Microbial Bioremediation of Uranium: an Overview

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Abstract

Uranium contamination is a worldwide problem. Preventing uranium contamination in the environment is quite challenging and requires a thorough understanding of the microbiological, ecological and biogeochemical features of the contaminated sites. Bioremediation of uranium is largely dependent on reducing its bioavailability in the environment. *In situ* bioremediation of uranium by microbial processes has been shown to be effective for immobilizing uranium in contaminated sites. Such microbial processes are important components of biogeochemical cycles and regulate the mobility and fate of uranium in the environment. It is therefore vital to advance our understanding of the uranium–microbe interactions to develop suitable bioremediation strategies for uranium contaminated sites.This article focuses on the fundamental mechanisms adopted by various microbes to mitigate uranium toxicity which could be utilised for developing various approaches for uranium bioremediation.

Introduction

Metal contamination cannot be destroyed but can only be concentrated and contained in solid form for final disposal. Bioremediation is an option that offers the possibility to destroy or render the various contaminants harmless using natural biological activity. Microorganisms harbor the potential to restore the metal contaminated environments inexpensively and effectively by employing a number of mechanisms including complexation, binding, reduction, precipitation and accumulation.

Uranium is the heaviest, naturally occurring element found in the earth's crust. It is an alpha emitter and a weakly radioactive element which shows both radiotoxicity and chemotoxicity. However, the chemical toxicity of dissolved uranium is of greatest environmental significance and poses a major concern for public health and safety. Ingestion of high concentrations of soluble uranium compounds manifest in chemotoxic effects on renal tissue leading to kidney failure [1]. Uranium is released in to the environment through its mining, disposal of tailings, nuclear power or weapon production and nuclear accidents. The mobility of uranium in the environment is dependent on its speciation and its redox state as well. It occurs as U(VI) under oxidizing conditions in the form of a) UO_2^{2+} below pH 2.5, b) or hydroxyl complexes below pH 6.5 or c) as uranyl carbonate at pH$>$ 7 [2]. In the reducing conditions, it exists as insoluble and immobile U(VI) as mineral uraninite. Uranium speciation in contaminated waters is critical for the selection of the treatment process and its successful application. The interactions between microbes and uranium play a very important role in controlling the latter's mobility in natural environment. These interactions can be stimulated to immobilize aqueous uranium thereby remediating the uranium contamination. In microbial systems, no specific mechanism has been attributed to uranium toxicity.

We came across some interesting mechanisms displayed by the microbes to resist uranium toxicity while investigating the microbial interactions with uranium in our laboratory. These mechanisms, harboured by microbes for detoxification of uranium, form the basis of utilization of these organisms for various bioremediation approaches and are discussed below.

Adsorption of uranium complexes onto the surface of a unicellular, marine cyanobacterium, *Synechococcus elongatus*

Microbial cells are able to interact with uranium in multiple ways due to diversity in their metabolism and cell surface structures. The latter provide a highly efficient matrix for metal complexation. The metal binding with surfaces of microbial cells is even more efficient than that with inorganic soil components like minerals. The high metal complexation ability of microbial cells is primarily based on two facts: the high surface-to-volume ratio and the usually large number of metal binding ligands, which are presented by the organic cell surface polymers, e.g. peptidoglycan, lipopolysaccharides, proteins and glycolipids. These ligands include functional groups, such as phosphate, carboxyl, hydroxyl, amino and sulfhydryl groups. The adsorption of aqueous metal cations onto these functional groups, the so called biosorption process is rapid, reversible and does not depend on the cell metabolism.

Most of bacterial surface uranyl adsorption studies have focused on low pH conditions where UO_2^{2+} is the predominant aqueous uranium species. Above pH 5, neutral and negatively charged uranyl carbonate predominates the aqueous uranium speciation in the marine environment. We investigated the uranium-binding abilities in a marine unicellular cyanobacterium, *Synechococcus elongatus* BDU 75042 from micromolar concentrations of uranyl carbonate at pH 7.8. These cells when exposed to 23.8 mg L^{-1} U (or 100 μM) at pH 7.8 bound 68-72 % U in less than 10 min resulting in a loading of 53.5 mg U g-1 dry weight [3]. Treatment of U-loaded cells with 0.1 N HCl showed 80 % of U desorption. Most of the bound uraniumwas found to be associated with the extracellular polysaccharides (EPS) of the cells. The amide groups and the deprotonated carboxyl groups harboured within EPS were likely to be involved in uranyl complexation as suggested by Fourier-transform infrared (FT-IR) spectroscopy. The X-ray diffraction (XRD) analyses revealed the identity of the uranium deposits associated with the cell biomass as uranyl carbonate hydrate. The uranyl-binding efficiency of the heat killed or the non-viable *Synechococcus* cells was similar to that of live cells, corroborating the metabolism independent bioadsorption of U in these cells [3].

Uranium concentration in surface associated polyphosphates in a filamentous, marine cyanobacterium, *Anabaena torulosa*

Uranium has no known biological function and is transported into microbial cells only due to increased membrane permeability (e.g. resulting from uranium toxicity) [4]. There is a lack of direct evidence for the presence of uranium transporters in microorganisms [4]. Therefore, intracellular accumulation of uranium is considered as metabolism-independent process. Bacterial cells have demonstrated several mechanisms to immobilize uranium once it is accumulated intracellularly. One of the known phenomena is uranium chelation by polyphosphate bodies. Inorganic polyphosphates (poly P), are linear polymers of inorganic phosphate (Pi) residues linked by phosphoanhydride bonds. There is a strong evidence for the incorporation of heavymetals into the polyphosphate granules/bodies in several microorganisms.

