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Visualization of bacteria embedded in their extracellular polymeric substances with environmental scanning electron microscopy

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Most of the microbial life on earth is thought to exist in biofilms, whereby self-produced extracellular polymeric substances (EPS) are surrounding the microbial cells, which facilitate adhesion among cells and to other surfaces or interfaces. It seems that the EPS matrix serve as a multifunctional element for microbial communities, including adhesion, structure integrity and protection mechanisms. Visualization techniques give us the opportunity to resolve the structure of biofilms which is necessary to understand their functionality and developing strategies. EPS-associated bacteria could be visualized via confocal laser scanning microscopy as well as electron microscopy. In soil systems, transmission electron microscopy has been used based on histochemical staining. However, for electron microscopic techniques the samples have to undergo a sample preparation including fixation and dehydration. Thus, most studies visualized the interface between microorganisms, EPS and e.g. soil in a dry and/or embedded state. This preparation, in particular the complete drying and fracturing cause a collapse of hydrated features like EPS and can lead to artefacts. However, analysis of EPS without disruption of their matrix is of great importance when studying its distribution and ecological role. Thus, there is great necessity to test visualization and preparation techniques to obtain visualization data under more real environmental conditions (e.g. hydrated states) to improve our knowledge of microbe-surfaces-interactions. Recent technological developments introduced the application of environmental scanning electron microscopy (ESEM), which is an advanced SEM equipped with a dedicated vacuum pump controlling vapor pressure, a gaseous secondary electron detector (GSED) that prevents charging and a thermoelectric cooling stage that allows temperature control. This technique enables us to maintain environmental conditions across a range of relative humidity's during sample detection, without desiccation and coating requirements. However, EPS visualization studies with ESEM is still challenging due the low electron density of EPS, comparable to water and air. The trapped water in hydrated biofilms cause limitations in resolution and can make the use of staining techniques with contrast agents containing high-atomic-number elements necessary. We visualized several bacterial species able to produce EPS with an environmental scanning electron microscope, to identify the most appropriate preparation and visualization setups. These results will be necessary to affectively visualize EPS as key to understand the functionality of EPS in heterogeneous soil habitats.