Interactions between Metals and Bacteria: Fundamental and Applied Research

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Soils, sediments, and waters heavily polluted with radionuclides and other toxic metals, are a reservoir of unusual bacteria well adapted to these toxic environments. These bacteria possess fascinating mechanisms for interaction with and bio-transformation of radionuclides and other heavy metals, thus regulating the mobility of the metals in the environment. This paper will give an overview on the different mechanisms of interaction between radionuclides/metals and bacterial strains isolated from different extreme habitats including uranium mining waste piles as well as groundwater of a radioactive repository. For this purpose, a combination of spectroscopic (EXAFS, XANES, TRLFS), microscopic (TEM), microbiological and wet chemistry techniques is used. Elucidating the interaction mechanisms microbe/metals is helpful for understanding the role which bacteria play in the transport and mobility of toxic metals in the environment as well as their biotechnological application in the bioremediation of heavy metal contaminated waters. Another application of the isolated bacterial cells and their biocomponents is in the field of nanotechnology. Thus, the surface layer (S-layer) protein of *Bacillus sphaericus* JG-A12, a bacterium isolated from a uranium mining waste pile near the town of Johanngeorgenstadt in Germany, is used as template for the formation of noble metal (Pd, Pt, Au, etc.) nanoparticles for industrial application (e.g. catalysis). The structure and the size of these metallic nanoparticles were characterized using synchrotron radiation-based methods such as X-ray absorption spectroscopy.

Keywords radionuclide/metal; bacteria; heavy metals contaminated sites; interaction mechanisms; spectroscopic and microscopic techniques; bioremediation; nanotechnology

1. Background

The increasing contamination of soil, sediment, and water with heavy metals by natural and industrial processes is a worldwide problem. Mining processes produced million tons of material contaminated with radionuclides such as U and different heavy metals as, for instance, Cd, Ni, and Pb [1]. For the prevention of an eventual release and migration of these contaminants into the environment all factors which can influence it such as ions, minerals and microorganisms must be considered [2, 3]. Bacteria which are ubiquitous in nature play an important role in the mobility of radionuclides and metals. To better understand the microbial mechanisms which influence the metal/radionuclide mobility and in order to establish bioremediation strategies for the contaminated sites, information on the distribution of the microorganisms in these extreme habitats are required. Because more than 99% of the microorganisms in the environment are uncultivable by using standard cultivation techniques, culture-independent molecular approaches based on analyses of 16S rRNA genes were used to explore microbial diversity in nature [4, 5].

During the last decade, a number of studies investigated microbial communities in radionuclide contaminated environments by using the 16S rRNA gene retrieval, in order to determine the phylogenetic placement of the microorganisms that are inhabiting these environments [1, 4, 6-10]. Differences in the composition of the bacterial communities contaminated environments were observed, which are site-specific and possibly connected to their different grade of contamination, to their different geographic and geologic origin as well as to their site history [6]. *Alphaproteobacteria* and *Acidobacteria* were found to be predominant applying the 16S rRNA gene retrieval with different primer sets in soil samples collected from different depths of the uranium mining waste pile Haberland, located near the town of

Johanngeorgenstadt in Germany [4, 6, 7]. In soil samples from the uranium mill tailings Gittersee/Coschütz in Germany no *Acidobacteria* were identified and the *Alphaproteobacteria* were not predominant [6, 8]. Instead, the number of *Gammaproteobacteria* especially of *Pseudomonas* spp. as well as the number of representatives of the *Bacteroidetes* phylum was extremely high in these samples [6, 8]. *Gammaproteobacteria* were also predominant in a soil sample from the uranium depository site Gunnison, Colorado, USA [6]. In contrast, the composition of the bacterial community in a soil sample from the uranium mill tailings Shiprock, New Mexico, USA was extremely complex and Gram-positive bacteria, especially *Bacillus* spp., green non-sulphur bacteria, and *Gammaproteobacteria* were found to be predominant [6]. Bacterial diversity was also investigated in water samples collected from several uranium mining wastes [9]. The analysis of the 16S rRNA gene retrieval revealed that *Nitrospina*-like bacteria are predominant in the uranium mill tailings Schlema/Alberoda, Germany, whereas *Pseudomonas* spp. and *Frateuria* spp. from *Gammaproteobacteria* were predominant in the uranium mill tailings Shiprock, New Mexico, USA [9].

In addition to the description of the distribution of 16S rRNA and other gene sequences in the environment, it is necessary to have information on the physiology of pure microbial cultures related to the natural populations identified via molecular methods [11]. Thus, there is a need to isolate bacteria from the uranium contaminated sites and to study their interactions with uranium and other heavy metals. Number of studies has shown that natural microorganisms, isolated from heavy metal- contaminated habitats [12-15] or from other extreme environments [16, 17], as well as some laboratory strains [18, 19, 20] effectively interact with toxic metals and radionuclides via direct and indirect mechanisms. These interactions can cause mobilization and/or immobilization of the metals [21, 4]. They encompass biotransformations such as oxidation or reduction of metals [22, 23], biosorption by cell surface polymers [12, 15], uptake of metals into the cells [24-26], induction of metal precipitation and generation of minerals [27, 28], or alteration of metal speciation caused by microbially induced redox changes in the environment [29]. The above described microbial activities strongly influence the fate of toxic metals in the environment. In context of bioremediation, solubilisation provides a route for removal from solid matrices such as soils, sediments, dumps and industrial wastes. Alternatively, immobilisation processes may enable metals to be transformed in *situ* into insoluble and chemically inert forms and are particularly applicable for removing metals from mobile aqueous phases.

