Vitality and growth rate of agar plate-cultivated Antarctic microautotrophs: Analysis of PSII functioning by chlorophyll fluorescence parameters

Anna Manová[1], Blanka Hýžová[1], Diana Darriba Canora[2], Ana Castrillo Antolin[3], Kristýna Dufková[4,5*]

[1]*Czech Collection of Microorganisms, Faculty of Science, Masaryk University, Department of Experimental Biology, Kamenice 5, 625 00 Brno, Czech Republic*
[2]*University of Girona, Faculty of Science, Campus Montilivi, 170 03 Girona, Spain*
[3]*University of Salamanca, Faculty of Biology, Campus Miguel de Unamuno, C/Donantes de sangre, 370 07 Salamanca, Spain*
[4]*Department of Internal Medicine - Haematology and Oncology, University Hospital Brno, Jihlavská 340/20, 625 00 Brno, Czech Republic*
[5]*Department of Internal Medicine - Haematology and Oncology, Faculty of Medicine, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic*

Abstract

In our study, we focused on the growth of three different microautotrophs isolated from Antarctic lichens (*Placopsis contortuplicata*, *Solorina spongiosa*) and cryptoendolithic algal vegetation. The isolates were purified and inoculated on agar plates, Bold´s Basal Medium (BBM). The growth of the cultures and the markers of physiological (photosynthetic) activity were monitored by chlorophyll fluorescence in 1 week intervals for 3 months after inoculation. For the assessment of photosynthetic activity, the method of slow Kautsky kinetics supplemented with saturation pulses was applied. Four chlorophyll fluorescence parameters calculated: (1) maximum quantum yield of PSII (F_V/F_M), (2) effective quantum yield of photosynthetic processes in PSII (Φ_{PSII}), (3) non-photochemical quenching of chlorophyll fluorescence, and (4) background chlorophyll fluorescence ratio ($F_0/F_0´$). Troughout the cultivation period, the maximum quantum yield of PSII (F_V/F_M) showed high values in all three autotrophs with only slight increase in the first part of the cultivation period, followed by slight decrease in the second part. The Φ_{PSII} values showed a rapid decline within the first 4 weeks of cultivation followed by more or less constant values in the isolates from *P. contortuplicata* and cryptoendolithic alga. Contrastingly, time course of Φ_{PSII} rather showed an increase followed by a decrease in *S. spongiosa* isolate. NPQ (related to the activation of protective mechanisms) increased in the second part of cultivation period, the rate of increase and maximum values were species-specific. The species-specific differences in chlorophyll fluorescence parameters are discussed as well as their potential for evaluation of photosynthetic performance of *in vitro* cultivated algal/cyanobacterial cultures on agar plates.

Key words: lichen photobionts, algal biotechnology, Kautsky kinetics

DOI: 10.5817/CPR2022-2-20

Received July 8, 2022, *accepted* January 3, 2023.
[*]Corresponding author: K. Dufková <423682@mail.muni.cz>
Acknowledgements: The VAN2022 (Czech Antarctic Research Infrastrucure, Czech Ministry of Education, Youth and Sports, Czech Republic) and ECOPOLARIS (CZ.02.1.01/0.0/0.0/16_013/0001708) are acknowledged.

Introduction

Microalgae are single-celled eukaryotic autotrophic organisms. They are ubiquitously present in a wide range of habitats on all continents. Thanks to their efective photosynthesis, they are important organic matter producers (Benedetti et al. 2018) and sink for atmospheric carbon dioxide (Vale et al. 2020). Therefore, they have been increasingly used for a variety of biotechnologies, including industrial-scale production of bioethanol (Kim et al. 2020), plant pigments and antioxidants (*e.g.* Cazzaniga et al. 2022), and pharmaceutically prospective secondary metabolites (for review see Sathasivam et al. 2019).

Stock cultures of microalgae are frequently cultivated on agar plots. During the cultivation, cultures grow in size and volume, and undergo several changes related to the cultivation conditions including physical (temperature, irradiation, moisture) and chemical factors (composition and changes of cultivation medium).

In biotechnologies of microalgae in liquid media, as well as laboratory experimental practices, several methods have been used for the detection and quantification of agal cultures growth. The widely used methods comprise mainly the following approaches (1) optical density measurements (*e.g.* Nielsen and Hansen 2019, Ziganshina et al. 2020), (2) flow cytometry (Habercorn et al 2021, Hyka et al. 2013), and (3) chlorophyll fluorescence methods (*e.g.* Malapascua et al. 2014). Estimation of algal cultures growth and vitality by a wide variety of chlorophyll fluorescence techniques is recently applied in many algal biotechnologies. Those comprise *e.g.* outdoor photobioreactors (Masojídek et al. 2021), indoor tubular photobioreactors (*e.g.* Schoeters et al. 2022), small-volume tabular photobiorectors installed in the field in polar regions (*e.g.* Šnokhous 2021) or in a laboratory (Balarinová et al. 2013), and even microtiter plates (Albrecht et al. 2022).

