Mechanisms of iron biomineralization by neutrophilic bacteria. Influence on inorganic pollutant scavenging.

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Bacteriogenic iron oxides (BIOS) associated to iron-related bacteria are widespread in the environment because they are produced by both neutrophilic and acidophilic bacteria. Because of their high surface area and reactivity, these BIOS have been shown to play a key role in natural attenuation of pollutants, via sorption and coprecipitation of heavy metals, metalloids and radionuclides. Although BIOS have been recognized to have great potential for remediation in acidic mining environments, the importance of such bioremediation processes in near-neutral pH conditions -which are more frequent in nature- is less documented (Gadd 2004; Ferris 2005).

The aim of the present work is to study the parameters that control iron biomineralization and therefore the formation of BIOS and the immobilization of inorganic pollutants by two selected bacterial strains, *Sphaerotilus natans* and *Leptothrix cholodnii*, both characteristic of neutrophilic habitats (Siering & Ghiorse 1996; Spring et al. 2006). The environmental implications of the study will especially concern the evaluation of the potential role of these iron related-bacteria in natural attenuation of heavy metal and radionuclide pollution in impacted environments.

For this purpose, preliminary tests have been performed up-to-date on *S. natans*. Oxygen conditions tested demonstrated that this strain is able to grow not only aerobically but also in microaerophilic and anaerobic conditions. Fluorescence *in situ* hybridization (FISH) was used to confirm the purity of these cultures, as *S. natans* growth has never been reported before on anaerobic conditions. These results suggest that *S. natans* might be able to ferment some carbon source present in the medium and/or it could use nitrate as an electron acceptor during its anaerobic respiration. On going experiments will shed light on the metabolic processes of *Sphaerotilus*.

In addition, iron tolerance has been investigated at two different concentrations, 0.05 and 1 mM Fe^{2+}. Bacterial growth was observed under all culture conditions at 0.05 mM Fe^{2+} after 24h and in the case of 1mM Fe^{2+}, growth was observed after 4 days in aerobic, microaerophilic and anaerobic with nitrate culture conditions. These results shown that *S. natans* is able to resist minimum 1mM Fe^{2+} and suggest that nitrate might play a key role in the iron metabolism when it is present at elevate doses. Specific growth rates and filamentation kinetics will be determined in the following months by using quantitave polymerase chain reaction (qPCR). Scanning

electron microscope (SEM) images of those cultures shown that microorganisms grew not only as free-cells but also in filamentous form. Both morphologies were encrusted only after 24h of culture in all conditions tested. X-ray diffraction and Mössbauer spectroscopy experiments will be conducted during future months in order to identify the mineral precipitate.

Finally, as a brief outlook, filamentation kinetics and growth rates will be determined by using qPCR while mineralogy studies will be performed using XRD and Mössbauer spectroscopy in order to identify the mineral precipitated on the bacterial surface. Thereafter, transmission electron microscopy coupled to an energy dispersive X-ray spectrometer (TEM-EDX) will be used to test intracellular precipitates. Additionally, iron speciation and scavenging of heavy metals and radionuclides will be analyzed by using different synchrotron based X-ray spectroscopies in order to be able to evaluate possible future applications of those bacteria on bioremediation processes.

References

Ferris, F.G. (2005) Biogeochemical properties of bacteriogenic iron oxides. *Geomicrobiol. J.*, 22, 79-85.

Gadd, G.M. (2004) Microbial influence on metal mobility and application for bioremediation. *Geoderma*, 122, 109-119.

Siering, P.L.; Ghiorse, W.C. (1996) Phylogeny of the *Sphaerotilus-Leptothrix* group inferred from morphological comparisons, genomic fingerprinting, and 16S ribosomal DNA sequence analyses. *Int. J. Syst. Bacteriol.*, 46(1), 173-182.

Spring, S.; Dworkin, M.; Falkow, S.; Rosenberg, E.; Schleifer, K.; Stackebrandt, E. (2006) The genera *Leptothrix* and *Sphaerotilus*. In Dworkin, M.; Falkow, S.; Rosenberg, E.; Schleifer, K.H.; Stackebrandt, E. (Eds.) The Prokaryotes Vol. 5 (758-777). *Springer Science+Bussiness*, New York, USA.