Early research by Beverdige and Murray [30] related the bacterial metal interactions to the anionic character of specific functional groups situated on membrane components. These authors considered the major cell wall components (e.g. teichoic acids, peptidoglycan, phospholipids) as principally responsible for the overall bacterial reactivity. The determined reactive anionic functional groups were essentially carboxyl groups ($R-COOH$), phosphomonoesters ($R-OPO_3H_2$), phosphodiesters ($(RO)_2 -P(OH)_2$), amines ($R-NH_3^+$), and hydroxyls ($R-OH$). On this basis, Fein et al. [31] developed a bacterial sorption model simplifying the variety of these groups to there main site groups differing in acid base reactivity: acidic sites involving carboxyl and phosphodiester groups ($pK < 4.7$), neutral sites involving phosphomonoester groups ($pK \approx 7$), and basic sites involving hydroxyl and amine groups ($pK > 8$). Using such approaches, the reactivity of various heavy metals (Cu, Cd, Zn, Al, Pb, etc.) with numerous bacterial substrates was assessed [31, 32]. Yee and Fein [32] found that the metal sorption capacity and reactivity of different Gram-positive and –negative bacteria roughly compare. This finding led them to develop a universal adsorption edge model, which considers similar reactivity for all bacteria. Although helpful in practice, this approach appears surprising on a first look, specifically with regard to the different structures involved in the cell wall of Gram –positive and –negative bacteria. To validate this conceptualization, innovative approaches coupling macroscopic, microscopic and spectroscopic studies are needed to assess both reactivity and structure of reactive sites on bacterial membranes. In the following section an overview of the use of a multidisciplinary approach combining spectroscopy, microscopy, wet chemistry and microbiology to elucidate the interaction mechanisms between uranium and bacterial strains isolated from uranium contaminated sites and other extreme habitats will be given.

2. Interaction mechanisms between bacteria and metals: fundamental research

The present work describes the use of a combination of wet chemistry, spectroscopic, and microscopic methods to characterize the environment of uranium around the cells of bacterial strains isolated form extreme habitats as well as to elucidate the interaction mechanisms of these bacteria with this radionuclide. We begin with a short discourse of the different spectroscopic and microscopic methods used. In the remaining portion selected examples of interaction of bacteria with uranium at molecular scale performed at our Group will be presented.

2.1 X-ray absorption spectroscopy (XAS)

Sine X-ray absorption spectroscopy (XAS) was introduced over 30 years ago as an element-specific local structure probe [33], it has become the technique of choice for determining the local coordination environment of specific elements in solids (both amorphous and crystalline) and liquids at low concentrations, where X-ray and neutrons diffraction methods are relatively insensitive. Because of this attribute and the ability to examine samples under *in situ* conditions with minimal preparation, XAS spectroscopy has become the most important method for determining the speciation of environmental pollutants such as uranium, zinc, lead and arsenic, which are often present in complex, multiphase, natural samples at concentrations ranging from greater than a weight percent to less than a ppm in a variety of chemical forms [34]. XAS is a nondestructive method, and no sample reduction or digestion is required which would alter the chemistry of the element of interest. This synchrotron-based technique has been used to determine the oxidation state (X-ray absorption near edge spectroscopy, XANES) and to identify the number of atoms, and their distances in the local structural environment (extended Y-ray absorption fine structure spectroscopy, EXAFS) of metals within a variety of microbial samples [14-16, 25].

2.2 Time resolved induced laser fluorescence spectroscopy (TRLFS)

TRLFS is an excellent tool for study the complex formation of radionuclides (e.g. uranium, americium, and curium) and lanthanides (e.g. europium) with various ligands at relatively low concentrations. TRLFS is a very sensitive method which can give insight into complexation reactions, even with poorly defined polyfunctional ligands such as living bacterial cells. This method is inappropriate for metal ions which show no change in their spectroscopic proprieties with complexation. TRLFS provide information on life time and spectral characteristics of species, which allows deriving information on the number of different species and their spectral identity. It is based on the fact that the measured fluorescence lifetime and intensity of the electronic transition of the excited metal ions are dependent on their molecular environment [35]. This method may therefore provide information complementary to XAS spectroscopy. The use of this technique in studying the interaction of metal ions with bacteria [36] and plants [37] is well documented.

2.3 Transmission electron microscope (TEM)

TEM is a useful technique that can help to localize and to identify metals/radionuclides deposited within or around microbial cells. Identification of the site of accumulation is important as it can give clues to the biochemical mechanisms driving metal accumulation. A high-energy electron beam, when passed through a sample in a TEM, is deflected by high mass elements including metals and radionuclides through the electron shells or nuclei. Biological materials which are largely composed of light elements, such as C, N, H, O, P and S, do not deflect the electron beam to the same degree. Thus it is possible to visualize radionuclides (and metals) against the faint image of a bacterial cell [38].

2.4 Interactions of bacterial isolates with uranium

Cultivation-based methods were used in our Group to isolate bacteria form extreme habitats including uranium mining waste piles, heavy metals contaminated waters as well as the ground water of the deep-well monitoring site S15 of the Siberian radioactive waste depository Tomsk-7, Russia [4, 5, 12-15, 17]. Different bacterial strains belonging to different groups were cultivated and physiologically characterized.

Chemolithoautrophic bacteria, such as *Acidithiobacillus ferrooxidans* were isolated from different uranium mining waste piles [4, 6, 13, 14]. The mechanisms and genetic basis for sequestration of uranium by eco-types of this bacterium were investigated using a combination of spectroscopic (XAS, Infrared, TRLFS), microscopic (TEM) and microbiological techniques as well as sorption and tolerance studies.

