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Screening of bacterial strains isolated from uranium mill tailings porewaters for bioremediation purposes

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ABSTRACT

The present work characterizes at different levels a number of bacterial strains isolated from porewaters sampled in the vicinity of two French uranium tailing repositories. The 16S rRNA gene from 33 bacterial isolates, corresponding to the different morphotypes recovered, was almost fully sequenced. The resulting sequences belonged to 13 bacterial genera comprised in the phyla Firmicutes, Actinobacteria and Proteobacteria. Further characterization at physiological level and metals/metalloid tolerance provided evidences for an appropriate selection of bacterial strains potentially useful for immobilization of uranium and other common contaminants. By using High Resolution Transmission Electron Microscope (HRTEM), this potential ability to immobilize uranium as U phosphate mineral phases was confirmed for the bacterial strains Br3 and Br5 corresponding to *Arthrobacter* sp. and *Microbacterium oxydans*, respectively. Scanning Transmission Electron Microscope- High-Angle Annular Dark-Field (STEM-HAADF) analysis showed U accumulates on the surface and within bacterial cytoplasm, in addition to the extracellular space. Energy Dispersive X-ray (EDX) element-distribution maps demonstrated the presence of U and P within these accumulates. These results indicate the potential of certain bacterial strains isolated from porewaters of U mill tailings for immobilizing uranium, likely as uranium phosphates. Some of these bacterial isolates might be considered as promising candidates in the design of uranium bioremediation strategies.

1. Introduction

Uranium, a naturally occurring radioactive element, exists as a trace element in natural environments such as soil, sediment and water. Uranium mining is crucial in the production of electricity through nuclear power, becoming a high-impact economic activity. In order to limit the environmental consequences of uranium-related industry, continuous monitoring/remediation approaches taking into account a large number of abiotic and biotic factors are desirable.

Influence of microbial communities on uranium behavior in the environment, including uranium mining sites, has been previously demonstrated (Francis, 1998). Thus, the study of microbial processes occurring in these disturbed environments might be an effective approach to determine the associated impact and to gain insights into the factors governing uranium fate and behavior in nature. Previous studies have explored the indigenous microbial diversity from uranium-rich environments through culture-dependent and -independent approaches (Akob et al., 2007; Chang et al., 2001; Fields et al., 2005; Hemme et al., 2010; Mondani et al., 2011; Radeva and Selenska-Pobell, 2005; Schippers et al., 1995; Selenska-Pobell et al., 2001; Wolfaardt et al., 2008).

Microbes may adopt various strategies for adapting to uranium-rich environments by employing different mechanisms including biosorption at the cell surface (Merroun et al., 2005), biomineralization (Beazley et al., 2007; Macaskie et al., 2000), intracellular

Abbreviations: MM, metals/metalloid; BRU, Brugeaud; BZN, Bellezane; HRTEM, High resolution transmission electron microscope; STEM-HAADF, Scanning transmission electron microscope-High angle annular dark-field; EDX, Energy dispersive X-ray.

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accumulation (Merroun et al., 2002); Strandberg et al., 1981), and redox transformations either by reduction leading to the formation of insoluble U(IV) (Lovley et al., 1991), or by oxidation into mobile U(VI) (DiSpirito and Tuovinen, 1982), which form the basis of the utilization of these organisms for uranium immobilization. Effects of these bacteria–uranium interactions and consequences at different levels linked to bioremediation actions have been extensively studied by *in situ* investigations (Cardenas et al., 2008; Holmes et al., 2002; Hwang et al., 2009; Michalsen et al., 2007; Nevin et al., 2003; North et al., 2004; Suzuki et al., 2003; Xu et al., 2010). Some of these works, which investigated the molecular scale speciation of U associated with particular bacterial strains, have revealed the capacity of indigenous bacteria to mediate the immobilization of uranium, suggesting potential benefits in their application for bioremediation purposes (Merroun and Selenska-Pobell, 2008).

Screening for those bacterial isolates that offer higher performance on uranium immobilization emerges as a prerequisite for optimal design of bioremediation strategies. This selection process must be based on certain defined criteria like the ability to cope with a wide variety of constraining conditions (high concentrations of U and other metals normally co-occurring in mining sites and waste piles, nutrients limitation, acidity/alkalinity, etc.) or presence of particular features (morphological and/or physiological, like specific enzymatic activities), which allow an effective immobilization of U(VI), and consequently make them promising candidates for remediation.

The present study aims to retrieve bacterial strains from environments rarely studied, as porewaters of two uranium mill tailings repositories, and to characterize them at different levels (molecular taxonomic classification, enzymatic potential, metals/metalloid [MM] tolerance and eventual interaction with uranium) in order to predict their role in the biogeochemical cycle of uranium and to screen for those with potential for bioremediation purposes. To address this objective, we conducted a multidisciplinary analysis of porewaters collected through monitoring wells located in the vicinity of former uranium mines, in the region of Limousin (France).

