Enzymatic extracellular superoxide in microbial Mn(II) oxidation

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Manganese (Mn) oxide minerals are among the strongest sorbents and oxidants in the environment, controlling the fate of contaminants, degradation of recalcitrant carbon, and cycling of nutrients. We have recently shown that a common marine bacterium, *Roseobacter* sp. AzwK-3b, oxidizes Mn (II) through an indirect pathway, via enzymatic production of extracellular superoxide during exponential growth. Superoxide production and Mn (II) oxidation in cell-free filtrate was inhibited in the presence of proteases, suggesting that superoxide production is due to a soluble protein. NADH enhanced Mn (II) oxidation and inhibition by the oxidoreductase inhibitor DPI suggested that an NADH oxidase is involved in extracellular superoxide production, and subsequent Mn (II) oxidation, by this species.

Here we present new findings revealing the proteins and genes involved in extracellular superoxide production by *Roseobacter* AzwK-3b. A soluble fraction of proteins demonstrated the ability to oxidize Mn (II), which was also substantially enhanced by the presence of NADH and inhibited by superoxide dismutase (SOD). Global protein analysis using GeLC-MS/MS identified several soluble proteins related to oxidoreductases (e.g. ferredoxin-NADH reductase, NADH ubiquinone oxidoreductase). These types of proteins have previously been implicated in the production of extracellular superoxide by pathogenic bacteria. These proteins are currently being targeted by site-directed transposon mutagenesis. In-gel assays of this soluble protein fraction also showed a protein band active for Mn (II) oxidation. Surprisingly, we observe differences in Mn (II)-oxidizing proteins when *Roseobacter* is grown under different nutrient conditions. In the presence of a minimal medium, Mn (II)-oxidizing proteins are only detected when *Roseobacter* is grown in the presence of Mn (II). Using a comparative protein approach, we are identifying differences in the proteins produced under these two conditions, which will identify the protein involved in Mn (II) oxidation.

In combination, our past and current efforts have identified a new pathway for Mn (II) oxidation that is linked to a putative NADH oxidoreductase. Our current efforts will reveal the protein and genes involved in extracellular superoxide production by *Roseobacter*. Considering the abundance of *Roseobacter* in marine environments as well as the versatility of superoxide to act as both a metal oxidant and reductant, this research has large implications in the processes driving metal cycling within marine waters.

Measuring $\delta^{13}C$ in siderite and organic matter of lake sediments

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The evolution of Earth surface biogeochemistry is still not completely understood. Among the various tracers available, the isotope compositions of C and N in Archean rocks are increasingly used but their interpretations remain ambiguous. The study of present stratified water body (oxic at the surface and anoxic in the deeper part) and of their sediments may help us to better interpret the isotopic signal from old sediments. Lake Pavin (French Massif Central) is permanently stratified with anoxic Fe-rich bottom water and can be regarded as an analogue of Archean oceans before the Great Oxidation Event (~2.5 Ga). We aim at analyzing C and N isotope of particulate matter sinking in the water column together with sediment cores from various water depths, in order to understand modifications related to early diagenesis within the water column and surface sediments. In this preliminary study, we develop protocols allowing the measurement of $\delta^{13}C$ values in both organic matter and siderite (Fe-carbonate) from sediment samples.

For organic matter analysis, the removal of siderite is necessary. Previous studies have shown that carbonate dissolution with HCl should not be used because immature organic matter can be partially attacked. Diluted HCl attacks (0.5, 1 and 2M) were tested on two siderite-free Pavin sediment samples. Despite a loss of carbon, no fractionation of C isotopes occurred. A siderite-rich sediment sample will be treated in the same way to check whether siderite is completely dissolved or not.

For C and O isotope analysis of siderite, the presence of organic matter might be problematic. Tests on mixtures of yeast with siderite show that organic matter reacts with phosphoric acid and modifies C and O isotopes signal of siderite. Organic matter removal with NaOCl 3.5% and H_2O_2 30% has been tested on the mixtures. While both reactants removed efficiently organic matter, siderite was also partially oxidized, but without $\delta^{13}C_{carb}$ and $\delta^{18}O_{carb}$ modifications. The chemical treatments thus improved greatly the siderite analysis. Vacuum pyrolysis and low temperature plasma ashing will also be tested to check if they can remove organic matter without destabilizing siderite.