The uranium sorption studies demonstrated that the strains from these types possess different capability to accumulate and tolerate uranium. The amount of uranium bound to the biomass increases in the order *A. ferrooxidans* type I, type III and type II. Interestingly, the strains belonging to the types I and III are resistant to 8 and 9 mM of uranium, respectively, whereas those of the type II does not tolerate more than 2 mM of uranium [13]. On the basis of the results presented one may speculate that the strains of the type I and III are more resistant to uranium, probably because they possess a mechanism which limits the uranium binding below the lethal amounts. Such physiological differences (tolerance to uranium) between the different *A. ferrooxidans* types could explain their heterogeneous distribution in the uranium mining waste piles: The group of isolates belonging to types I and III were predominant in more contaminated samples from greater depths, while the representatives of the type II were distributed in less contaminated area preferably close to the surface. In uranium mining waste piles, all *A. ferrooxidans* types are present together, by increasing the concentration of uranium and other heavy metals, adaptation of the type I and III in these environments occurs by natural selection, consequently the later two types outgrow its competitors (representatives of the type II) and dominate the population. There are many reports on the adaptation and dominance of microbial population in acidic environments [39, 40]. These reports have tended to focus on competition between mesophilic iron-oxidising chemolithotrophs (*A. ferrooxidans* and *Leptospirillum ferrooxidans*) for ferrous iron and mineral oxidation [41]. Because of its greater affinity for ferrous iron, tolerance of very low pH (< 1.8) and greater tolerance of ferric iron, *L. ferrooxidans* tends to be more effective when leaching ores (e.g. gold concentrates) which are rich in pyrite, or in environments where ferrous iron concentration and/or pH are low. In contrast, the faster growth rate of *A. ferrooxidans* generally results in this iron-oxidiser dominating situations (such as enrichment cultures used frequently to isolate iron-oxidising acidophiles) where ferrous iron concentrations are relatively high and/or pH is greater than 2 [42].
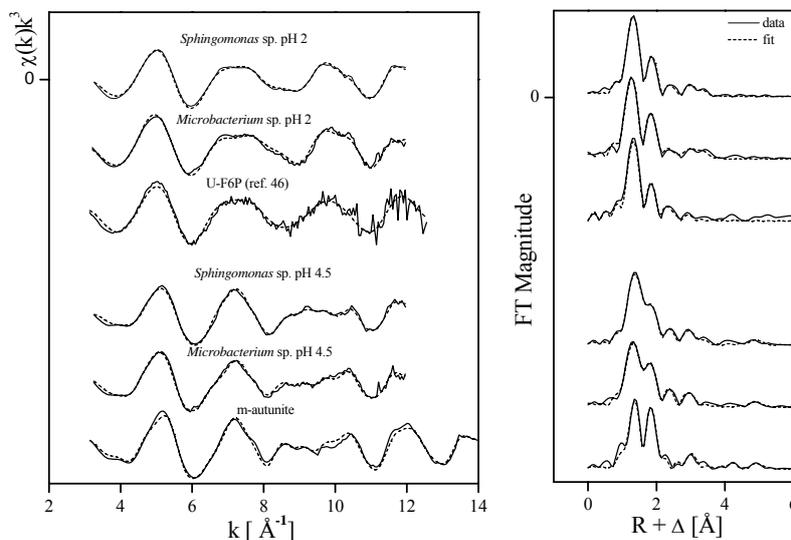
Fig. 1 Uranium L_{III}-edge k^3–weighted EXAFS spectra (left) and the corresponding fourier transforms (FT) (right) of the uranium complexes formed by the cells of *Microbacterium* sp. at pH values 2, 3 and 4.5, as well as of the reference compounds (m-autunite and U-fructose(6)P [46]).

The cellular localization of the uranium bound by the cells of three types of *A. ferrooxidans* was studied using TEM. The results indicated that the accumulated uranium is located mainly within the extracellular polysaccharide (EPS) and on the cell wall. However, some intracellular uranium accumulates associated with the polyphosphate bodies were also identified in the cytoplasm of the bacterial cells [14, 24]. TRLFS studies demonstrated that the uranium complexes built by the three eco-types of *A. ferrooxidans* have different lifetimes. These differences could be explained by the fact that the eco-type II forms very strong complexes (higher formation constants) with uranium and the lifetime of the corresponding uranyl complexes obtained are longer in comparison with the eco-types I and III which form significantly weaker uranium complexes with shorter lifetimes [43]. In addition, the amount of the uranium bound increases increasing the formation constants values (mass action law). Therefore the amount of uranium accumulated by the three bacterial eco-types increases with the increase of lifetime of the corresponding uranium complexes [43]. The energy of the emission bands of the U/*A. ferrooxidans* complexes (496, 517, 541, 566 nm) are almost identical with the energies obtained for the uranyl organic phosphate compounds such as (U(VI)-ATP) (495, 517, 540, 565 nm) and (U(VI)-AMP) (497, 519, 542, 569 nm) and different from those obtained for the uranyl inorganic phosphate compounds [44]. These results are in agreement with those obtained by EXAFS spectroscopy, which demonstrated that the cellular organic phosphates are the main groups implicated in the binding of uranium. In addition, no structural differences were found between the uranium complexes formed by representatives of three eco-types of *A. ferrooxidans* at pH 2, 3 and 4.5.

However, the structure uranium complexes formed by the cells of other bacterial strains (*Microbacterium sp.* and *Sphingomonas* sp.) isolated from S15 deep-well monitoring site, located at the Siberan radioactive subsurface depository Tomsk-7, Russia are dependent of the pH (Fig. 1). Thus, EXAFS studies demonstrated that at pH 2 uranium formed complexes with organically bound phosphate at the cell surface such uranium complexes formed with fructose 6-phosphate [45], while at pH 4.5 , this radionuclide was precipitated with inorganic phosphate as meta-autunite like precipitates. At pH 3, both organic and inorganic phosphate uranyl species occurred together, dominating the inorganic phase. To our knowledge, there are only a few spectroscopic studies on the effect of pH on the speciation and association of U to bacterial cells. Kelly et al. [46] used EXAFS spectroscopy to study the effect of this environmental parameter on the complexation of U with the soil bacterium *B. subtilis*. The authors found that at pH 1.67, U is bound to the phosphate groups of the cell surfaces, in agreement with our studies at pH

2. However, at the higher pH value of 4.5 the complexation of U by the cells of *Microbacterium* strain differed significantly from those by the *Bacillus* cells.