Maintanance cultivations of microalgae are performed on solid (agar) media for their further re-inocultion to liquid culture, thus the agar cultivation itself is mainly focused on survival of strains and subsequent regrowth in liquid media. Recently, the study of Yee et al. (2019) showed that majority of microalgal cells restore even after 24 month cultivation on agars. Physiological status of the agar plate-cultivated strains is, however, not checked regularly. The changes in the functioning of photosynthetic apparatus in chloroplasts, photosystem II (PSII) in particular, with the time of cultivation are not evaluated. Therefore, the extent of the change in PSII functioning is generally unknown. Study of Dufková et al. (2019) suggested that both slow Kautsky kinetics as well as derived chlorophyll fluorescence parameters change with the time of cultivation and emphasized the importance of more detailed study in this field.

Chlorophyll fluorescence imaging has been used to study heterogeneity of chlorophyll fluorescence signal emitted from agar plates-grown cultures in *Synechocystis* mutants (Ozaki et al. 2007, Ozaki and Sonoike 2009) and Antarctic microautotrophs (Dufková et al. 2019). In these studies, slow Kautsky kinetics of chlorophyll fluorescence and derived chlorophyll fluorescence parameters were evaluated and atributed to the growth phase of the cultures. The latter study brought an analysis of the changes in the shape of slow Kautsky kinetics of chlorophyll fluorescence with cultivation time.

Previous study done with microalgae cultivated on agar plates (Dufková et al. 2019) focused on several algal strains from polar regions. In our study, we studied several algal/cyanobacterial species extracted from Antarctic lichens in order to evaluate the changes in their vigor and primary photosynthesis characteristics during the first 13 weeks after inoculation on agar

plates. We hypothesized that with growth time, majority of primary photosynthetic parameters will decline due to intristic reasons (Petri dish microenvironment). However, we expected species-specific sensitivity to such decline. Therefore, the aim of this study was to find the species showing lowest sensitivity to the decline and analyze the underlying physiological mechanisms.

Material and Methods

Collection of samples

Samples of autotrophic organisms used in this study were isolated from lichens and cryptoendolithic collections (James Ross Island, Antarctica). In the following text, the cultures of autotrophic organisms are abbreviated according to the original collection, a source of photobionts, *i.e.* PL (lichen *Placopsis contortuplicata*, algal photobiont isolated from marginal part of thallus), SO (lichen *Solorina spongiosa*, algal photobiont isolated from apothecium), CE (cryptoendolitic microbiological mat isolated from microcraks in a stone). Particular samples were collected from deglaciated areas of the Ulu peninsula: (1) PL – 63° 52′51′′ S, 58° 04′ 52′′ W, (2) SO – 63° 52′ 54′′ S, 57° 47′ 30′′, and (3) CE – 63° 48′ 28′′ S, 57° 51′ 12′′ W. After the collection, samples were naturally dried in a shady place outside the Mendel station, then trasported in a portable fridge with inserted freezer packs to the Masaryk University (Brno, Czech Republic), where they were stored in a refrigerator (5°C) before the experiments.

Isolation of autotrophs and cultivation

Isolation of lichen autotrophs from PL and SO samples was done from homogenized material of lichen thalli. After isolation from lichen thalli, the cultures were cultivated on agar plates in the facilities of the Extreme Enviroment Life Laboratory (EEL, Brno, Czech Republic). All the cultures were grown on Bolds Basal Medium (BBM, for composition see *e.g.* Khan et al. 2018) on Petri dishes at the temperature of 5°C.

Chlorophyll fluorescence measurements

The chlorophyll fluorescence measurements of the above-specified experimental species grown on agar plates started four weeks after inoculation and were repeated in 7 d interval for 13 weeks. In the following text, the first measurement is denoted as Day 0. The chlorophyll fluorescence measurements consisted of the record of slow Kautsky kinetics induced by continuous light and the measurements of peak values of chlorophyll fluorescence induced by a saturation puls applied in dark- and ligh-adapted state of the samples. The measurements were done by a FluorCam (HFC-010, Photon Systems Instruments, Czech Republic) and resulted in the images of particular chlorophyll fluorescence parameters (*see* below) over an area of a Petri dish (*see* Fig. 1).

