ROBL-CRG	**Experiment title:** **Interaction between Pseudomonas sp. and uranium using EXAFS**	**Experiment number**: **20-01-065**
Beamline: BM 20	**Date of experiment**: 6.3.-8.3.05; 17.7.-19.7.05; 17.9.-20.9.05; 15.4.-18.4.06; 23.7.-24.7.06	**Date of report**: 12.01.07
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Report: Three oligotrophic bacterial strains were cultured from the ground water of the deep-well monitoring site S15 of the Siberian radioactive waste depository Tomsk-7, Russia. They were affiliated with *Actinobacteria* from the genus *Microbacterium*. The cells of all three strains accumulated high amounts of uranium, i.e. up to 240 mg U (g dry biomass)$^{-1}$ in the case of *M. oxydans* S15-M2. X-ray absorption spectroscopy (XAS) analysis showed that this strain precipitated U(VI) at pH 4.5 as a meta-autunite-like phase. At pH 2, the uranium formed complexes with organically bound phosphate groups on the cell surface. The results of the XAS studies were consistent with those obtained by transmission electron microscopy (TEM) and energy dispersive X-ray (EDX) analysis [1].

Experimental*:* Bacterial cells grown to mid-exponential phase were harvested by centrifugation at 8,600 x *g* for 20 min at 4 ^{o}C and washed twice with 0.1 M $NaClO_4$. Washed cells were resuspended and shaken for 48 h in 10 ml of uranium solution (119 mg L^{-1}) at pH values of 2, 3 and 4.5. We used 0.1 M $NaClO_4$ as a background electrolyte. After contact with the uranium solution, cells were harvested and washed with 0.1 M $NaClO_4$. The pelleted samples were dried in a vacuum incubator at 30 ^{o}C for 24 h and powdered.

Results: XANES analysis (data not shown) demonstrated that the oxidation state of uranium species bound to the cells was unchanged indicating the bacteria do not reduce U(VI). Information on the local environment of uranium atoms in the uranium treated bacterial samples was provided by analysis of EXAFS data. Uranium L_{III}-edge EXAFS spectra of the uranium species formed at pH 2, 3 and 4.5

by the cells of *M. oxydans* S15-M2 and their corresponding Fourier transforms (FT`s) are presented in Fig. 1. Quantitative fit results (data not shown) indicate that the adsorbed U(VI) has the common linear trans-dioxo structure: two axial oxygens at about 1.75 - 1.77 Å, and an equatorial shell of 4 to 5 oxygens at 2.27 - 2.33 Å. The U-O_{eq1} bond distance is within the range of previously reported values for phosphate bound to uranyl [2]. The FT peak, which appears at R+Δ ~ 3 Å (radial distance R = 3.59 - 3.62 Å) is a result of the back-scattering from phosphorus atoms. This distance is typical for a monodentate coordination of U(VI) by phosphate. For the studied *M. oxydans* S15-M2 strain, at pH 3 and 4.5, an FT shell corresponding to a distance of R = 5.19-5.20 Å can be related to a U-U contribution.. At pH 2, however, the U-U peak is absent indicating a lower site symmetry around uranium. All known U(VI) complexes with organic phosphates show a $N_{Oeq} \geq 5$ and are related to a longer U-O_{eq} distance, e.g. 2.32 Å in the case of fructose 6-phosphate (F6P) used as reference in Fig. 1. Interestingly, we have found that EXAFS spectra of the samples at this pH have high similarities to those of organic phosphate ligands complexed with U such as fructose 6-phosphate [3].

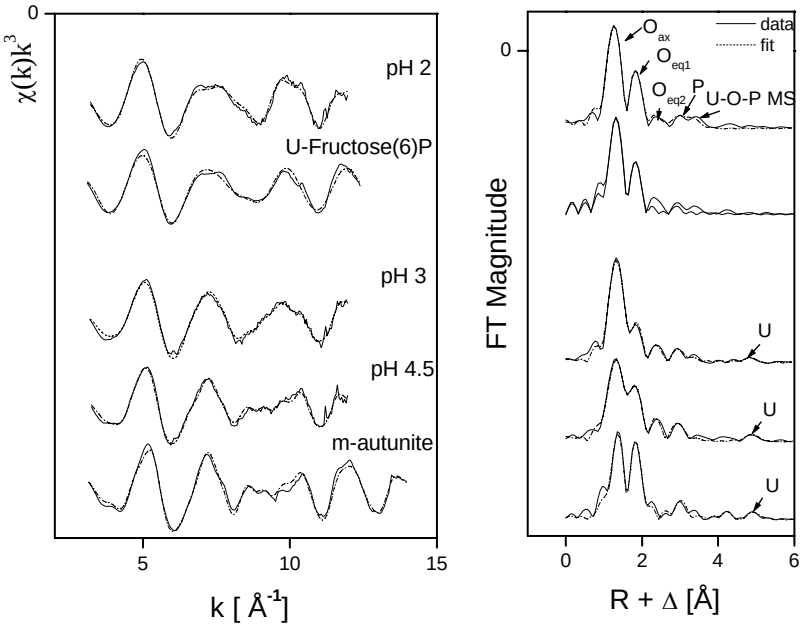

Fig.1: Uranium L_{III}-edge k^3–weighted EXAFS spectra (left) and the corresponding fourier transforms (FT) (right) of the uranium complexes formed by the cells of *M. oxydans* S15-M2 at pH values 2, 3, and 4.5, as well as of the reference compounds (meta-autunite and U-Fructose(6)Phosphate).

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