Changes of chloroplast structure in lichenized algae

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Introduction

Lichens are symbiotic organisms composed of a fungal partner (mycobiont) and photosynthetic partner represented by green alga or cyanobacteria (photobiont). Both symbionts undergo a variety of structural, physiological and biochemical modifications as a result of lichenization (Galun, 1988). Photobionts are more or less changed by the influence of the fungal partner. The most frequent changes are reduction of cell size, changes of reproductive strategies and various modifications of some organelles and structures on subcellular level (chloroplast, thylakoids, pyrenoid, pyrenoglobuli, dictyosomes, etc.) (e.g. Bubrick, 1988; Ahmadjian, 1992; Tschermak-Woess, 1995). However, the changes of the algal cells are not permanent and when the cells are freed from the fungal hyphae, they resume after several divisions their original size and characteristics (Ahmadjian, 1992).
The most frequently occuring phycobiont in lichens is *Trebouxia* (*Asterochloris* resp.). A basic character in systematic of trebouxioid algae represents chloroplast morphology. However, in different ontogenetic, physiological and ecological stages, chloroplast may markedly vary.

Objectives

- Isolation and identification of photobionts from four lichens with different type of thalli and systematic position (collected on a single locality)
- **Detailed investigation of chloroplast morphology in different ecological and physiological conditions, made by laser scanning confocal microscopy.**

Results

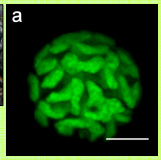
Identity of photobionts:

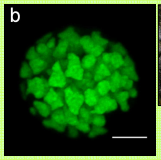
- Only two species of symbiotic algae were distinguished in four lichens based on morphology and ITS sequences: ***Asterochloris* sp.** (*C. foliacea*, *Lepraria* sp.) and ***Trebouxia incrustata* Ahmadjian ex Gärtner** (*L. fuscoatra*, *X. conspersa*).

Chloroplast structure:

- Confocal microscopy revealed obvious differences between chloroplast structure of algae within **dessicated and hydrated thalli** and between **lichenized and free-living** (cultured) form of photobionts.
- During the **liberation of photobiont** from the lichen thallus, chloroplast changes significantly in its shape and structure. The intensity of these changes was more expressive in *Asterochloris* sp. compared with *Trebouxia incrustata*.
- The pairs of genetically identical strains show certain differences in the morphology and ontogeny of chloroplasts (Fig. 1: compare a with b, c with d).

Cladonia foliacea (thallus heteromerous dimorphic; Cladoniaceae)





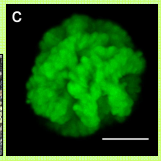
***Lepraria* sp.** (homoiomerous leprose; Stereocaulaceae)

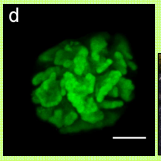
Asterochloris sp.

One locality - four lichens – two photobionts

Lecidea fuscoatra (heteromerous crustose; Lecideaceae)

Trebouxia incrustata

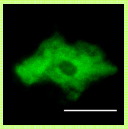




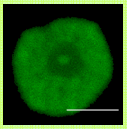
Xanthoparmelia conspersa (heteromerous foliose; Parmeliaceae)

Effect of hydration to chloroplast structure

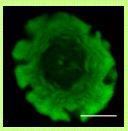
In a **dessicated lichen thallus** (herbarium specimen), chloroplast is strongly deformed (often „star-like" shaped), distinctly compressed within dehydrated protoplast.



Immediately after hydration of dry thallus, chloroplast is changing very fast to globular compact form without lobes, pyrenoid is well observable including penetrating thylakoids.

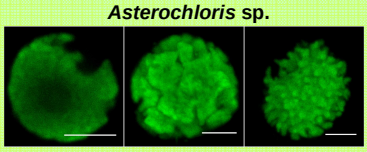


Within a **wet (living) lichen thallus**, chloroplast occurs in several morfological stages with different degree of lobation. Pyrenoid is distinctly penetrated by thylakoids.



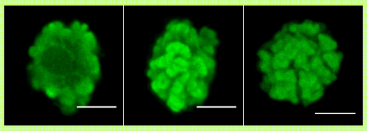
(all pictures: *Asterochloris* sp. from *C. foliacea*).

