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Introduction

Uranium (U) is a chemical toxicant for Humans, naturally present in soils but also susceptible to be disseminated by industrial activities. In France the Bessine region, in Haut Limousin, has been identified as a contaminated region in which soils contain high concentrations of U. Soil plays a key role in toxic metals bioavailability, by modulating their transfer to surface waters and plants. These transfer properties depend on multiple parameters and among them the presence of bacterial populations. The purpose of our project is to evaluate the potential role of soil bacteria in complex phenomena of immobilization or transfer of U to the foodchain. A library of bacterial strains, isolated from U-contaminated soils sampled near Villard and Vénachat (15000 ppm and 4700 ppm of U, respectively), in Bessine region, has been constructed. Some of them are able to grow in a medium containing up to 5 mM of U. One mechanisms of this high resistance can be their ability to modify U speciation, which would preclude their ability to modify U bioavailability in soils. In a previous experiment, U speciation in the most resistant strains, exposed separately, was identified. When bacteria were exposed in culture medium, only U-phosphate complexes were detected. Electron microscopy observation showed that these complexes were precipitated extracellularly or in cell walls. When bacteria were exposed to U in water rather than in culture medium (this experiment had only been performed on two strains), U speciation differed, suggesting that in absence of phosphate, U speciation can be governed by an intracellular process. In natural soils, phosphate concentration is lower than in bacterial culture medium. This U metabolization process may thus govern U speciation modification in natural conditions. Finally in this first experiment, we identified U speciation in Bessine soils from where these resistant bacteria have been isolated (V. Chapon et al., submitted). The present experiment was designed to evaluate U speciation modification in conditions which were more representative of bacterial activity in natural environments. Since bacteria are rarely isolated in soils, and more probably grouped into communities, we worked with artificially reconstituted bacterial consortia, rather than with isolated strains. Moreover these consortia were exposed to U either prepared in water or prepared in soil lixiviates, experimentally contaminated with depleted U, rather than in laboratory bacterial culture medium.

Experimental method

The more resistant bacteria isolated from the U-rich Bessin soils were grown separately. When reaching the exponential growth phase, the same amount of each strain was sampled, were mixed together, rinsed with ultrapure water and exposed to U, either aerobically or anaerobically. U exposure solutions were prepared either from U-acetate salts dissolution in water (pH2), or from lixiviation of Bessine soils with a bicarbonate solution. This procedure led to a lixiviate containing 200-500 µM of U. Exposed bacteria were collected after 1, 2 or 3 days, then rinsed with ultrapure water, collected by centrifugation, freeze-dried and pressed as 5-mm pellet which were packed and sealed. U exposure solutions were also sampled, and placed in quartz capillaries for analysis. XANES and EXAFS spectra of these samples were collected at U L_{III} edge on BM30B beamline, in fluorescence mode and at room temperature, using the 30 elements solid state Ge detector (Canberra). 3-8 spectra (depending on the uranium concentration of the sample) were recorded for

each sample and averaged to improve the statistics. EXAFS oscillations were isolated from raw, averaged data by removal of the pre-edge background, approximated by a first-order polynomial, followed by μ_0-removal *via* spline fitting techniques using Athena software. The resulting EXAFS curves in the wavevector (k) space were weighted by k^3 and qualitatively compared to reference curves.

Results

Oscillations were extracted from EXAFS spectra of soil lixiviates, exposure media and bacterial pellets, and compared to spectra recorded on reference compounds. As shown in Figure 1A, lixiviation of Bessine soils with a bicarbonate solution leads to solubilization of U in a complexed form which might be U-carbonate (fitting need still to be refined).

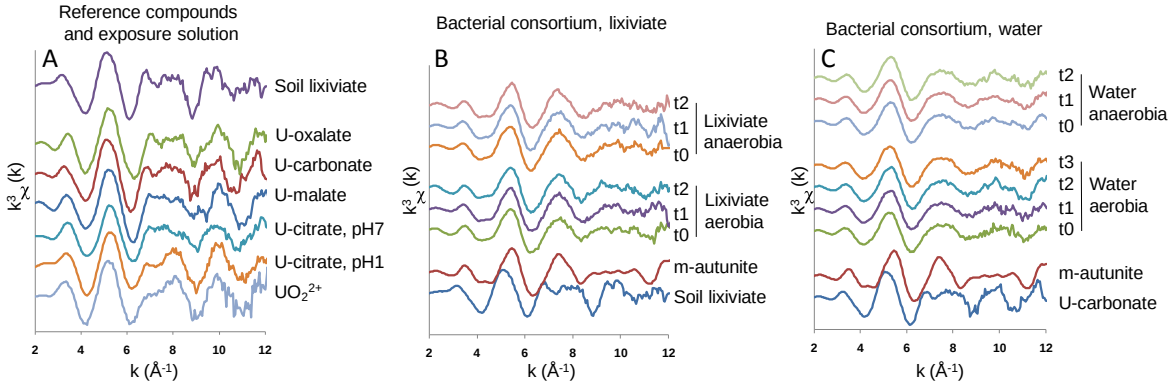

Figure 1. U LIII-edge k3 weighted EXAFS spectra of exposure solution and reference compounds (A), bacterial consortium exposed to U prepared in soil lixiviate (B) and bacterial consortium exposed to U prepared in water (C).

Our data lead to several major conclusions. First, U speciation in bacteria differs when they are exposed in soil lixiviates as compared to exposure to U prepared in water (Figure 1B and 1C). In both cases, U is in the U(VI) oxydation state. Extracted EXAFS spectra clearly show differences in the 6.5-8.5 $Å^{-1}$ region, with a sharp peak upon exposure in soil lixiviate (Figure 1B), whereas the peak is larger upon exposure in water (Figure 1C). Comparison with reference spectra suggests that when exposed to U prepared in soil lixiviate, U speciation in bacteria is dominated by a U-phosphate complex. When exposed to U prepared in water, U speciation could be a combination of U-phosphate and U-carboxylate, as previously observed in plants (Laurette et al., submitted). These data prove that U speciation is transformed by bacterial activity. This transformation is very rapid, since in the t0 time points (bacteria and exposure solution are mixed, then bacteria are immediately centrifuged and lyophilized, this procedure lasts 2-5 min) U speciation has already been transformed. Another conclusion is that after this first transformation, U speciation does not change, and remains the same even after several days of exposure. The presence of oxygen (aerobic vs. anaerobic exposure condition) has no influence on U final speciation. Finally, comparison with the results of our previous experiment show that bacteria organization as a consortium has no influence on their ability to modify U speciation.

Conclusions and perspectives

Lixiviation of U-containing soils with bicarbonate is sufficient to render U bioavailable and to permit its mobilization by bacteria. Bacteria would then reduce its availability by modifying its speciation, for instance by inducing its precipitation as U-phosphate. Bacterial organization in consortium does not modulate their ability to transform U speciation, but it may quicken the transformation process. In parallel to this experiment, we studied the evolution of bacterial consortium upon exposure to U (the more resistant bacteria may have take advantage over other ones). In perspective, and since we also work on plant response to U exposure and the influence of U speciation on plant accumulation and distribution, the next step will be to further complexify our system, by studying synergistic response of bacteria and plants face to U exposure. The final goal of this project will be to understand U mobilization in natural environments, but also to optimize exposure conditions which would lead to efficient bio- and phyto-remediation.

Bibliography

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