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EPS composition and calcification potential of tufa-dominating cyanobacteria investigated by Scanning Transmission X-ray Microscopy (STXM) and Laser Scanning Microscopy (LSM)

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Tufa deposits in freshwater habitats are the result of calcium carbonate precipitation within interfacial microbial ecosystems. Calcite precipitation is influenced by the saturation index and the occurrence of extracellular polymeric substances (EPS) which are produced by a variety of microorganisms. In theory, the first important step of biologically induced calcification processes is the adsorption of calcium ions by extracellular polymeric substances (EPS) produced by cyanobacteria. In the present study we take advantage of Laser Scanning Microscopy (LSM) and combine it with Synchrotron imaging using Scanning Transmission X-ray Microscopy (STXM). STXM represents a technique that allows simultaneous analysis of inorganic and organic constituents as a scale of 50 nm. By means of STXM it is possible to differentiate between calcium carbonate phases at the Ca L-edge. Furthermore, STXM has also been used at the C K-edge to map the major biomolecules (proteins, lipids, and polysaccharides). The purpose of this study is to find out if there are differences in calcium adsorption depending on specific composition of the EPS produced by filamentous cyanobacteria isolated from a German hard water creek (Westerhöfer Bach, Harz Mountains). The goal was to elucidate the potential of biofilms constituents, including microbial cell surfaces as well as extracellular polymeric substances, in triggering the formation of calcium carbonate in tufa systems. For this purpose three filamentous cyanobacteria (Pseudanabaena sp., Leptolyngbya sp. and Nostoc sp.) were cultivated in creek-adapted as well as standard media (BG11) on polycarbonate slides. In situ EPS composition was detected by means of fluorescence lectin-binding approach (FLBA) using 23 commercially available lectins with different specificities for mono- and disaccharides and amino sugars. For CaCO3 nucleation experiments cyanobacterial biofilms grown on polycarbonate slides were deposited in NaHCO3/CaCl2 solutions supersaturated 10 times with respect to calcite for 48, 72 and 144 hours. For the STXM experiment on beamline 10-ID1 at the Canadian Light Source (CLS), the biofilm samples were scrapped off, suspended in a slurry and deposited carefully on a Si3N4 window. In order to obtain quantitative speciation maps of cyanobacterial sheath EPS, image sequences (stacks) were recorded at the C-1s (280-320 eV) and Ca-2p (340-360 eV) edges. Data analysis was done by using the software aXis2000, and energy spectra were fitted with available reference spectra. Nearly the same lectins specific for fucose, mannose, N-acetylgalactosamine and N-acetylglucosamine, as well as sialic acid bound preferentially to the EPS of cyanobacterial sheaths of Pseudanabaena sp. and Leptolyngbya sp. Surprisingly, in case of Nostoc sp. only two lectins specific for fucose, and N-acetylgalactosamine showed a clear binding to the EPS of sheaths. Qualitative, lectin-specific EPS composition was not influenced by nutrient concentrations within the medium during cultivation. In order to biochemically characterize the CaCO3 nucleation sites within the sheaths of the cyanobacteria investigated, carbon maps of the most abundant organic components were derived from C-1s image sequences. The sheaths of the cyanobacteria contained mainly polysaccharides followed by proteins, and a small amount of lipids. The highest amount of polysaccharides was detected in EPS produced by Pseudanabaena sp., whereas in Nostoc sp. only one-fifth was found. All samples investigated contained spectral signatures of Ca2+ adsorbed to EPS. Aragonite-like CaCO3 was detected in close association with the cell surface of Leptolyngbya sp. only. Highest amount of adsorbed Ca to EPS was found in Pseudanabaena sp., whereas only one-third was detected within the EPS of sheaths in Leptolyngbya sp. and Nostoc sp. Results of this combined approach show that the cyanobacteria investigated are may be involved in calcification processes to different degrees.