Comparative analysis of heterogeneity of primary photosynthetic processes within fruticose lichen thalli: Preliminary study of interspecific differences

Short Communication

Stefano Conti[1], Jana Hazdrová[2], Josef Hájek[2], Petra Očenášová[2], Miloš Barták[2*], Kateřina Skácelová[2], Paola Adamo[1]

[1]*University of Naples Federico II, Department of Agricultural Sciences, Via Università 100, 80055 Portici (NA), Italy*
[2]*Department of Plant Physiology and Anatomy, Institute of Experimental Biology, Faculty of Science, Masaryk University, Kamenice 5, 625 00 Brno, Czech Republic*

Abstract
Two species of fruticose lichens from different habitats and of distinct color, *Usnea antarctica* and *Stereocaulon vesuvianum*, were compared using chlorophyll fluorescence imaging in order to study the distribution of primary photosynthetic processes within the thalli. The thallus of *U. antarctica* is yellow with black tips: in this species chlorophyll containing cells were mostly located in the middle region of the thallus and the highest PS II efficiency was detected in the middle to basal region, as shown by the F_V/F_M and Φ_{PSII} values. No chlorophyll fluorescence was detected in the apical part of the thallus, indicating that little or no photosynthesis takes place in these tissues. The lichen *S. vesuvianum* is homogeneously pale grayish green and chlorophyll containing cells are distributed along the thallus with maximum concentration in the middle region. In *S. vesuvianum*, the highest PS II efficiency was detected in the apical to middle region of the thallus, while the basal portion was found to have the lowest efficiency of primary photochemical reactions. Quenching analysis data confirmed the uneven patterns of primary photosynthetic processes within the thalli of these fruticose lichens.

Key words: chlorophyll fluorescence, Kautsky kinetics, quenching analysis, *Usnea antarctica*, *Stereocaulon vesuvianum*

DOI: 10.5817/CPR2014-2-15

Received December 1, 2014, *accepted* December 31, 2014.
[*]Corresponding author: Miloš Barták <*mbartak@sci.muni.cz*>
Acknowledgements: The authors thank the CzechPolar project for research infrastructure and the PASSEB project for funding traveling to co-ordination meetings of team members.

Introduction

In experimental plant biology, the technique of chlorophyll fluorescence imaging is used for the evaluation of plant primary photosynthetic processes and it allows the researcher to study their distribution within the photosynthesizing organs. This technique has a wide range of applications (Govindjee et Nedbal 2000) and it has been used to study the peculiarities of anatomical structures in lichens, such as *e.g.* fruiting bodies (Jensen et al. 1999), or the sensitivity of different parts of the thallus to photoinhibition (*e.g.* Singh et al. 2013), as well as the effects of low or freezing temperature on photosynthesis (Barták et al. 2007). Visualization of residual photosynthetic activity in dehydrated lichen thalli is another typical application (see *e.g.* Barták et al. 2000, 2005). It is well established that the intrathalline heterogeneity of primary photosynthetic processes, as defined by chlorophyll fluorescence parameters, is dependent on the particular growth pattern of each species, as well as on the age, physiological activity and distribution of symbiotic algae within the thallus. Several studies have been published on the heterogeneity of chlorophyll fluorescence in foliose lichens (*e.g.* Barták et al. 2000), whose planar thalli are relatively easy to handle but little research has investigated the spatial heterogeneity within the thallus of fruticose lichens. A recent study (Balarinová et al. 2014) reported interspecific differences in distribution of photosynthetic activity within the thalli of two Antarctic species of *Usnea*, as well as different sensitivities to photoinhibition. In this study, primary photosynthetic processes were found to be more efficient in the basal and middle part of branching thalli (yellow colored) while very low values were detected in the apical (dark colored) parts. Interestingly, unlike most of the other fruticose lichens, *Usnea* sp. lichens are characterized by contrastingly colored apical and basal parts of the thallus. Therefore, it is also possible that different patterns of primary photosynthetic processes may be observed when comparing *Usnea* to other fruticose lichen species. In order to test this hypothesis, comparative measurements of chlorophyll fluorescence parameters were done in this study. Two fruticose lichen species were selected for such a purpose: *Usnea antarctica* and *Stereocaulon vesuvianum*. These two species differ in thallus color: while the thallus of *U. antarctica* is yellow with black tips, *S. vesuvianum* is uniformly pale grayish green. The different colors of these two species are attributed to secondary metabolites located mainly in the upper cortex of the lichen: usnic acid in the case of *U. antarctica* or atranorin, norstictic acid, stictic acid in *S. Vesuvianum* (*e.g.* Walker et Lintott 1997). In the latter species several secondary metabolites were found (*e.g.* Caccamese et al. 1986), some of them with antimicrobial activity (Ingólfsdóttir et al. 1985, Kim et al. 2013).