To measure and evaluate chlorophyll fluorescence parameters, earlier published method was used (for details *see* e.g. Marečková et al. 2019). Here we bring just a brief overview. Each sample was dark-

adaptated for 10 min. before the chlorophyll fluorescence measurements. The time of darkening was long enough to fully open the reaction centres (RCs) of photosystem II (PSII), as tested before (not shown here). Then, background chlorophyll fluorescence (F_0), induced by a weak light of 1 μmol photons m^{-2} s^{-1} of photosynthetically active radiation PAR) was measured. The maximum chlorophyll fluorescence level induced by a saturation puls (SP, 5000 μmol photons m^{-2} s^{-1} PAR) applied in the dark-adapted state (F_M) was evaluated to enable the calculation of the maximum quantum efficiency of PSII (F_V/F_M, *see* Eqn. 1). Chlorophyll fluorescence record of slow Kautsky kinetic was done during a 5 min. exposition of the sampels to continuous light (60 μmol photons m^{-2} s^{-1} PAR). At the end of the period, another SP was applied in order to reach the maximum chlorophyll fluorescence level in light-adapted state (F_M'). It allowed the calculation of the effective quantum yield of PSII electron transport (Φ_{PSII}) according to Equation (2). Non-photochemical quenching (NPQ), a measure of the activation of protective mechanisms in chloroplast apparatus exposed to a sterssor, was calculated using Equation (3). Quencing of background chlorophyll fluorescence qF_0, a parameter evaluating the changes happening in light harvesting complexes (LHC), was calculated according to Equation (4).

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

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

$$NPQ = (F_M - F_M')/ F_M' \qquad \textit{Eqn.}\ 3$$

$$F_0 / F_0' \qquad \textit{Eqn.}\ 4$$

Data were presented as (1) the change of shape and timecourse of the slow Kautsky kinetics in response to the cultivation time, and (2) the time courses of the above-specified chlorophyll fluorescence parameters.

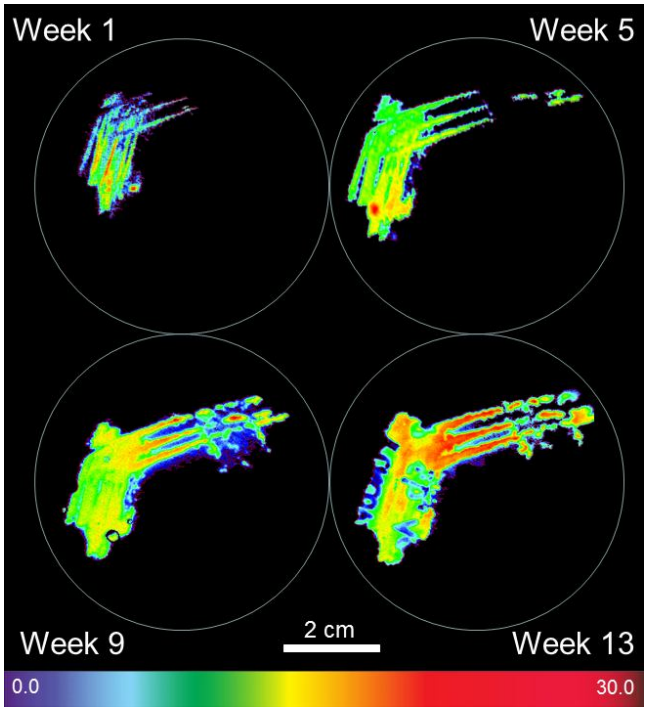

Fig. 1. Images of chlorophyll fluorescence parameter (F_S – steady state fluorescence, *see* the bottom false colour scale) recorded by a kinetic fluorometric camera over the area of Petri dish with the algal photobiont isolated from *Placopsis contortuplicata*.

Statistical analysis

If not stated otherwise, the statistically significant differences related to the species and/or the time of cultivation after the inoculation were calculated by one-way ANOVA.