TEM analyses, which demonstrated the cellular localization of the accumulated uranium in the studied *Microbacterium* and *Sphingomonas* strains, supported the findings from the EXAFS studies. The cells were able to precipitate uranium in the bulk phase at pH 4.5. However, the cellular localization of U precipitates on the cells of the two bacterial isolates are different as was shown by TEM analysis (Fig. 2) suggesting that different processes were possibly involved in uranium immobilization by the two types of bacteria. TEM observations of *Sphingomonas* sp. cells exposed to uranium solution allowed detection of electron-dense accumulates essentially within the cells as electron-dense granules and at the cell membranes as uranium-bearing precipitates. We suggest that intracellular granules correspond to polyphosphate bodies since the composition of these granules is comparable to that of poly P in other microorganisms studied by TEM [14, 26]. It has been proposed that polyphosphate sequesters the heavy metals, thereby reducing their intracellular concentration, and the other hand, that the hydrolysis of polyphosphate detoxifies the metals [47]. Renninger *et al*. [28] demonstrated that controlled polyphosphate metabolism can be used to sequester large quantities of phosphate in the form of intracellular polyphosphate, degrade the polyphosphate, secrete the resulting phosphate from the cell, and precipitate uranyl phosphate on the cell wall. These results are in line with those found in this work using EXAFS spectroscopy and TEM analysis indicating that the cells of *Sphingomonas sp*. precipitate U at the cell membrane as uranyl phosphate as phosphate was released from the cells after degradation of polyphosphate bodies.
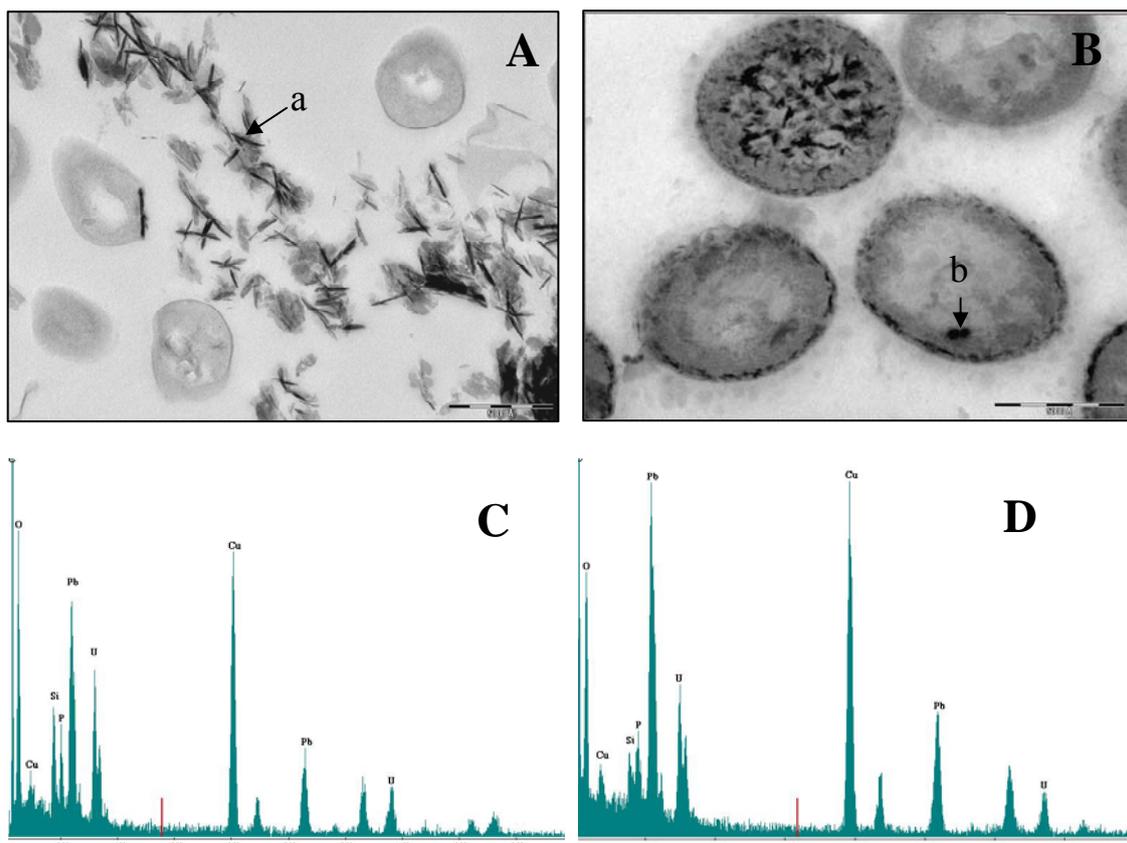

Fig. 2 Transmission electron micrographs of thin sections of *Microbacterium* sp. (A) and *Sphingomonas* sp. (B) cells treated with uranium at pH 4.5. Scale bars are: A and B = 0.5 μm.
C, and D are the energy dispersive X-ray spectra of the U accumulates marked with arrowheads (a) and (b) respectively.