Usnea antarctica is widely distributed over coastal ecosystems in maritime Antarctica (see *e.g.* Lamb 1964, Osyczka et Olech 2005) and several photosynthetic studies based on chlorophyll fluorescence parameters in this species have already been published. Potential quantum yield (F_V/F_M) and effective quantum yield (Φ_{PSII}) of photosystem II photochemistry, are sensitive indicators of efficiency of the photosynthetic apparatus. As such, these parameters are readily affected as a result of excess (Barták et al. 2004, Barták et al. 2012) or medium physiological light doses (Balarinová et al. 2014) or by freezing temperatures (Barták 2014).

Contrastingly, only a few reports on the physiology of *Stereocaulon vesuvianum* have been published to date. These studies have been devoted to the photobiont classification using molecular biology approaches (Anuforo 2012), to the chemical weathering of mineral substrates due to the

excretion of lichen metabolites (Adamo et al. 1997, Adamo et Violante 2000, Vingiani et al. 2013) or to radionuclide accumulation in the thallus (Adamo et al. 2004).

Some particular ecophysiological aspects of species of the *Stereocaulon* genus have been studied such as *e.g.* tolerance to long-term submersion in melt water ponds (Sadowsky et al. 2012). Peksa et Škaloud (2011) reported a specific association with algae of the genus *Asterochloris* (reclassified *Trebouxia)* as the primary photobiont, while a secondary stigonemoid photobiont was reported in cephalodia by Boekhout (1982).

Material and Methods

Lichen species

For a comparative study of the intrathalline distribution of primary photosynthetic processes, two lichens species with fruticose morphology of the thallus were chosen. *Usnea antarctica* is a typical representative of fruticose lichens in coastal regions of Antarctica with characteristic black hairy tips of individual thalli. This lichen grows on the upper parts of stones and rocky outgrowths forming a shrub-like structure. *Stereocaulon vesuvianum* has a widespread distribution at all latitudes on Earth. The thallus is pale grey with tufts of ascending to erect branches (pseudopodetia) and numerous globose clusters of fruiting bodies (soredia) are formed along the axis and at the tips of the pseudopodetia.

Collection of samples

In February 2013, lichen thalli of *Usnea antarctica* were collected at the James Ross Island (Antarctica) at several sites in Halozetes Valley (63°48′57″ S, 57°50′30″ W, 260 m a.s.l.). The collection sites were distributed within a 300 × 100 m area located at hyaloclastite breccia boulders forming an upper layer of ice-cored moraine. Thalli of *Stereocaulon vesuvianum* (Pers) were collected in November 2014 from a species-rich site on lavas of Mount Vesuvio, 12 km south-east of Naples (southern Italy). The samples reported in this study were collected from a monospecific lichen cover growing on recent tefritic leucitic lava flows (1944 eruption) in the Valley of the Giant (40°49'59" N 14°25'45" E, 885 m a.s.l.). After collection, lichen thalli were air dried under local climatic conditions, they were subsequently transferred to the laboratory and stored at 5°C in the dark.

