Confocal Laser Scanning Microscopy to study the bacterial communities associated with different species of lichens

M. Cardinale[1], J. Steinova[2], J. Rabensteiner[3], M. Grube[3], and G. Berg[1]

1. Institute for Environmental Biotechnology, Graz University of Technology, Petersgasse 12, Graz, 8010 (Austria).
2. Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic.
3. Institute for Plant Sciences, Karl-Franzens University, Holteigasse 6, Graz, 8010, Austria

m.cardinale@tugraz.at

Keywords: Confocal Laser Scanning Microscopy, Bacterial communities, Lichens, 3D-rendering, Image analysis

Lichens are organisms formed by the synergistic cooperation of a fungus and a green alga or cyanobacteria. Since they are slow growing but extremely long living, they indeed represent a stable niche for further environmental microorganisms. While "lichenicolous fungi" were studied largely, the bacteria associated with lichen received only a few attention [1]. Here we describe our results obtained by both taxonomic-specific and non-specific fluorescent staining coupled with Confocal Laser Scanning Microscopy (CLSM). Lichen samples of different species, representing the major lichen grow-types, were collected in the mountain regions near Graz (Austria) and fixed with ethanol or paraformahldeyd the same day of collection. Cryosections of 15 to 30 µm were stained by incubation with acridine orange or by fluorescence *in situ* hybridization (FISH) with both bacterial universal probes and specific probes targeting major bacterial phyla. Confocal stacks composed of 10 to 80 optical slices (Z-step= 1µm) were taken with a Leica TCS SP1 or a Leica TSC SPE (Leica Microsystems, Germany)

The results showed that a very dense bacterial community is living in association with all the analyzed lichens. Based on cell counting of acridine orange-stained bacteria, we calculated a density between 4.5×10^5 and 1.7×10^6 bacteria per mm^3 of lichen volume (depending on the lichen species). The FISH analysis showed that the bacterial communities were dominated by uncultivable *Alphaproteobacteria* (30-82% of the total FISH-stained bacteria, depending on the lichen species). Other groups are less abundant (Fig. 1). Further analysis based on different methods (for example 16S rRNA gene clone library) will be necessary to identify the fraction of the bacterial communities not addressed by FISH-CLSM.

The colonization pattern was studied by 3D-rendering of the confocal stacks. The software Imaris 6.0 (Bitplane, Switzerland) was used to visualize volume rendering or to transform the fluorescent objects in isosurfaces and dots. The bacteria showed different colonization patterns, both species-specific and tissue-specific (Fig. 2). The lichen *Cladonia arbuscula* harbors biofilm like structures on the internal surface of its podetia [2], while leaf-like lichen, as *Lobaria* and *Umbilicaria* spp. show major bacterial colonization on the external surfaces [3]. Bacteria belonging to different taxonomic groups grow intermingled.

The roles of the bacteria in the lichen still remain to be understood. Nevertheless, the specificity, the abundance and the difficulty to cultivate the bacteria belonging to the major group (*Alphaproteobacteria*) suggest an active involvement in the lichen symbiosis. Some observation and previous works suggest a possible involvement in the N_2 fixation [4].