2. Materials and methods

2.1. Study sites and sampling conditions

Porewaters samples were collected from the Brugeaud (BRU) and Bellezane (BZN) mill tailings repository sites, located near Bessines-sur-Gartempe (Limousin, France). This region was one of the main uranium mining areas in Europe due to the presence of high-grade natural uranium deposits. As mining operations advanced, the mill tailings were stored in the open pits already exploited. During reclamation, a rock cover was set up on those mill tailings. Concurrently, water collection systems were built for monitoring and treatment of uranium and radium-226 (see AREVA, 2004).

Brugeaud, in the immediate vicinity of Bessines-sur-Gartempe, is part of the industrial platform of Bessines. In this case, the characteristics of the sample might be altered by the contribution of flowing granitic groundwaters in contact with target mill tailings repository porewaters. The second sampling site, BZN, where remediation works ended in 1996, is located 3 km southeast of Bessines-sur-Gartempe. This former mining site consisted of several open pit mines and underground mines. Besides mill tailings produced directly in these mining sites, some other mill tailings produced between 1989 and 1993 in other mining sites nearby were stored in the former open pit mines 68 and 105 of BZN area (Fig. 1). Complementary information about the sampling sites is available in a report published by AREVA (2004).

In March 2012, 10–15 L of porewater were collected from a monitoring well in both tailings repositories, BRU and BZN, at a depth of 35 and 25 m respectively, by using an inertial water-pump (WaTerra Pumps Ltd.) and sterilized high density polyethylene (HDPE) tubing and storing HDPE containers (Fig. 1). Initially, each piezometer was purged in order to access to the porewaters. The purge was complete at BZN (equivalent to 1 volume of the well), but not at BRU monitoring well where the water renewal rate was very high. Then, this sample was interpreted as a mix of mill tailings porewaters and granitic groundwaters presenting different characteristics. Collected samples were stored at approximately 4 °C in a cool-box for transportation to the laboratory where chemical and microbiological analyses were performed.

2.2. Physico-chemical analyses

Immediately after the water samples collection, physico-chemical parameters (Eh, pH, conductivity and dissolved oxygen) were determined *in situ* using a flow-through cell containing a multi-parameter probe (HORIBA multiprobes #W-22XD) calibrated prior to measurements. Water samples intended for chemical analyses were filtered immediately after sampling, using a Nalgene vacuum filtration device with Sartorius 0.2-μm-pore PES membrane. Alkalinity was measured just after sampling by H_2SO_4 titration using colorimetric test (bromocresol green/methyl red). Total organic and inorganic carbon contents were assayed by Carbon Analyzer TOC Shimadzu apparatus. Samples dedicated for cations, metals and radioactivity measurements were acidified to pH 2 with ultrapure 70% HNO_3. Major cations (Na^+, Ca^{2+}, Mg^{2+}, K^+) contents were determined by Flame Atomic Absorption (240AA Varian) and NH_4^+ concentration by UV Spectrophotometer (Carry 300). Major anions (Cl^-, F^-, SO_4^{2-}, NO_3^-, PO_4^{3-}) contents were determined by Ionic Chromatography (DX 500 Dionex). Trace elements (Al, As, Fe, Mn) and Si contents were measured by ICP-AES (720 Varian) and uranium concentrations by ICP-MS (Thermo X7). ^{226}Ra was determined by measuring daughters ^{214}Pb and ^{214}Bi by gamma spectrometry after one month equilibrium. Filters were analyzed in a second stage for insoluble uranium and ^{226}Ra contents.

2.3. Bacterial isolation and cultivation

In order to isolate a wide range of bacterial strains, a cultivation strategy based on the use of media containing different types and concentrations of nutrients was conducted. In total, five aerobic culture media for heterotrophic bacterial growth were used: LB broth (rich-nutrient medium; Scharlau Chemie, SA), LB10 (LB broth 10-fold diluted), TSB (rich-nutrient medium; Scharlau Chemie, SA), R2A oligotrophic medium (low-nutrient medium; Reasoner and Geldreich, 1985), and CYP medium containing 0.01% Bacto casaminos acids, 0.01% Bacto yeast extract and 0.03% peptone (low-nutrient medium; modified from Bollmann et al., 2007). Low-nutrient media were used to stimulate the growth of slow-growing oligotrophic species. For solid cultures, 1.5% agar was used as a solidifying agent.

The inoculation and incubation of the proposed media was approached by two distinct methods to obtain a higher diversity. Thus, for the first approach, one mL of each water sample was used to inoculate 50 mL of each culture medium. After two to five days of incubation, 10-times dilution series were made from each growing medium in 0.9% NaCl; 0.1-mL portions of the dilutions 10^{-3} to 10^{-7} were spread with a sterile glass rod on agar plates containing the same medium used in the previous step and incubated again for 2–3 days at 28 °C in the dark. For the second method, 2 L of each water sample were filtered under sterile conditions through filter

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