Chlorophyll fluorescence imaging

Prior to the chlorophyll fluorescence measurements, dry thalli were allowed to rehydrate for 48 h over wet filter paper in Petri dishes under dim light at 5ºC. After rehydration and full activation of primary photochemical processes (maximum F_V/F_M – data not shown), the lichen samples were subjected to the measurements of chlorophyll fluorescence imaging so that heterogeneity of chlorophyll fluorescence parameters could be determined. Lichen thalli were pre-darkened for 10 min in the measuring compartment of a FluorCam HFC-010 fluorometer (Photon Systems Instruments, Czech Republic). Then Kautsky kinetics supplemented with quenching analysis were measured (Fig. 1). The method is described elsewhere (*e.g.* Barták et al. 2004) and consists of measurements of chlorophyll fluorescence curves induced by an actinic light supplemented with saturation pulses during the period when the light is on (F_M') or off (F_M''), respectively. Using a FluorCam 7 software, effective quantum yield (Φ_{PSII}) and non-photochemical

quenching (NPQ) were calculated and visualized. Subareas of high and low photosynthetic activity were then identified within the thallus projection area and their particular shares were calculated.

Statistical analysis

For each species, at least 16 replicate measurements of chlorophyll fluorescence parameters were recorded and means and standard deviations were calculated. The Student's t-test was used to determine the significance of the differences between F_V/F_M or Φ_{PSII} values in the apical and basal regions of the thalli.

Results and Discussion

The Kautsky kinetics differed between the two lichen species, both in the absolute values of chlorophyll fluorescence and in the curve shapes (Fig. 1). The higher values observed in *S. vesuvianum* may be due to its higher chlorophyll content and/or to lower content of light absorbing compounds in the upper cortex of the thallus. Unlike *U. antarctica*, in *S. vesuvianum* an apparent secondary peak of chlorophyll fluorescence, higher than F_P, was recorded during actinic light period (Fig. 1, 70 s). In *S. vesuvianum* this phenomenon could be attributed either to less effective reoxidation of plastochinone pool or to contribution of F_0 (during actinic light) to the overall chlorophyll fluorescence signal (Roháček et Barták 1999) in *S. vesuvianum*. Further study will, therefore, be focused on F_0 changes in this species during actinic light period.

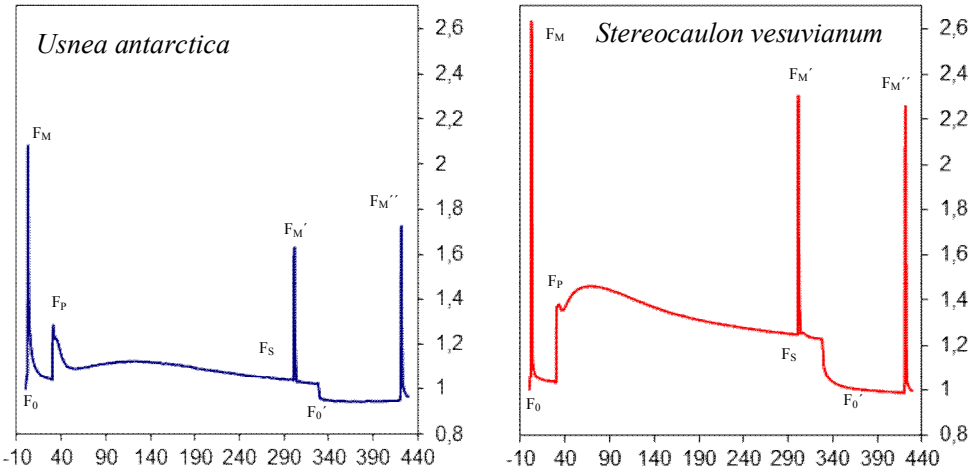

Fig. 1. Slow Kautsky kinetics of chlorophyll fluorescence supplemented with maximum values reached after saturation pulses. The curves are means of 12 replicates, normalized to background chlorophyll fluorescence (F_0). The following chlorophyll fluorescence parameters are indicated: F_M – maximum fluorescence on dark adapted thalli, F_M' – maximum fluorescence on light-adapted thalli, F_M'' – maximum fluorescence measured in dark when actinic light is off, F_0, F_0' – background chlorophyll fluorescence, F_P – peak fluorescence during actinic light period, F_S – steady state fluorescence.