Results

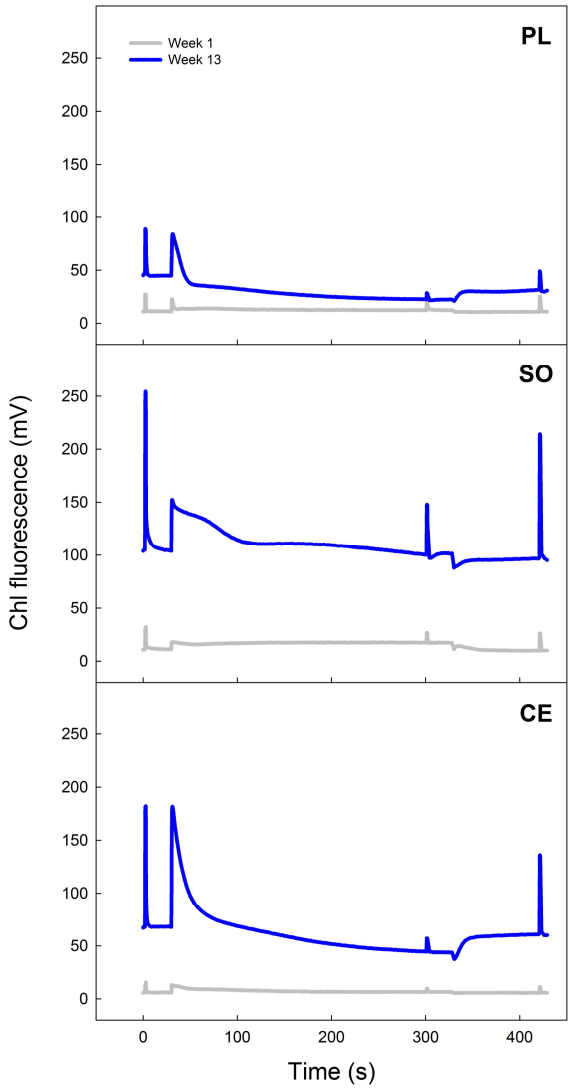

Fig. 2. Slow Kautsky kinetics of chlorophyll fluorescence recorded from the photobionts isolated from the samples of *Placopsis contortuplicata* (PL), *Solorina spongiosa* (SO), and a cryptoendolithic autotrophs (CE). The Kautsky kinetics were taken during the 1st and 13th week of chlorophyll fluorescence measurements.

F_V/F_M was found high at the begining of the chlorophyll fluorescence measurements (day 0, week 1), however, showed somewhat lower value for CE (0.565) than for the other two photobionts (PL – 0.595, SO – 0.650) – *see* Figs. 3-5. Then, F_V/F_M increased slightly in all species with the time of cultivation reaching maximum values in week 4 (*see* an arrow in Fig. 3). The peaks were comparable in all samples having the value slightly above or below 0.700. During following cultivation, F_V/F_M tended to decline and reached lower than initial values at the end of chlorophyll fluorescence measurements (week 13). This was apparent in PL and SO samples. In CE sample, the decline in F_V/F_M was much less pronounced. Therefore, final F_V/F_M value was higher than the initial one. In all cases, however, F_V/F_M remained relatively high, indicating that potential processes in PSII were negatively affected by the cultivation on agar plots to only limited extend (*see* Discussion).

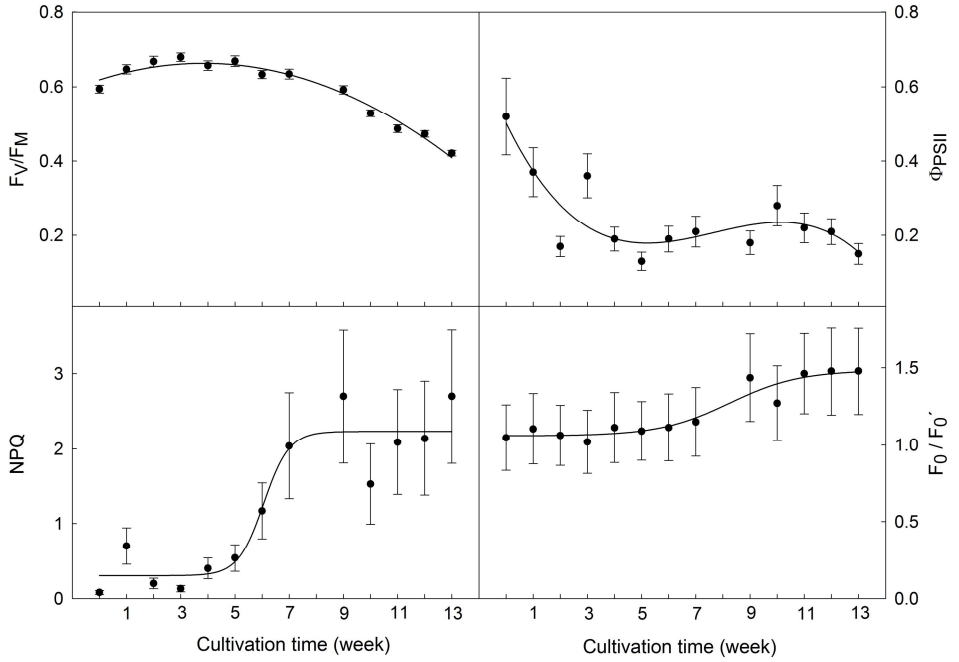

Fig. 3. Time courses of selected chlorophyll fluorescence parameters (F_V/F_M, Φ_{PSII}, NPQ, F_0 / F_0') for the photobiont isolated from *Placopsis contortuplicata.*

Effective quantum yield of photosynthetic processes in PSII (Φ_{PSII}) showed an exponential decline in PL and CE samples in the first part of the monitoring period (a decline to about 0.2, *see* Fig. 3, 4) followed either by a no change or a slight increase found at the end of the monitoring period. The SO samples, however, showed different time course of Φ_{PSII}. Significant increase from initial (0.371) to maximum value (0.579) was found at a half of monitoring period. Then, with ongoing cultivation time, Φ_{PSII} decreased to a minimum (0.234), followed by a slight increase at the end of monitoring period ($\Phi_{PSII} > 0.300$).