In the case of *M. oxydans*, no intracellular accumulation of U was detected, excluding the implication of polyphosphate bodies in the interaction with this metal, which is precipitated via phosphate groups in a form of m-autunite extracellularly and at the cell surface, probably by the activity of nonspecific microbial acidic phosphatase. Precipitation of uranium and other heavy metals, through the use of membrane-bound acidic phosphatase was reported for *Citrobacter* sp. [27, 48].

In addition to the above described isolates, we could cultivate a *Bacillus sphaericus* strain, JG-A12, from a uranium mining waste pile near the town of Johanngeorgenstadt in Saxony, Germany. The interaction of this strain with 19 heavy metals (Al, Ba, Cd, Co, Cr, Cs, Cu, Fe, Ga, Mn, Ni, Rb, Si, Sn, Sr, Ti, U, and Zn) was investigated. The results of these studies demonstrated that this strain selectively and reversibly accumulates U, Cu, Pb, Al, and Cd [12]. The cells of this bacterium are enveloped by a highly ordered crystalline proteinaceous surface layer (S-layer) that differs significantly in its primary structure from the other *B. sphaericus* S-layers studied to date [49]. Purified and recrystallized S-layer proteins of this bacterium were shown to be phosphorylated by phosphoprotein-specific staining, inductive coupled plasma-mass spectrometry (ICP-MS) analysis, and a colorimetric method. Compared to the S-layer protein of the uranium mining waste pile *B. sphaericus* JG-A12, the S-layer protein of its closed relative, the reference strain *B. sphaericus* NCTC 9602, contains about one sixth as much phosphorus [15]. This difference may reflect an adaptation of *B. sphaericus* JG-A12 to the extensive uranium contamination of its environment. The high affinity of phosphate groups on the surface of the cell may allow *B. sphaericus* JG-A12 to selectively bind large amounts of uranium before being damaged by this toxic radionuclide.

We used extended X-ray absorption fine structure (EXAFS) spectroscopy to determine the structural parameters of the uranium complexes formed by purified and recrystallized S-layer sheets of *B. sphaericus* JG-A12. In addition, we investigated the complexation of uranium by the vegetative bacterial cells. The EXAFS analysis demonstrated that in all samples studied (cells and purified S-layer protein), the U(VI) is coordinated to carboxyl groups in a bidentate fashion with an average distance between the U atom and the C atom of 2.88 ± 0.02 Å, and to phosphate groups in a monodentate fashion with an average distance between the U atom and the P atom of 3.62 ± 0.02 Å[15].

In the case of isolated S-layer proteins, previous studies [50] revealed that the amino acid composition of this surface protein included a high content of glutamic and aspartic acids, and of other amino acids such as serine and threonine. The C-terminal part especially consists of stretches of glutamic acid and aspartic acid (both residues with carboxyl groups) and of serine and threonine (both residues with hydroxyl groups), the latter being potential phosphorylation sites. The carboxyl groups and phosphate groups of these amino acids are probably implicated in the complexation of uranium. The participation of the carboxyl groups of this S-layer in the binding of palladium has been demonstrated using EXAFS spectroscopy and ATR-FT-Infrared spectroscopy [15].

TEM analysis showed that U accumulated by the cells of *B. sphaericus* JG-A12 is located at the cell surface (S-layer sheets and cell wall). No intracellular accumulation of U was detected. Because of the ability of S-layer to self-assemble and replace the "older" S-layer sheets on the cell surface, one can speculate about the mechanism of its protective function against uranium and other toxic metals. The saturation with metals (in this case uranium, as was demonstrated by TEM studies) may lead to denaturation of the S-layer lattice, which is then replaced by freshly synthesized protein monomers.

Summarizing, the present study gives an overview on different kinds of interaction mechanisms between U and bacterial strains isolated from extreme habitats. The combination of different spectroscopic, microscopic and microbiological methods indicated that bacterial isolates interact with uranium in different ways. The main interaction mechanisms included, binding to organic phosphate and carboxyl groups (*B. sphaericus* JG-A12), chelation by polyphosphate bodies (*Sphingomonas* sp., *A. ferrooxidans*), precipitation of U as inorganic phosphate mineral phase (*Microbacterium* sp., *Sphingomonas* sp. etc.). The latter mechanism (uranium precipitation) has the advantage of producing chemically stable forms, and its use is not limited to reducible metals. The information obtained is useful in predicting the fate and transport of radionuclides in near-surface environments as well as in using these bacteria for bioremediation of actinide-contaminated sites and in the field of nanotechnology.

3. Interactions metals/bacteria: applied research

Investigation of the microbe-metal interactions provides insight into the potential of micro-organisms to alter toxicity of radionuclides and heavy metals, and to influence their behavior in the environment. Understanding the underlying mechanisms of these interactions is important for the development of bioremediation strategies [51] as well as the use of microbes and their biocompenents as templates for the formation of metallic nanoparticles with industrial applications. In this work, two examples of the use of isolated bacterial strains and their surface layer proteins in the bioremediation of heavy metal contaminated waters and in the filed of nanotechnology will be summarized.