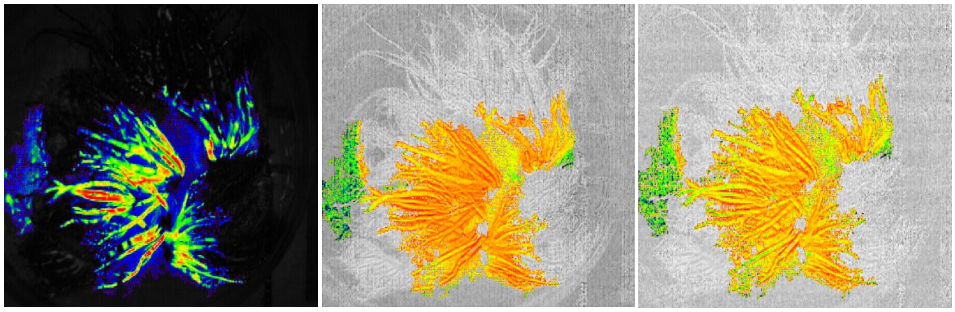

Fig. 2. Intrathalline distribution of chlorophyll fluorescence parameters in *Usnea antarctica*. Basic chlorophyll fluorescence (F_0, left panel) showed that chlorophyll containing cells were mostly located in the middle part of the thalli. Potential (F_V/F_M, central panel) and effective (Φ_{PSII}, right panel) efficiency of PS II were found to have the same values in the middle and in the basal parts of thalli. No chlorophyll fluorescence was detected in the apical parts of thalli, thus resulting in the absence of imaging false colors.

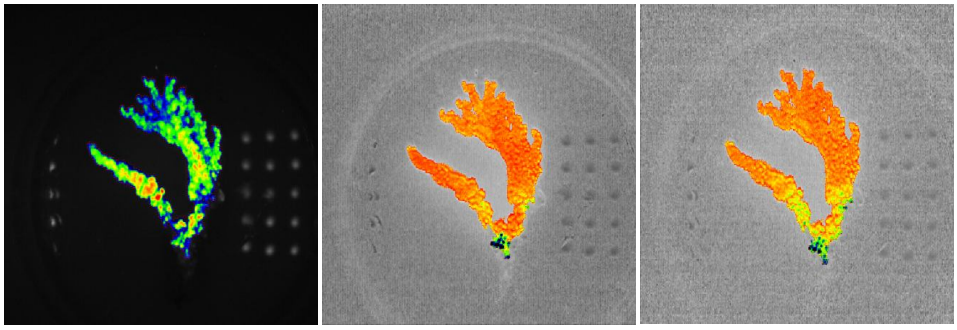

Fig. 3. Intrathalline distribution of chlorophyll fluorescence parameters in *Stereocaulon vesuvianum*. Basic chlorophyll fluorescence (F_0, left panel) indicates that the highest concentration of chlorophyll containing cells was observed in the middle part of the thalli, while a lower signal could be measured in the apical and basal portions. Potential (F_V/F_M, central panel) and effective (Φ_{PSII}), right panel) yield of PS II were high in the apical and middle portions of thalli and they were found to decrease in the basal parts.

Chlorophyll fluorescence imaging showed species-specific intrathalline distribution of primary photochemical processes. In *U. antarctica* (*see* Fig. 2), the middle and basal parts of the thallus were found to be the most photosynthetically effective (Φ_{PSII} within a range of 0.405-0.375). This result was in accordance with earlier studies (Očenášová et al. 2014, Balarinová et al. 2014), which also found the highest photosynthetic activity to be located within the basal portion of the thallus. As expected, little or no fluorescence could be detected in the dark colored apical portion of thalli. This could be due to the abundance of pigments which interfered with the measurements and/or to the small number of the symbiotic algal cells in these tissues. The basal part of the thallus exhibited a lower F_V/F_M (0.375) value compared with the

middle part (0.522) (Fig. 4). Fluorescence signals were found to be stronger in the middle and basal region of the thallus, resulting in higher F_V/F_M and Φ_{PSII} values. This was possibly due to the abundance of algal cells in the subcortical photobiont layer, whose thickness was reported to be in the order of as much as 10^1 to 10^2 micrometers (Gielwanowska et Olech 2012, Barták 2014).