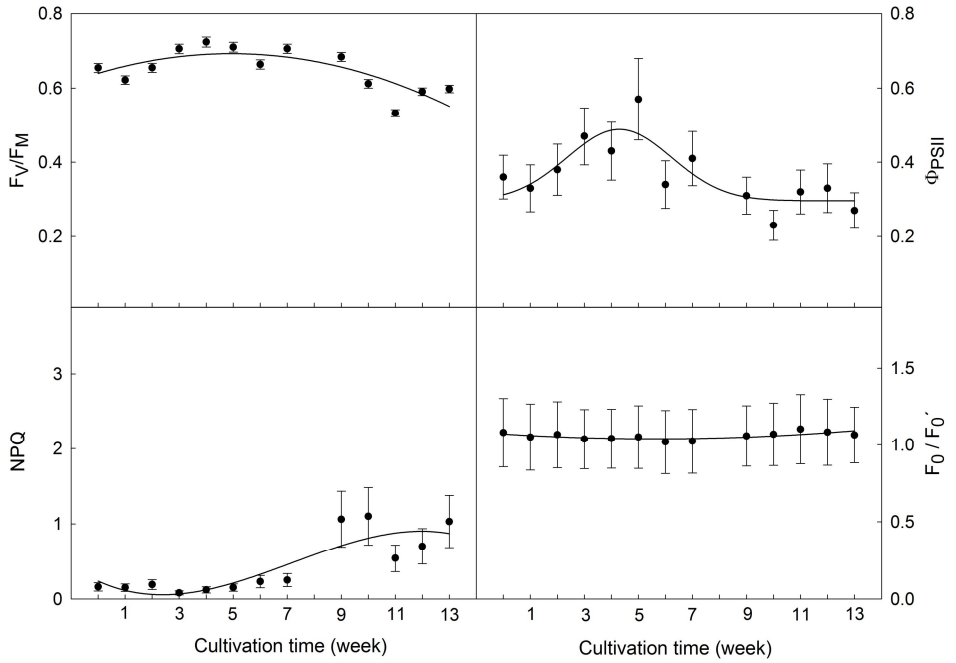

Fig. 4. Time courses of selected chlorophyll fluorescence parameters (F_V/F_M, Φ_{PSII}, NPQ, F_0 / F_0') measured in the photobiont isolated from *Solorina spongiosa.*

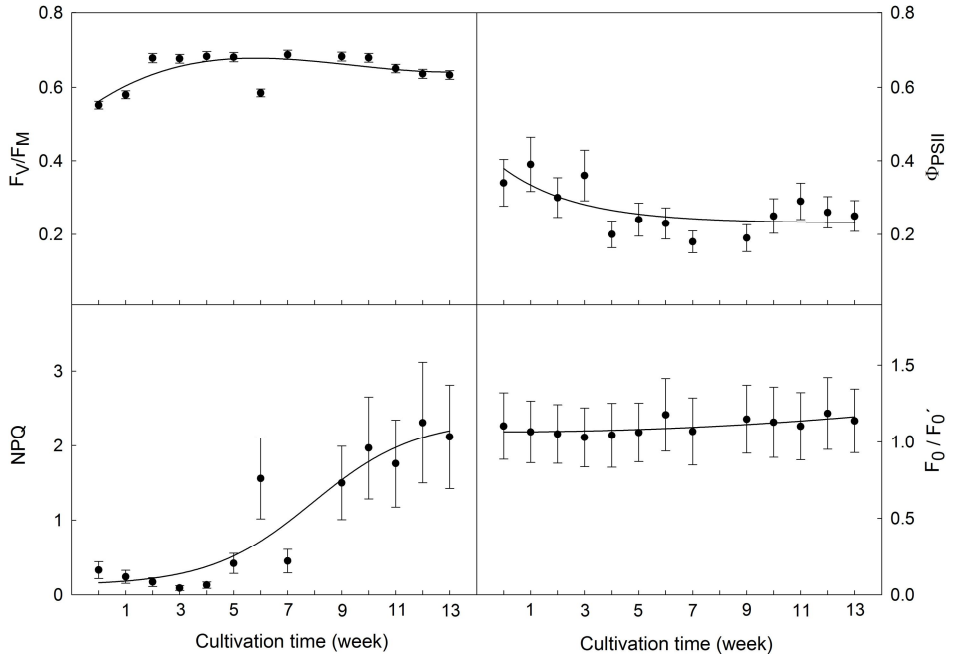

Fig. 5. Time courses of selected chlorophyll fluorescence parameters (F_V/F_M, Φ_{PSII}, NPQ, F_0 / F_0') measured in the photobiont isolated from a cryptoendolithic sample.

The NPQ values were similarly low at the begining of experiement (*i.e.* below 0.3 on Day 0) but increased with the time of cultivation in a species-specific manner. The PL and CE samples showed an exponential growth until the week 7-9 and then reached more or less constant NPQ values of about 2.0. For the SO samples, NPQ remained low (below 0.3) for a majority of cultivation time. Then (after week 9), NPQ increased rapidly reaching a peak value of 1.1 followed by a rapid decrase to about 55 % of maximum.