1.   M. Cardinale et al., FEMS Microbiol. Ecol. 57 (2006) p484.
2.   M. Cardinale et al., FEMS Microbiol. Ecol. 66 (2008) p63.
3.   M. rube et al., ISME J. (2009). *In revision*.
4.   C.M. Liba et al., J. Appl. Microbiol. 101 (2006) p1076.
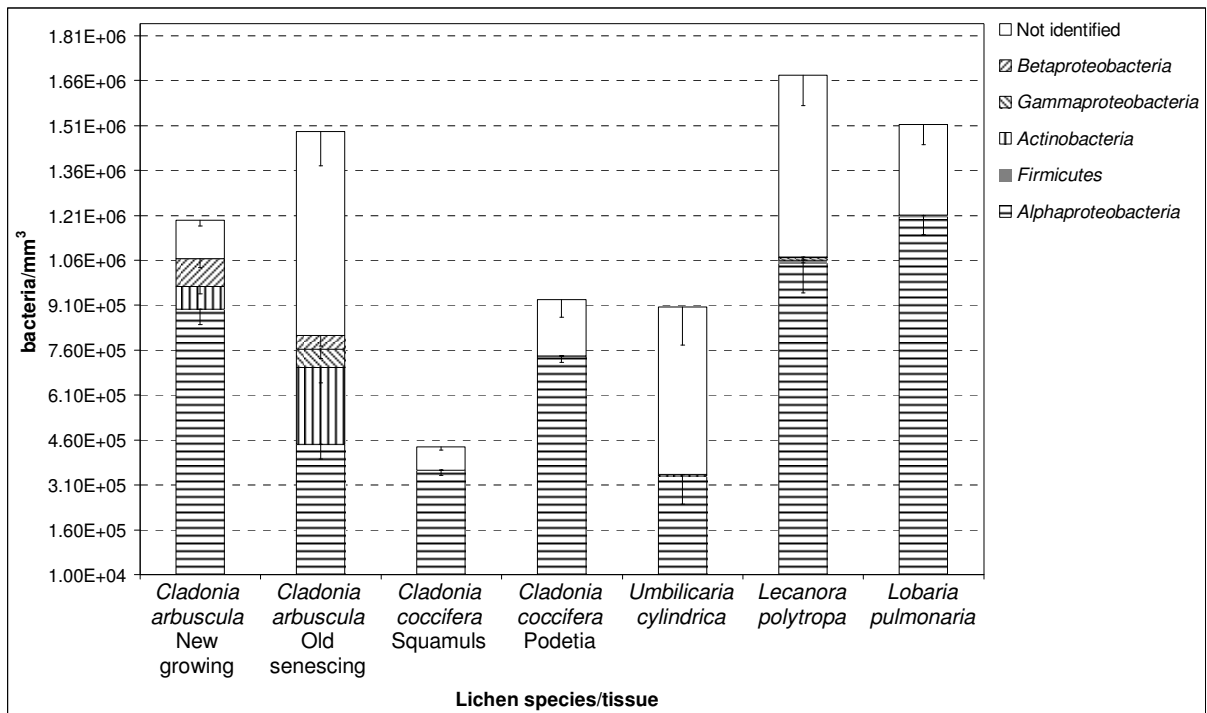5.   This research was supported by Fonds zur Förderung der wissenschaftlichen Forschung – FWF.

Figure 1. Abundance and taxonomic structure of the bacterial communities associated with different lichen species/tissues assessed by FISH-CLSM.
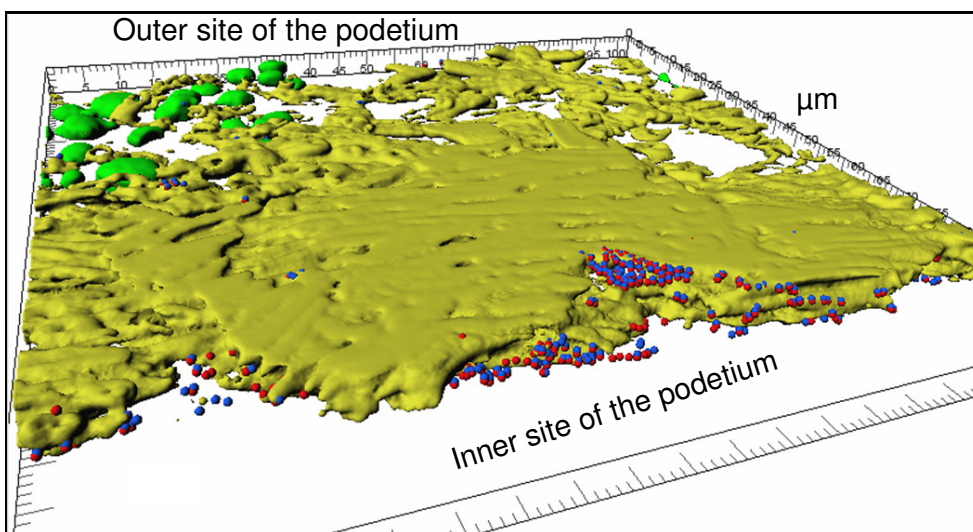

Figure 2. 3D rendering of a confocal stack showing the bacterial colonization of the lichen *Cladonia arbuscula*. Green: algae; yellowish: fungi; red/blue dots: *Alphaproteobacteria*; red dots: other bacteria