In *S. vesuvianum* (Fig. 3), the apical to middle regions of the thallus were found to have the highest maximum theoretical efficiency of PS II, with a measured F_V/F_M value of 0.616 (Fig. 4). The maximum efficiency was recorded in the basal region of the thallus, with a mean F_V/F_M value of 0.500. The overall efficiency of PS II followed the same pattern, with a measured Φ_{PSII} value of 0.498 in the apical thalli regions and a lower efficiency in the basal parts of the thalli, with a mean Φ_{PSII} value of 0.344. These data closely matched the chlorophyll content imaging results (Fig. 3) if the intrathalline distribution of basal chlorophyll fluorescence (F_0) and steady-state chlorophyll fluorescence were considered (data not shown here). Thus, most of the chlorophyll content could be expected to be found in the middle region of *S. vesuvianum* thalli. Since information on *S. vesuvianum* cross sections are missing in literature, further research on thallus anatomy and on the intrathalline distribution of symbiotic algae is needed, in order to interpret the chlorophyll fluorescence data in the light of the anatomical structure of this lichen. On the basis of these data, it could also be possible to understand why lower values of F_V/F_M and Φ_{PSII} were recorded in the basal part compared to the middle and apical region of the thallus.

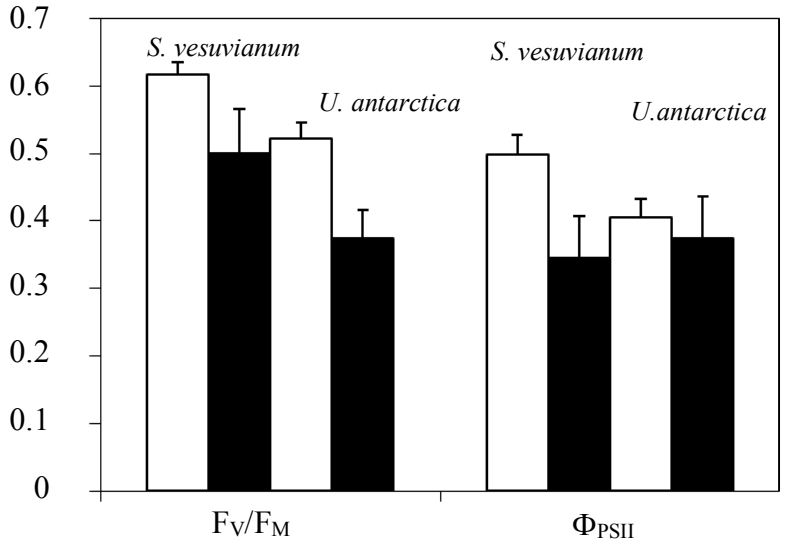

Fig. 4. Potential (F_V/F_M) and effective (Φ_{PSII}) quantum yield of photochemical processes in the apical (white bars) and basal regions (black bars) of *Stereocaulon vesuvianum* thalli. In the case of *Usnea antarctica*, data refer to middle (white bars) and basal region of thalli (black bars), instead. Means were calculated from at least 16 replicate measurements, error bars represent standard deviations.

Results of quenching analysis are summarized in Table 1. In *U. antarctica*, photochemical quenching (qP) and photochemical quenching recorded in the dark (qP-d) did not differ between middle and basal portions of the thalli, indicating that primary photochemical processes were similar between these tissues. Such conclusion might be supported by the Φ_{PSII} values that did not differ either (Fig. 4). Both non-photochemical quenching (NPQ) and non-photochemical quenching recorded in dark (NPQ-d) when actinic light was switched off, were higher in the basal than in the middle region of the thalli, although these differences did not appear to be statistically significant due to high data variability.

In *S. vesuvianum* NPQ did not differ significantly between the apical and the basal region of the thallus. These data suggest that an effective quenching system is present in both regions of the thallus, which may protect the photosynthetic apparatus from adverse environmental condition, as previously reported *e.g.* by Nayaka et Saxena (2014) in the case of light stress, or by Singh et al. (2013) in the case of water stress. Photochemical quenching (qP), however, was higher in the apical region, indicating that photosynthetic performance in these tissue may be higher than at the base the thalli, confirming the F_V/F_M and Φ_{PSII} data. The NPQ-d parameter was found to be significantly higher in the basal compared with the apical part of the thalli. Similarly to qP, the qP-d parameter was found to be significantly higher in the apical part of *S. vesuvianum* thalli.