The F_0 / F_0' ratio showed the values higher than 1 indicating the activation of chlorophyll fluorescence quenching during exposition of the samples to light which resulted in $F_0 > F_0'$ (*see* also F_0, F_0' in Fig 2). The timecourses of the F_0 / F_0' ratio showed similar shape in all three samples: slight decrease at the begining of the monitoring period followed by a gradual increase with maximum value found at the end: 1.42 (PL), 1.10 (SO), and 1.19 (CE).

Discussion

Data presented in Fig. 2 show the changes in a shape of the slow Kautsky kinetics as well as an increase in absolute values of particular chlorophyll fluorescence signals (F_M, F_P, F_S). The latter characteristic, especially the steady state chlorophyll fluorescence (F_S), is associated with culture growth in the time of cultivation (Albrecht et al. 2022). In our study, F_S increased with time indicating the growth of cultures. It is consistent with previous studies since in the initial phases of algal cuture growth after innoculation, F_S is a proxy of biomass growth (*see* e.g. Orekhova at al. 2018 for *Heterococcus* sp. strain). In this concept, the early biomass growth on agar plates is linearly related to F_S.

In the three samples of Antarctic autotrophs used in our study, F_0 was always higher than F_0', which is associated with the phenomenon of quechning of background chlorophyll fluorescence (q_0, relative change of minimum chlorophyll fluorescence – Roháček 2002). In our study, F_0 quenching mechanism was light-dependent, because F_0' value increased with the following dark period (in an exponential manner) after the actinic light was switched off. Therefore, a presence of a strong quencher might be expected activated by actinic light and light-induced protective mechanisms (reviewed by Ruban (2016). The values of F_0/F_0' over 1 indicate a presence of a strong quencher that decreases value of F_0 / F_0'. The quencher is likely associated with the response of LHCII to actinic light which was stronger (60 μmol photons m^{-2} s^{-1} PAR) than that applied during the cultivation (15 μmol photons m^{-2} s^{-1} PAR). Therefore, the cultures of the PL, SO, and CE autotrophs were low light-acclimated during cultivation. When actinic light was applied during the chlorophyll fluorescence measurements, part of the LHCII antenna was uncoupled from core of RCs. This is typically associated with increased levels of F_0 quenching as reported by Ruban (2016), as well as an increase in overal non-photochemical quenching with the time of the sample exposition to actinic light.

The non-photochemical quenching (NPQ) increase with the cultivation time (Figs. 3-5) might be associated with the changes in the shape of the slow Kautsky kinetics that is modulated by the actual proportion of photochemical quenching (photosynthetic processes) and non-photochemical quenching components. They comprise several photochemical processes acting in thylakoid membrane: (1) energy-dependent quenching associated with the formation of transthylakoidal pH gradient and acti-

vation of xanthophyll cycle pigments conversion (*e.g.* Štroch et al. 2004, Thaipratum et al. 2009), (2) photoinhibition-induced changes in PSII functioning (*e.g.* Nikolaou et al. 2015) and (3) state transition (Kodru et al. 2015), *i.e.* a regulatory adjustment of the distribution between of the excitation energy PSI and PSII (Rochaix 2014, Bhatti et al. 2021). State transitions are light-acclimative processes that enlarge or diminish the size of light-harvesting complexes that feed excitation to the core complexes of PSII and PSI (for review *see* Allen and Mullineaux 2004). Appart of biophysical primary processes in thylakoid membrane, the rates of biochemical photosynthetic processes, such as CO_2 fixation, photorespiration, and chlororespiration may act as the factors affecting the shape of the slow Kautsky kinetics (reviewed by *e.g.* Stirbet et al. 2014).

Decline of PSII functioning with the time of cultivation of the samples on agar plots (*see* F_V/F_M in Figs. 3-5) was found consistent with the earlier study done in Antarctic species (Dufková et al. 2019). For Φ_{PSII}, only few comparative data are available since the chlorophyll fluorescence parameter is not generally monitored during the cultivation of microalgae on agar plots. Among the studies exploiting agar samples, Küpper et al. (2004) immobilized mobile flagellate cells by 1 – 3% agar or agarose during slow Kautsky kinetics measurements.

References

ALBRECHT, M., KHANIPOUR ROSHAN, S., FUCHS, L., KARSTEN, U. and SCHUMANN, R. (2022): Applicability and limitations of high-throughput algal growth rate measurements using *in vivo* fluorescence in microtiter plates. *Journal of Applied Phycology*, 34: 2037-2049. doi: 10.1007/s10811-022-02778-z

ALLEN, J. F., MULLINEAUX, C. W. (2004): Probing the mechanism of state transitions in oxygenic photosynthesis by chlorophyll fluorescence spectroscopy. Kinetics and imaging. *In*: G. C. Papageorgiou, Govindjee (eds): Chlorophyll fluorescence: A signature of photosynthesis. Advances in photosynthesis and respiration vol 19. Springer, Dordrecht, The Netherlands, pp. 447–461.