3.1 Bioremediation of heavy metal contaminated waters

Water pollution due to toxic heavy metals remains a serious environmental and public problem. Strict environmental regulations on the discharge of heavy metals make it necessary to develop various efficient technologies for their removal. The main techniques that have been used to reduce the heavy metal content of effluents include chemical precipitation, ion exchange, adsorption onto activated carbon, membrane processes, and electrolytic methods. These methods have been found to be limited, because they often involve high operational costs or may also be insufficient to meet strict regulatory requirements as for chemical precipitation [52]. Therefore numerous approaches have been studied for the development of cheaper and effective metal sorbents, such as microbial cells. However, Microbial biomass in its native form is not suitable for large-scale process utilization [53]. It may be necessary to immobilize the biomass, especially when microorganisms of small particle are to be used [54]. Immobilization procedure converts biomass to particulate form for use as a conventional adsorbent with desired size range, high porosity, and better chemical and physical performance. Agents used for immobilization of biosorbents include alginate, silica, and polyacrylamide [55]. To use biocomponents as effective parts of filter materials in bioremediation processes for cleaning radionuclide and heavy metal contaminated drain waters of different environments, the pH-stability of the biological components, the possibility of a repeated use of the filter material and the immobilisation of the biocomponents is of special importance. Sol-gel technology allows the immobilisation of various biomolecules or microorganisms without loosing their activity and structure [56, 57]. Other advantages of sol-gel ceramics are a high mechanical, thermal and photochemical stability, biological and toxical intertness and controlled matrix porosity. This technology was applied to produce a metal selective filter material for bioremediation processes. Cells, spores and S-layers of *B. sphaericus* JG-A12, strain isolated from a uranium mining waste, were embedded in silica gels using an aqueous sol-gel process to produce a porous filter matrix with a homogeneous structure and completely immobilized biocomponents (biocer) [58]. After the encapsulation of the different JG-A12 biocomponents, the biomaterial still retained its metal accumulating capacities. The produced biocers were successfully used to remove copper and uranium from contaminated water. The binding of the metals is reversible and both metals can be completely removed by using aqueous citric acid. Cells, spores and S-layers of JG-A12 were found to be stable in acidic drain water at pH 4 and below. In conclusion, due to the high stability of the biocers, the safe immobilization of the biocomponents, the high metal binding capacity and the simple and complete removal of the bound metals, the biocers are well suited for the reversible usage for bioremediation purposes without influencing the binding capacity.

3.2 Nanotechnology

The area of nanotechnology encompasses the synthesis of nanoscale materials, the understanding and the utililization of their often exotic physicochemical and optoelectronic properties, and the organization of nanoscale structures into predefined superstructures. Thus, nanotechnology promises to play an increasingly important role in many key technologies of the new millenium [59]. As for the synthesis of nanoparticles, there is an ever-growing need to develop clean, non-toxic, and environmentally friendly

("green chemistry") synthetic procedures. Consequently, researchers in the field of nanoparticle preparation have been looking at biological systems for inspiration. Several biological materials have been successfully used for the fabrication of ordered nanoparticles arrays. Silver, gold, palladium, platinum or copper nanoparticles have been deposited on DNA templates by the reduction of metal ions or metal complexes associated with DNA [60, 61]. Other promising biological templates for the production of metal nanoclusters are the regularly structured paracrystalline surface layers of bacteria and archaea [62]. The crystalline bacterial cell S-layers represents the outermost cell envelope component of many bacteria and archaea [63]. S-layers are generally composed of identical protein or glycoprotein subunits, and they completely cover the cell surface during all stages of bacterial growth and division. Most S-layers are 5-15 nm thick and possess pores of identical size and morphology in the range of 2 to 6 nm. The regular distributed pores of the crystalline arrays work as bonding sites for various metals and offer ideal structures for the formation of regular distributed nanoclusters of a defined size. S-layers have been used as templates for the fabrication of different inorganic nanocrystals arrays. They act as template for the nucleation of ordered two-dimensional arrays of CdS nanocrystals [64]. The S-layer lattices of B. sphaericus CCM 2177 were used to produce gold nanoclusters by exposing the S-layer lattices, in which thiol groups have been introduced before, to a tetrachloroauric(III) acid solution under electron radiation [62]. The cells and the purified S-layer sheets of B. sphaericus JG-A12 (natural isolate recovered from a uranium mining waste pile near the town of Johanngeorgenstadt in Saxony, Germany) are able to complex Pd(II) from a solution of Na2PdCl4[65]. The bound Pd(II)-complexes can be reduced to Pd(0)-nanoclustes by the addition of H_2 as an electron donor. The formation of these Pd nanoclusters was confirmed by EXAFS analysis. The fabricated nanoparticles consisted of 19-43 atoms with a diameter range between 0.85 - 1 nm formed at a layer of nearest neighbors around a central atom [66]. TEM analysis indicated that the produced Pd nanoclusters are localized mainly at the cell surface (Fig. 3). The formed Pd-nanoparticles were used as catalysts to reduce toxic Cr(VI) to Cr(III). S-layer supported nanoparticles showed a significantly higher catalytic activity in comparison to cells supported Pd(0) or bulk Pd(0) [67].

In addition, the S-layer of this bacterium were used also as templates for the deposition of metallic platinum nanoclusters using dimethyl amino borane (DMAB) as reducing agent. XAS analysis indicated that the formed Pt nanopartciles have a mean diameter of about 2.5 to 3.5 nm (personal communication). In the case of gold, XANES, EXAFS, UV-vis spectroscopy and X-ray powder diffraction showed that in the presence of H_2 as electron donor, the cells and purified S-layer protein of B. sphaericus JG-A12 are able to form gold nanoclusters. EXAFS studies indicated that the sizes of these clusters are about 1 nm [68].
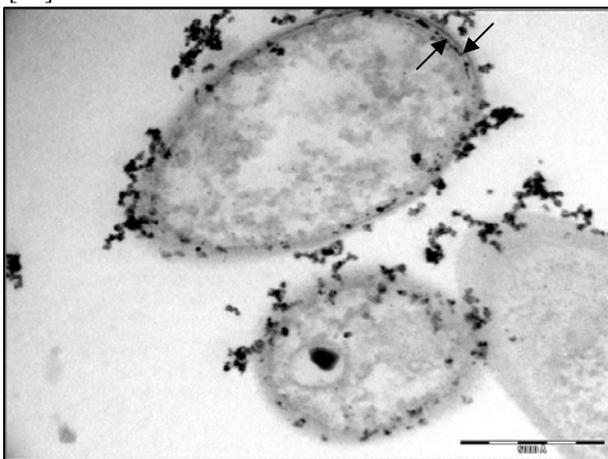

Fig. 3 TEM image of a thin section of Pd treated *B. sphaericus* JG-A12 cells after addition of H_2 as reducing agent. The Pd nanoparticles are localized mainly at the cell surface. Scale bar: 0.5 μm.