		NPQ	qP	NPQ-d	qP-d
U. antarctica	middle	**0.228**±0.060	**0.856**±0.058	**0.257**±0.061	**0.971**±0.040
	basal	**0.452**±0.203	**0.825**±0.049	**0.432**±0.171	**0.952**±0.038
S. vesuvianum	apical	**0.150**±0.044	**0.855**±0.069	**0.226**±0.064	**0.498**±0.029
	basal	**0.132**±0.042	**0.723**±0.061	**0.500**±0.065	**0.344**±0.063

Table 1. Quenching parameters derived from chlorophyll fluorescence analysis for *Stereocaulon vesuvianum* and *Usnea antarctica*. The differences between apical/middle and basal parts of the thalli. Means and standard deviations were calculated from at least 16 replicates.

Concluding remarks

In this preliminary study, chlorophyll fluorescence imaging and quenching analysis data confirmed that the efficiency of primary photosynthetic processes was unevenly distributed within the thallus of each lichen species. Moreover, this study provided evidence that the patterns of distributions of primary photosynthetic processes within the thallus may differ among species. Future studies will focus anatomy of these lichen species in order to investigate whether the chlorophyll fluorescence parameters are related to frequency, distribution and physiological activity of symbiotic algae.

References

ADAMO, P., COLOMBO, C. and VIOLANTE, P. (1997): Iron oxides and hydroxides in the weathering interface between *Stereocaulon vesuvianum* and volcanic rock. *Clay Minerals*, 32: 453-461.

ADAMO, P., ARIENZO, M., PUGLIESE, M., ROCA, V. and VIOLANTE, P. (2004): Accumulation history of radionuclides in the lichen *Stereocaulon vesuvianum* from Mt. Vesuvius (south Italy). *Environmental Pollution*, 127: 455-461.

ADAMO, P., VIOLANTE, P. (2000): Weathering of rocks and neogenesis of minerals associated with lichen activity. *Applied Clay Science*, 16: 229-256.

ANUFORO, Ó. U. U. (2012): Cyanobacteria in lichens and mosses. PhD thesis, University of Iceland, Háskóli Íslands, Reykyavik, 75 p.

BALARINOVÁ, K., BARTÁK, M., HAZDROVÁ, J., HÁJEK, J. and JÍLKOVÁ J. (2014): Changes in photosynthesis, pigment composition and glutathione contents in two Antarctic lichens during a light stress and recovery. *Photosynthetica*, 52: 538-547.

BARTÁK, M. (2014): Lichen Photosynthesis. Scaling from the Cellular to the Organism Level. *In*: M. F. Hohmann-Marriott (ed.): The Structural Basis of Biological Energy Generation. Series: Advances in Photosynthesis and Respiration. Dordrecht, Springer, pp. 379-400.

BARTÁK, M., GLOSER, J. and HÁJEK, J. (2005): Visualized photosynthetic characteristics of the lichen *Xanthoria elegans* related to daily courses of light, temperature and hydration: a field study from Galindez Island, maritime Antarctica. *Lichenologist*, 37:433–443.

BARTÁK, M., HÁJEK, J. and OČENÁŠOVÁ, P. (2012): Photoinhibition of photosynthesis in Antarctic lichen *Usnea antarctica*. I. Light intensity- and light duration-dependent changes in functioning of photosystem II. *Czech Polar Reports*, 2: 42-51.

BARTÁK, M., HÁJEK, J. and GLOSER, J. (2000): Heterogeneity of chlorophyll fluorescence over thalli of several foliose macrolichens exposed to adverse environmental factors: Interspecific differences as related to thallus hydration and high irradiance. *Photosynthetica*, 38: 531-537.

BARTÁK, M., HÁJEK, J., VRÁBLÍKOVÁ, H. and DUBOVÁ, J. (2004): High-light stress and photoprotection in *Umbilicaria antarctica* monitored by chlorophyll fluorescence Imaging and changes in zeaxanthin and glutathione. *Plant Biology*, 6: 333-341.