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(1): 19-27. doi: 10.5817/CPR2013-1-4

BENEDETTI, M., VECCHI, V., BARERA, S. and DALL'OSTO, L. (2018): Biomass from microalgae: The potential of domestication towards sustainable biofactories. *Microbial Cell Factories*, 17: 173. doi: 10.1186/s12934-018-1019-3

BHATTI, A. F., KIRILOVSKY, D., VAN AMERONGEN, H. and WIENTJES, E. (2021): State transitions and photosystems spatially resolved in individual cells of the cyanobacterium *Synechococcus elongatus*. *Plant Physiology*, 186: 569-580. doi: 0.1093/plphys/kiab063

CAZZANIGA, S., PEROZENI, F., BAIER, T. and BALLOTTARI, M. (2022): Engineering astaxanthin accumulation reduces photoinhibition and increases biomass productivity under high light in *Chlamydomonas reinhardtii*. *Biotechnology for Biofuels and Bioproducts*, 15(1): 77. doi: 10.1186/s13068-022-02173-3

DUFKOVÁ, K., BARTÁK, M., MORKUSOVÁ, J., ELSTER, J. and HÁJEK, J. (2019): Screening of growth phases of Antarctic algae and cyanobacteria cultivated on agar plates by chlorophyll fluorescence imaging. *Czech Polar Rep*orts, 9(2): 170-181. doi: 10.5817/CPR2019-2-15

HABERKORN, I., OFF, C. L., BESMER, M. D., BUCHMANN, L. and MATHYS, A. (2021): Automated online flow cytometry advances microalgal ecosystem management as *in situ*, high-temporal resolution monitoring tool. *Frontiers in Bioengineering and Biotechnology*, 9: 642671. doi: 10.3389/fbioe.2021.642671

HYKA, P., LICKOVA, S., PŘIBYL, P., MELZOCH, K. and KOVAR, K. (2013): Flow cytometry for the development of biotechnological processes with microalgae. *Biotechnology Advances*, 31(1): 2-16. doi: 10.1016/j.biotechadv.2012.04.007

KHAN, A., HABIB, M., HOSSAIN, M. and MIAH, M. (2018): Culture of *Chlorella vulgaris* in press mud media as sugar mill waste. *International Journal of Fisheries and Aquatic Research*, 3(2): 41-45.

KIM, E. J., KIM, S., CHOI, H. G. and HAN, S. J. (2020): Co-production of biodiesel and bioethanol using psychrophilic microalga *Chlamydomonas* sp. KNM0029C isolated from Arctic Sea ice. *Biotechnology for Biofuels*, 13: 20. doi: 10.1186/s13068-020-1660-z

KODRU, S., MALAVATH, T., DEVADASU, E., NELLAEPALLI, S., STIRBET, A., SUBRAMANYAM, R. and GOVINDJEE (2015): The slow S to M rise of chlorophyll *a* fluorescence reflects transition from state 2 to state 1 in the green alga *Chlamydomonas reinhardtii*. *Photosynthesis Research*, 125(1-2): 219-231. doi: 10.1007/s11120-015-0084-2

KÜPPER, H., ŠETLÍK, I. and HLÁSEK, M. (2004): A versatile chamber for simultaneous measurements of oxygen exchange and fluorescence in filamentous and thallous algae as well as higher plants. *Photosynthetica*, 42: 579-583.

MALAPASCUA, J. R. F., JEREZ, C. G., SERGEJEVOVÁ, M., FIGUEROA, F. L. and MASOJÍDEK, J. (2014): Photosynthesis monitoring to optimize growth of microalgal mass cultures: Application of chlorophyll fluorescence techniques. *Aquatic Biology*, 22: 123-140.

MAREČKOVÁ, M., BARTÁK, M. and HÁJEK, J. (2019): Temperature effects on photosynthetic performance of Antarctic lichen *Dermatocarpon polyphyllizum*: A chlorophyll fluorescence study. Polar *Biology*, 42: 685-701. doi: 10.1007/s00300-019-02464-w

MASOJÍDEK, J., ŠTĚRBOVÁ, K., REARTE, A., CELIS-PLÁ, P., TORZILLO, G., BENAVIDES, S., NEORI, A., ÁLVAREZ-GÓMEZ, F., LUKEŠ, M., CAPORGNO, M., ABDALA DÍAZ, R., MIAZEK, K., MASSOCATO, T., CARMO, J., ATZMUELLER, R., MAHROUQUI, H., SUÁREZ-ESTRELLA, F. and LOPEZ FIGUEROA, F. (2021): Changes in photosynthesis, growth and biomass composition in outdoor *Chlorella* g120 culture during the metabolic shift from heterotrophic to phototrophic cultivation regime. *Algal Research*, 56: 102303. doi: 10.1016/j.algal.2021.102303

NIELSEN, S. L., HANSEN, B. W (2019): Evaluation of the robustness of optical density as a tool for estimation of biomass in microalgal cultivation: The effects of growth conditions and physiological state. *Aquaculture Research*, 50(9): 2698-2706.

