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Isolation and Analyses of Uranium Tolerant *Serratia marcescens* Strains and Their Utilization for Aerobic Uranium U(VI) Bioadsorption

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Enrichment-based methods targeted at uranium-tolerant populations among the culturable, aerobic, chemoheterotrophic bacteria from the subsurface soils of Domiasiat (India's largest sandstone-type uranium deposits, containing an average ore grade of 0.1% U_3O_8), indicated a wide occurrence of *Serratia marcescens*. Five representative *S. marcescens* isolates were characterized by a polyphasic taxonomic approach. The phylogenetic analyses of 16S rRNA gene sequences showed their relatedness to *S. marcescens* ATCC 13880 (≥99.4% similarity). Biochemical characteristics and random amplified polymorphic DNA profiles revealed significant differences among the representative isolates and the type strain as well. The minimum inhibitory concentration for uranium U(VI) exhibited by these natural isolates was found to range from 3.5-4.0 mM. On evaluation for their uranyl adsorption properties, it was found that all these isolates were able to remove nearly 90-92% (21-22 mg/L) and 60-70% (285-335 mg/L) of U(VI) on being challenged with 100 μM (23.8 mg/L) and 2 mM (476 mg/L) uranyl nitrate solutions, respectively, at pH 3.5 within 10 min of exposure. his capacity was retained by the isolates even after 24 h of incubation. Viability tests confirmed the tolerance of these isolates to toxic concentrations of soluble uranium U(VI) at pH 3.5. This is among the first studies to report uranium-tolerant aerobic chemoheterotrophs obtained from the pristine uranium ore-bearing site of Domiasiat.

Keywords: uranium-rich area, uranium-tolerant bacteria, *Serratia marcescens*, adsorption

Domiasiat (25°30′N, 91°30′E), located 130 km southwest of Shillong (capital city of Meghalaya) in North East India, forms a part of the Cretaceous Mahadek basin covering roughly an area of 13000 sq. km. It is one of the largest sandstone-type uranium deposits in India containing 9.22 million tonnes of ore reserves, with an average ore grade of 0.1% U_3O_8 (Raju *et al.*, 1989; Sengupta *et al.*, 1991).

Radionuclides/metal-enriched reserves, as the one mentioned above, are known to be colonized by metal-resistant and/or accumulating microbial strains (Francis *et al*., 2004; Schmidt *et al*., 2005; Ohnuki *et al.*, 2005; Pollmann *et al*., 2006). Such environments result in adaptation of microorganisms, which influences the migration of radionuclides/heavy metals through biosorption, bioprecipitation, extracellular sequestration, transport mechanisms, and/or chelation. These mechanisms serve as the basis for bioremediation of metal-contaminated areas (Haferburg and Kothe, 2007). Radionuclides like uranium are potentially toxic to microbes due to their chemical and radiological activity. However, in the case of highly radiation-tolerant *Deinococcus radiodurans,* actinides have been proven to be less toxic than transition metals such as cadmium, chromium, zinc, copper, etc. (Ruggiero *et al*., 2005). The chemical tolerance of microbes to radionuclides/heavy metals rather than radiation tolerance is therefore preferable for remediation of metal contamination. The naturally occurring organisms in metal-contaminated sites possess the radiation tolerance required for their survival and growth in those sites. Natural isolates exhibiting (a) multimetal and actinide tolerance, (b) acid tolerance, and (c) aerobic metabolism hold promise for *in situ* bioremediation of actinide and metal-contaminated areas. Immobilisation of metals/radionuclides by bioadsorption is a possible remediation strategy in such areas (Choudhary and Sar, 2009).

The present investigation was aimed at isolating 5 *Serratia marcescens* strains representing the uranium-tolerant *Serratia* population recovered from the subsurface soils of a pristine uranium ore-bearing site. A polyphasic taxonomic approach was undertaken to characterize these 5 *S. marcescens* isolates and compare them to a type strain, *S. marcescens* ATCC 13880. The minimum inhibitory concentration (MIC) for uranium was determined for these isolates. A working hypothesis relating to the adaptation of the naturally occurring microorganisms in metal/radionuclide-contaminated sites, through their resistance mechanisms leading to the development of superior metal-resistant variants, was followed in this investigation. Our study was further extended to examine the efficiency of such isolates for uranyl adsorption followed by evaluation of their viability at toxic concentrations of uranium. To the best of our knowledge, this is the first study to report uranium tolerance of aerobic chemoheterotrophic *S. marcescens* strains isolated from subsurface soils of the uranium ore-bearing site at Domiasiat in North East India.

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Materials and Methods

Source of bacterial isolates

Subsurface soils (15 cm deep to the top layer of the soils) were collected from the uranium-rich Domiasiat (25°30′N, 91°30′E) area of Meghalaya in North East India. The Upper Cretaceous lower Mahadek sandstone forms a major lithological unit at Domiasiat. Rich uranium deposits were reported in this lower Mahadek sandstone formation in the late 1980s by the Atomic Mineral Directorate for Exploration and Research, India (Raju *et al.*, 1989; Sengupta *et al.*, 1991). Uranium-containing host rock in this region is arkose (feldspar-rich) sandstone with an age of about 65-135 Ma (Upper Cretaceous age group). The main uranium ores are pitchblende and coffinite. This region is a proposed mining site for uranium exploration and the soil samples were collected from designated mining sites of the Uranium Corporation of India Ltd. The pH of the soil was acidic and within the range of 4.3-6.3.

