT, S. (2016): Metabolic response to desiccation stress in strains of green algal photobionts (*Trebouxia*) from two Antarctic lichens of southern habitats. *Phycologia*, 55: 703-714.

SEABURG, K. G., PARKER, B. C, WHARTON, R. A. and SIMMONS, G. M. (1981): Temperature-growth responses of algal isolates from Antarctic oases. *Journal of Phycology*, 17: 353-360.

SEHNAL, L., VÁCZI, P. and BARTÁK, M. (2014): Effect of temperature and increased concentration of CO_2 on growth and photosynthetic activity of polar alga *Trebouxia* sp. *Czech Polar Reports*, 4: 47-56.

SCHOFIELD, E., AHMADJIAN, V. (1972): Field observations and laboratory studies of some Antarctic cold desert cryptogams. *In*: G. Llano (ed.): Antarctic Terrestrial Biology, Antarctic Research Series, Vol. 20, American Geophysical Union, Washington, DC, pp. 97-142.

STIBAL, M., ELSTER, J. (2005): Growth and morphology variation as a response to changing environmental factors in two Arctic species of *Raphidonema* (Trebouxiophyceae) from snow and soil. *Polar Biology*, 28: 558-567.

TEOH, M.-L., CHU, W.-L., MARCHAND, H. and PHANG, S.-M. (2004): Influence of culture temperature on the growth, biochemical composition and fatty acid profiles in six Antarctic microalgae. *Journal of Applied Phycology*, 16: 421-430.

TSCHERMAK-WOESS, E., FRIEDMANN, E. I. (1984): *Hemichloris antarctica*, gen. et sp. nov. (Chlorococcales, Chlorophyta), a cryptoendolithic alga from Antarctica. *Phycologia* 23 (4): 443-454.

TUBA, Z., CSINTALAN, Z. and PROCTOR, M.C.F. (1996): Photosynthetic responses of a moss, *Tortula ruralis*, ssp. *ruralis*, and the lichens *Cladonia convoluta* and *C. furcata* to water deficit and short periods of desiccation, and their ecophysiological significance: a baseline study at present-day concentration. *New Phytologist*, 133: 353-361.

VÁCZI, P., BARTÁK, M. (2006): Photosynthesis of lichen symbiotic alga *Trebouxia erici* as affected by irradiance and osmotic stress. *Biologia Plantarum*, 50 (2): 257-264.

WELLBURN, A. R. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. *Journal of Plant Physiology,* 144: 307-313.