Our recent studies on the interactions of uranium with a filamentous, heterocystous, nitrogen-fixing marine cyanobacterium, *Anabaena torulosa*, revealed that this strain could sequester uranium in acid soluble polyphosphates, which could be extracted from the cells upon acidification with 1 N HCl at 100°C [5]. Polyphosphate bodies are generally known to be localized intracellularly in cyanobacterial cells. In the present study, observations using light, fluorescence and electron microscopy-based imaging, coupled with Energy Dispersive X-ray (EDX) spectroscopy and spectrophotometric analyses, have revealed (a) the presence of novel surface associated polyphosphate

bodies (SAPBs) in the filamentous cyanobacterium *A. torulosa* and (b) the interaction of such SAPBs with uranium [5]. When challenged with 23.8 mg L^{-1} or 100μM $UO_2(CO_3)_2^{2-}$ for 24 h at pH 7.8, under phosphate limited conditions, *A. torulosa* cells bound 65% (15.47 mg L^{-1}) of the inputU (23.8 mg L^{-1}) resulting in a loading of 77.35 mg U g^{-1} dry wt. [6]. Backscattered electron SEM image of the uranium loaded cells revealing high contrast spots and the co-occurrence of U and P in the EDX spectra exhibiting such discrete spots suggested the concentration of uranium in such polyphosphate bodies [5]. The co-localization of uranium with the polyphosphate bodies and detachment or extraction of such bodies resulting in large craters on the cell surface accompanied by loss of U and Pi upon HCl based desorption without causing cell lysis, further substantiates their surface association and acid solubility. Uranium immobilization by suchsurface associated polyphosphate bodies (SAPBs), reported in cyanobacteria for the first time, demonstrated a novel uranium sequestration phenomenon [5].

Uranium bioprecipitation by chemo-heterotrophic bacteria isolated from uranium rich deposits

An alternative strategy for bioremediation under oxygenated conditions is the immobilization of uranium due to the precipitation of hardly soluble inorganic uranium compounds. A well-known mechanism for bioprecipitation of uranium is based on the activity of non-specific phosphatases, in particular, acid and alkaline phosphomonoesterases, which are commonly generated by active soil microorganisms. These enzymes were expressed by a large variety of aerobic and anaerobic bacteria and release inorganic orthophosphate from organic phosphate compounds[7]. As a consequence of this, the released orthophosphate interacts with uranium and causes the precipitation of inorganic uranyl phosphate phases, directly at the cell surface or in the surrounding aqueous system. Beazley and co-workers [7] demonstrated that the hydrolyzation of organophosphate by aerobic heterotrophic bacteria could play an important role in bioremediation of uranium-contaminated sites.

Domiasiat located in the west Khasi hill district of Meghalaya in Northeast India is one of the largest sandstone-type uranium (U) ore deposits in India containing 9.22 million tonnes of ore reserves withan average ore grade of around 0.1 % U_3O_8[8]. This geographically distinct U deposit of Domiasiat is un-mined and harbours diverse group of bacteria surviving the stressful environmental conditions prevalent in the ore deposit. Various adaptive features exhibited by the indigenous bacteria from such contaminated sites for their survival under conditions of toxic concentrations of radionuclide and heavy metals have been studied. Representative bacteria (130 isolates- 76 Gram-positive and 54 Gram-negative) were isolated from sub surface soils of such uranium rich deposit and analysed by 16S rRNA gene sequencing [9]. They were affiliated to Firmicutes (51%), Gammaproteobacteria (26%), Actinobacteria (11%), Bacteroidetes (10%) and Betaproteobacteria (2%). Overall, 76% of the characterized isolates revealed phosphatase positive phenotype (phosphatase activity was checked on phenolphthalein diphosphate (PDP) and methyl green agar) and 53% had PIB-type ATPase genes (that detoxify the cytoplasm by effluxing heavy metal ions) [9]. The cultivable bacteria have been reported to tolerate substantial concentrations of U (4 mM) and other metals (Cu, Cd, Pb) and showed potent capacity for binding of U[9,10]. Representative strains removed more than 90% and 53% of U from 100 μM and 2 mM uranyl nitrate solutions, respectively, at pH 3.5 within 10 min of exposure[9]. Two of the representative strains (from the phylum Proteobacteria), Serratia marcescens belonging to Gamma proteobacteria and *Burkholderia arbores* belonging to Betaproteobacteria, displayed phosphatase activity. *In vitro* zymogram assay was attempted for both the strains using cell free extracts under acidic and alkaline conditions separately. Under

acidic conditions, active bands at ~80kDa were observed for both the strains whereas under alkaline conditions, active bands at ~58kDa and ~29kDa were seen for *Serratia marcescens* and *Burkholderia arbores* respectively (unpublished results). Assays performed with *Serratia marcescens*(OD_{600}~2) with 2mM uranyl nitrate at pH 3.5 in 2mM acetate buffer and 2mM uranyl carbonate at pH 8 in 2mM bicarbonate buffer showed 94% and 80% precipitation of uranium respectively within 72h. Whereas *Burkholderia arbores under* similar conditions precipitated 95% and 89% of U under acidic and alkaline conditions respectively in 72h (unpublished results). Enzymatic precipitation of uranium demonstrated by these naturally occurring, sub surface bacterial isolates from uranium rich deposits reveal the detoxification phenomenon adopted by these isolates to limit uranium toxicity in U and metal contaminated soils.

Conclusion

Our studies have identified different mechanisms employed by a variety of microorganisms for alleviating uranium toxicity. A fundamental understanding of these mechanisms will prove useful for utilization of these organisms for developing various approaches to uranium bioremediation.

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