***Asterochloris* sp.**

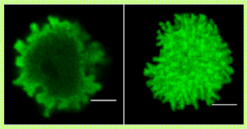


Changes of chloroplast structure during the liberation of photobiont from the lichen thallus

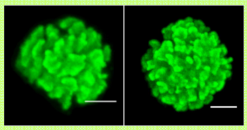
Trebouxia incrustata

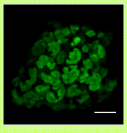


Fresh wet lichen thali
Active photobiont cells contain compact to simple lobed chloroplast. Pyrenoid is penetrated by one or more thylakoids and surrounded by starch sheath.

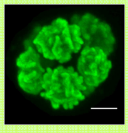


7th–11th day after inoculation of thallus fragments to agar slants
Strongly lobate chloroplast is formed. Algae adapted themselfs to new light and hygric conditions (= **lag phase**) and decreasing influence of mycobiont, which withers away due to unsuitable conditions (continual moisture within Petri dish). Chloroplast contains a large volume of starch (dark area around the black pyrenoid on the left picture).

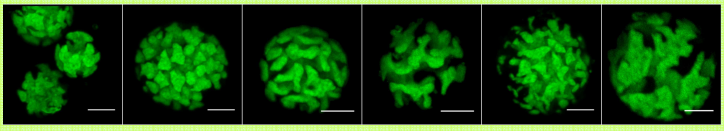


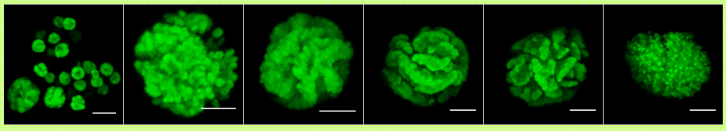


20th day
The phase of intesive division, characterized by the dominance of autospore (*Trebouxia*) or aplanospore (*Asterochloris*) packages.



30th and 40th day
Mature cells of the new generation are fully liberated from mycobiont influence. Several ontogenetic stages with different morphology of chloroplast occur in the cultures, reproduction mode is changed compared with lichenized generation (*Asterochloris* sp. forms aplanospores and zoospores, *T. incrustata* autospores, aplanospores and zoospores).





Conclusions

Chloroplast morphology is dependent mainly on physiological status (dryness/dampness), degree of lichenization (lichenized/free-living) and the species level (*Asterochloris* sp./*Trebouxia incrustata*). Ontogenetical changes are obvious especially in cultured (free-living) form of photobiont.

Very closely related algae (on species as well as population level) may show number of individual differences in their behaviour. We observed distinct differences in the chloroplast structure and reproduction mode between closely related species of *Asterochloris* and *Trebouxia*. Moreover, even genetically identical strains isolated from different lichen species exhibited different morphology and ontogenetical development of chloroplast.

Confocal microscopy constitutes a convenient technique to demonstrate details in chloroplast structure of green algae.

Acknowledgments
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Methods

Isolation and cultivation of the photobionts: Photobionts were isolated by the thallus fragment method (Ahmadjian, 1993). All lichens were collected from the single rocky steppe slope, at the „Máslovická stráň" protected area in Central Bohemia, Czech Republic. Algae were cultivated on agar slants of Bold´s basal medium modified by adding more nitrogen (BBM 3N) at 18 °C, under an illumination of 20-30 mmol m^{-2} s^{-1} and 16:8 h light:dark cycle.
Microscopical observation: The pure algal samples and fragments of lichen thalli were investigated by laser scanning confocal microscope Leica TCS SP2 equipped with an Argon-Krypton laser using the 488 nm excitation line. Photobionts were observed within a dessicated fragments of the lichen thalli and subsequently immediatelly after hydration of the fragments, further within a hydrated thalli (fresh thalli incubated 3 days in the same conditions as the isolates and cultures), during the process of their liberation (isolation) from lichen thalli (after 3, 7, 11, 20, 30 and 40 days after putting of thallus fragments to agar plates) and in the final cultures on BBM 3N and Trebouxia medium.
Molecular methodology: ITS rDNA sequences were obtained from four samples. Homology between sequences and those currently available in the NCBI database was assessed using BLAST (National Center for Biotechnology Information, NIH, http://www.ncbi.nlm.nih.gov/BLAST) score system.

References
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Scale bar in all pictures indicate 5 µm.