NIKOLAOU, A., BERNARDI, A., MENEGHESSO, A., BEZZO, F., MOROSINOTTO, T. and CHACHUAT, B. (2015): A model of chlorophyll fluorescence in microalgae integrating photoproduction, photoinhibition and photoregulation. *Journal of Biotechnology*, 194: 91-99. doi: 10.1016/j.jbiotec.2014.12.001

OREKHOVA, A., BARTÁK, M. and HÁJEK, J. (2018): Post rapid freezing growth of Antarctic strain of *Heterococcus* sp. monitored by cell viability and chlorophyll fluorescence. *Cryobiology*, 85: 39-46.

OZAKI, H., IKEUCHI, M., OGAWA, T., FUKUZAWA, H. and SONOIKE, K. (2007): Large-scale analysis of chlorophyll fluorescence kinetics in *Synechocystis* sp. PCC 6803: Identification of the factors involved in the modulation of photosystem stoichiometry. *Plant and Cell Physiology*, 48(3): 451-458.

OZAKI, H., SONOIKE, K. (2009): Quantitative analysis of the relationship between induction kinetics of chlorophyll fluorescence and function of genes in the cyanobacterium *Synechocystis* sp. PCC 6803. *Photosynthesis Research*, 101(1): 47-58.

ROCHAIX, J. D. (2014): Regulation and dynamics of the Light-Harvesting System. *Annual Review of Plant Biology*, 65: 287-309.

ROHÁČEK, K. (2002): Chlorophyll fluorescence parameters: The definitions, photosynthetic meaning, and mutual relationspips. *Photosynthetica*, 40: 13-29.

RUBAN, A.V. (2016): Non-photochemical chlorophyll fluorescence quenching: Mechanism and effectiveness in protecting plants from photodamage. *Plant Physiology*, 170: 1903-1916.

SATHASIVAM, R., RADHAKRISHNAN, R., HASHEM, A. and ABD ALLAH, E. F. (2019): Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences*, 26(4): 709-722. doi: 10.1016/j.sjbs.2017.11.003

SCHOETERS, F., SPIT, J., AZIZAH, R. N. and VAN MIERT, S. (2022): Pilot-scale cultivation of the snow alga *Chloromonas* typhlos in a photobioreactor. *Frontiers in Bioengineering and Biotechnology*, 10: 896261. doi: 10.3389/fbioe.2022.896261

STIRBET, A., GOVINDJEE (2016): The slow phase of chlorophyll *a* fluorescence induction in silico: Origin of the S–M fluorescence rise. *Photosynthesis Research*, 130: 193-213.

STIRBET, A., RIZNICHENKO, G. Y., RUBIN, A. B. and GOVINDJEE (2014): Modeling chlorophyll *a* fluorescence transient: Relation to photosynthesis. *Biochemistry*, 79: 291-323.

ŠNOKHOUS, K. (2021): Optimalization and design of cultivation system for microalgae production under polar climate conditions. Thesis, Czech Technical University. https://dspace.cvut.cz/handle/10467/96964

ŠTROCH, M., ŠPUNDA, V. and KURASOVÁ, I. (2004): Non-radiative dissipation of absorbed excitation energy within photosynthetic apparatus of higher plants. *Photosynthetica*, 42: 323-337. doi: 10.1023/B:PHOT.0000046149.97220.18

THAIPRATUM, R., MELIS, A., SVASTI, J. and YOKTHONGWATTANA, K. (2009): Analysis of non-photochemical energy dissipating processes in wild type *Dunaliella salina* (green algae) and in zea1, a mutant constitutively accumulating zeaxanthin. *Journal of Plant Research*, 122: 465-476.

VALE, M. A., FERREIRA, A., PIRES, J. C. M. and GONÇALVES A. L. (2020): Chapter 17 - CO_2 capture using microalgae. *Advances in Carbon Capture. Methods, Technologies and Applications*, 381-405. doi: 10.1016/b978-0-12-819657-1.00017-7

YEE, W., TANG, S. G. H., PHUA, P. S. P. and MEGAWARNAN, H. (2019): Long-term maintenance of 23 strains of freshwater microalgae on solid microbiological culture media: A preliminary study. *Algal Research*, 41: 101516. doi: 10.1016/j.algal.2019.101516

ZIGANSHINA, E. E., BULYNINA, S. S. and ZIGANSHIN, A. M. (2020): Comparison of the photoautotrophic growth regimens of *Chlorella sorokiniana* in a photobioreactor for enhanced biomass productivity. *Plants-Basel*, 9(7): 169. doi: 10.3390/biology9070169