Natural microbial communities in the uranium contaminated environments are complex and dynamic, and many of the components organisms are known to have profound effects on the geochemical behaviour of metallic elements including uranium. Certain bacterial strains can facilitate the precipitation of

solid U phases (e.g. m-autunite phase) reducing the bioavailability of this radionuclide in the environment. Other strains bind it at the cell surface through phosphate and carboxylic groups, thereby increasing its mobility. Understanding the mechanisms of interactions opens up new prospects for the development of applications in bioremediation and nanotechnology. Therefore, the cells and surface layer of a uranium mining waste with the ability to accumulate toxic metals such as uranium make this strain and its surface protein a good candidate for the development of a filter material (biocer), based on a sol-gel matrix, to clean-up uranium contaminated waters. In addition, the cells and S-layers are able to bind metal ions and complexes from metal salt solutions such as Pd(II), Pt(II) and Au(III) that can be reduced to the corresponding metal nanoparticles by the addition of reducing agents such as H_2 or DMAB. Thus, the biomaterial can be used for the recovery of precious metals from industrial waste waters and for the synthesis of ordered nanoparticle arrays, which are promising for the development of novel catalysts.

References

[1] Y. Suzuki, S.D. Kelly, K.M. Kemner, and J.F. Banfield, Applied and Environmental Microbiology 71, 1797 (2005).
[2] S. Stroes-Gascoyne, and J.M. West, FEMS Microbiology Review*s* 20, 573 (1997).
[3] C. Anderson, and K. Pedersen, Geobiology 1, 169 (2003).
[4] S. Selenska-Pobell, Interactions of Microorganisms with Radionuclides (Elsevier Sciences Ltd Oxford, UK 2002), pp. 225-254.
[5] V. Torsvik, and L. Øvreås, Current Opinion in Microbiology 5, 240 (2002).
[6] S. Selenska-Pobell, K. Flemming, G. Kampf, G. Radeva, and G. Satchanska, Antonie van Leeuwenhoek 79, 149 (2001).
[7] G. Satchanska, E. Golovinsky, and S. Selenska-Pobell, Comptes Rendus de l´Académie Bulgare des Sciences 57, 75 (2004).
[8] G. Satchanska, and S. Selenska-Pobell, Comptes Rendus de l´Académie Bulgare des Sciences 58, 1105 (2005).
[9] G. Radeva, and S. Selenska-Pobell, Canadian Journal of Microbiology 51, 910 (2005).
[10] M.W. Fields, T. Yan, S-K, Rhee, S.L. Carroll, P.M. Jardine, D.B. Watson, C.S. Criddle, and J. Zhou, FEMS Microbiology Ecology 53, 417 (2005).
[11] D.R. Lovley, Nature Reviews Microbiology 1, 35 (2003).
[12] S. Selenska-Pobell, P. Panak, V. Miteva, I. Boudakov, G. Bernhard, and H. Nitsche, FEMS Microbiology Ecology 29, 59 (1999).
[13] M.L. Merroun, and S. Selenska-Pobell, Biometals 14, 171 (2001).
[14] M.L. Merroun, C. Hennig, A. Rossberg, T. Reich, and S. Selenska-Pobell, Radiochimica Acta 91, 583 (2003).
[15] M.L. Merroun, J. Raff, A. Rossberg, C. Hennig, T. Reich, and S. Selenska-Pobell, Applied and Environmental Microbiology 71, 5532 (2005).
[16] M.L. Merroun, M. Nedelkova, A. Rossberg, C. Hennig, and S. Selenska-Pobell, Radiochimica Acta 94, 723 (2006).
[17] M. Nedelkova, M.L. Merroun, A. Rossberg, C. Hennig, and S. Selenska-Pobell, FEMS Microbiology Ecology 59, 694 (2007).
[18] M.L. Merroun, K. Ben chekroun, J.M. Arias, and M.T. González-Muñoz, Chemosphere 52, 113 (2003).
[19] M.L. Merroun, N. Ben Omar, E. Alonso, J.M. Arias, M.T. González-Muñoz, Geomicrobiology Journal 18:2, 183 (2001).
[20] M.L. Merroun, M.L., N. Ben Omar, M.T. González-Muñoz, and J.M. Arias, Journal of Applied Microbiology 84, 63 (1998).

[21] A.J. Francis, Journal of Alloys and Compounds 271-273, 78 (1998).

[22] D. Lovley, Annual Review of Microbiology 47, 263 (1993).

[23] H.R. Beller, Applied and Environmental Microbiology 71, 2170 (2005).

[24] M.L. Merroun, C. Hennig, A. Rossberg, G. Geipel, T. Reich, S. Selenska-Pobell, Biochemical Society Transactions 30, 669 (2002).

[25] A. Francis, J.B. Gillow, C.J. Dodge, R. Harris, T.J. Beveridge, and H.W. Papenguth, Radiochimica Acta 92, 481 (2004).

[26] Y. Suzuki, and J.F. Banfield, Geomicrobiology Journal 21, 113 (2004).

[27] L.E. Macaskie, R.M. Empson, A.K. Cheetham, C.P. Grey, and A.J. Skarnulis, Science 257, 782 (1992).