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.

BOEKHOUT, T. (1982): Studies on Colombian cryptogams XVIII. The genus *Stereocaulon* (Schreber) Hoffmann (Lichenes). *Journal of the Hattori Botanical Laboratory*, 53: 483-511.

CACCAMESE, S., COMPAGNINI, A., TOSCANO, R. M. and CASCIO, O. (1986): Methyl β-Orcinolcarboxylate and Atranol from the Lichen *Stereocaulon vesuvianum*. *Journal of Natural Products*, 49:1159-1160.

GIELWANOWSKA, I., OLECH, M. (2012): New ultrastructural and physiological features of the thallus in Antarctic Lichens. *Acta Biologica Cracoviensia Series Botanica*, 54: 40–52.

GOVINDJEE, NEDBAL, L. (2000): Seeing is believing. *Photosynthetica*, 38: 481-482.

INGÓLFSDÓTTIR, K., BLOOMFIELD, S. F. and HYLANDS, P. J. (1985): *In vitro* evaluation of the antimicrobial activity of lichen metabolites as potential preservatives. *Antimicrobial Agents and Chemotherapy*, 28: 289-292.

JENSEN, M., CHAKIR, S. and FEIGE , G. B. (1999): Osmotic and atmospheric dehydration effects in the lichens *Hypogymnia physodes*, *Lobaria pulmonaria* and *Peltigera aphtosa*: an *in vivo* study of chlorophyll fluorescence induction. *Photosynthetica*, 37: 393-404.

KIM, M.-K., PARK, M. and OH, T.-J. (2013): Antimicrobial properties of the bacterial associates of the Arctic lichen *Stereocaulon* sp. *African Journal of Microbiology Research*, 7: 3651-3657.

LAMB, I. M. (1964): Antarctic Lichens. I. The genera *Usnea, Ramalina, Himantormia, Alectoria, Cornicularia*. *British Antarctic Survey Scientific Reports*, No. 38. 34 p.

NAYAKA, S., SAXENA, P. (2014): Physiological responses and ecological success of lichen *Stereocaulon foliolosum* and moss *Racomytrium subsecundum* growing in same habitat in Himalaya. *Indian Journal of Fundamental and Applied Life Sciences*, 4: 167-179.

OČENÁŠOVÁ, P., BARTÁK, M. and HÁJEK, J. (2014): Photoinhibition of photosynthesis in Antarctic lichen *Usnea antarctica*. II. Analysis of non-photochemical quenching mechanisms activated by low to medium light doses. *Czech Polar Reports*, 4: 90-99.

OSYCZKA, P., OLECH, M. (2005): The lichen genus *Cladonia* of King George Island, South Shetland Islands, Antarctica. *Polish Polar Research*, 26: 107-123.

PEKSA, M., ŠKALOUD, P. (2011) : Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga *Asterochloris* (Trebouxiophyceae). *Molecular Ecology*, 20: 3936-3948.

ROHÁČEK, K., BARTÁK, M. (1999): Technique of the modulated chlorophyll fluorescence: basic concepts, useful parameters, and some applications. *Photosynthetica*, 37: 339-363.

SADOWSKY, A., HUSSNER, A. and OTT, S. (2012): Submersion tolerance in a habitat of *Stereocaulon paschale* (Stereocaulaceae) and *Cladonia stellaris* (Cladoniaceae) from the high mountain region Rondane, Norway. *Nova Hedwigia*, 94: 323–334.

SINGH, R., RANJAN, S., NAYAKA, S., PATHRE, U. V. and SHIRKE, P.A.(2013): Functional characteristics of a fruticose type of lichen, *Stereocaulon foliolosum* Nyl. in response to light and water stress. *Acta Physiologiae Plantarum*, 35: 1605-1615.

VINGIANI, S., TERRIBILE, F. and ADAMO, P. (2013): Weathering and particle entrapment at the rock-lichen interface in Italian volcanic environments. *Geoderma,* 207–208: 244-255.

WALKER, J.R.L., LINTOTT, E.A. (1997): A phytochemical register of New Zealand lichens. *New Zealand Journal of Botany*, 35: 369-384.