[28] N. Renninger, R. Knopp, H. Nitsche, D.S. Clark, and J.D. Keasling, Applied and Environmental Microbiology 70, 7404 (2004).

[29] K. Bosecker, FEMS Microbiology Reviews 20, 591 (1997).

[30] T.J. Beveridge, and R. Murray, Journal of Bacteriology 141, 876 (1980)

[31] J.B. Fein, C. Daughney, N. Yee, and T.A. Davis, Geochimica et Cosmoschimica Acta 61, 3319 (1997).

[32] N. Yee, and J.B. Fein, Geochimica et Cosmoschimica Acta 65, 2037 (2001).

[33] D.E. Sayers, E.A. Stern, and F.W. Lytle, Physical Review Letters 27, 1204 (1971).

[34] G.E. Jr. Brown, and N.C. Sturchio, Reviews in Mineralogy and Geochemistry 49, 1 (2001)

[35] A. Rustenholtz, I. Billard, G. Duplatre, K. Lützenkirchen, and L. Sémon, Radiochimica Acta 89, 83 (2001).

[36] H. Moll, Th. Stumpf, M.L. Merroun, A. Roßberg, S. Selenska-Pobell, and G. Bernhard, Environmental Science & Technology 38, 1455 (2004).

[37] A. Günther, G. Bernhard, G. Geipel, T. Reich, A. Rossberg, and H. Nitsche, Radiochimica Acta 91, 319 (2003).

[38] J.R. Lloyd, and L.E. Macaskie, Environmental Microbe-Metal Interactions (American Society for Microbiology Press, Washington, DC 2002).

[39] P.L. Bond, G.K. Druschel, and J.F. Banfield, Applied and Environmental Microbiology 66, 4962 (2000).

[40] D. Fortin, M. Roy, J.P. Rioux, and P.J. Thibault, FEMS Microbiology Ecology 33, 197 (2000).

[41] P.R. Norris, D.W. Barr, and D. Hinson, Biohydrometallurgy: Proceedings of the International Symposium, Warwick 1987, UK, pp. 43-59.

[42] D.B. Johnson, FEMS Microbiology Ecology 27, 307 (1998).

[43] M.L. Merroun, G. Geipel, R. Nicolai, K-H. Heise, and S. Selenska-Pobell, BioMetals 16, 2 331 (2003).

[44] P. Panak, J. Raff, S. Selenska-Pobell, G. Geipel, G. Bernhard, and H. Nitsche, Radiochimica Acta 88, 71 (2000).

[45] A. Koban, G. Geipel, A. Roßberg, G. Bernhard, Radiochimica Acta 92, 903 (2004).

[46] S.D. Kelly, K.M. Kemner, J.B. Fein, D.A. Fowle, M.I. Boyanov, B.A. Bunker, and N. Yee, Geochimica et Cosmoschimica Acta 65, 3855 (2002).

[47] L.E. Macaskie, and A.C.R. Dean, Technology Letters 3, 49 (1982).

[48] C.D. Boswell, R.E. Dick, H. Eccles, and L.E. Macaskie, Journal of Industrial Microbiology and Biotechnology 26, 333 (2001).

[49] J. Raff, Ph.D. thesis. University of Leipzig, Leipzig, Germany, (2002).

[50] K. Pollmann, J. Raff, M. Schnorpfeil, G. Radeva, and S. Selenska-Pobell, Microbiology 151, 2961(2005).

[51] K. Finneran, R. Anderson, K. Nevin, and D. Lovley, Soil and Sediment Contamination 11, 339 (2002).

[52] Z. Reddad, C. Gerente, Y. Andres, and P. Le Cloirec, Environmental Science & Technology 36, 2067 (2002).

[53] M. Tzesos, Immobilization of Ions by Biosorption, (Ellis Horwood, Chischester 1986).

[54] S. Schiewer, and B. Volesky, (ASM Press, Washington, D.C. 2000).

[55] G.W. Bedell, and D.W. Darnall, (CRC Press, Inc., Boca Raton, Florida 1990).

[56] H. Böttcher, Journal für Praktische Chemie 342, 427 (2000).

[57] J. Livage, T. Coradin, and C. Roux, Journal of Physics: Condensed Matter 13, R673 (2001).

[58] J. Raff, U. Soltmann, S. Matys, S. Selenska-Pobell, H. Boettcher, and W. Pompe, Chemistry of Materials 15, 240 (2003).

[59] G. Schmid, Clusters and colloids from theory to applications, (Wiley-VCH, Weinheim, 1994).

[60] E. Braun, Y. Eichen, U. Sivan, G. Ben-Yoseph, Nature 391, 775 (1998).

[61] M. Mertig, R. Kirsch, W. Pompe, H. Engelhardt, European Physical Journal 9, 45(1999).

[62] S. Dieluweit, D. Pum, U.B. Sleytr, Supramolecular Science 5, 15 (1998).

[63] M. Sára, and U. B. Sleytr, Journal of Bacteriology 182, 859 (2000).

[64] W. Shenton, D. Pum, U.B. Sleytr, and S. Mann, Nature 389, 585 (1997).

[65] K. Fahmy, M.L. Merroun, K. Pollmann, J. Raff, O. Savchuk, C. Hennig, and S. Selenska-Pobell, Biophysical Journal 91, 996 (2006).

[66] M. Merroun, K. Pollmann, J. Raff, A.C. Scheinost, and S. Selenska-Pobell, FZR-Report 400, 25 (2003).
[67] K. Pollmann, J. Raff, A. Mücklich, S. Selenska-Pobell, FZD-Report 459, 47 (2007).
[68] M.L. Merroun, A. Rossberg, C. Hennig, A.C. Scheinost, and S. Selenska-Pobell, Materials Science and Engineering C. 27, 188 (2007).