Screening and isolation of subsurface uranium-tolerant bacteria

Duplicate soil samples (10 g) were inoculated in 100 ml of tryptone soy broth (diluted to 0.5%) (HiMedia, India) (pH 7) in Erlenmeyer flasks amended with 1 mM uranium U(VI) as $[UO_2(NO_3)_2{\cdot}6H_2O]$ and incubated at 32°C, at 150 rpm, for 24 h. Serial 10-fold dilutions of these enrichment cultures were inoculated onto the tryptone soy agar (diluted to 0.5%) (HiMedia) plates (Koch, 1981) supplemented with 1 mM U to isolate the uranium-tolerant populations. Plates were incubated at 32°C for 72 h. Colonies of uranium-tolerant bacteria were randomly picked from the plates. The purity of the cultures was confirmed by the streak plate method using nutrient agar medium and the cultures were preserved using 15% glycerol.

Phylogenetic analyses

Genomic DNA was extracted from the bacterial isolates. 16S rRNA gene sequences were amplified by PCR using 2 general bacterial 16S rRNA gene primers. PCR mixtures (25 µl) contained approximately 30 ng of DNA, 2 µM forward primer (27F), 2 µM reverse primer (1492R), 1.5 mM of $MgCl_2$ (Taq Buffer), deoxynucleoside triphosphates (250 µM each of dATP, dCTP, dGTP, and dTTP), and 0.6 U of Taq polymerase. DNA amplification was carried out in a Gene AMP PCR system 9700 (Applied Biosystems, USA). Approximately 1,500 nucleotides were amplified using PCR. Templates replaced with sterile water were always used as negative controls. Amplified products were purified using QIAquick Gel Extraction Spin kit (QIAGEN, Germany).

The purified PCR products were bi-directionally sequenced using the forward, reverse and internal primers corresponding to *Escherichia coli* positions 357F, 926F, 685R, and 1100R by genetic analyser ABI 3130XL (Applied Biosystems) with the Big Dye (3.1) terminator protocol. The sequencing reaction was performed with a 20-µl reaction mixture containing approximately 50 ng of template DNA and 1 pmol of sequencing primers. Post-reaction cleanup and resuspension were performed for removal of unincorporated dye terminators from the sequencing reaction by using 125 mM EDTA, 3 M sodium acetate and 70% ethanol. The Basic Local Alignment Search Tool (BLAST) was used initially to determine the phylogenetic neighbours from the nucleotide database of the National Centre for Biotechnology Information (NCBI) (Altschul *et al.*, 1997). For the 5 representative strains selected for the present investigation, the phylogenetic neighbours were obtained using the BLAST program against the database of type strains with validly published prokaryotic names (available online http://www.eztaxon.org/; Chun *et al*., 2007). Molecular Evolutionary Genetics Analysis software (MEGA version 4) was used for phylogenetic analyses (Tamura *et al*., 2007). The sequences of identified phylogenetic neighbours were aligned with the sequences of representative strains, including the sequence of the type strain *S. marcescens* ATCC 13880, by using the CLUSTAL W built into MEGA 4. *Xenorhabdus bovienii* DSM 4766 was used as the outgroup organism. The neighbour-joining method was employed to construct the phylogenetic tree with 1,000 bootstrap replications to assess nodal support in the tree.

Nucleotide sequence accession numbers

The partial 16S rRNA gene sequences of all 5 strains were submitted to the NCBI GenBank. The accession numbers obtained are as follows: *Serratia* sp. TS1, HM747949; *Serratia* sp. PKRS5, HM747950; *Serratia* sp. KMS4, GU270567; *Serratia* sp. PMS1, HM747955; and *Serratia* sp. OT4, HM747954. *Serratia marcescens* subsp. *marcescens* Bizio 1823 (ATCC 13880) was procured from American Type Culture Collection for comparative studies and further analyses.

Randomly amplified polymorphic DNA (RAPD) fingerprinting

Ten bacterial RAPD primers (RBa1, RBa2, RBa3, RBa4, RBa5, RBa6, RBa7, RBa8, RBa9, and RBa10), obtained from Bangalore Genei, India, were used for screening DNA samples (of all 5 *S. marcescens* strains and the type strain) to obtain RAPD fingerprints. Amplification reactions were performed in a total volume of 25 µl containing 20 ng of DNA extract as the template, each primer at a concentration of 2 µM; $MgCl_2$ (Taq Buffer) 1.5 mM; deoxynucleoside triphosphates, 0.25 mM; and 0.6 U of Taq DNA polymerase. Only 7 of the 10 primers used for fingerprinting provided consistent, reproducible banding patterns. The RAPD-PCR protocol was followed as per the manufacturer's instructions and was run thrice to evaluate and check the reproducibility of the generated fingerprints. The presence (1) or absence (0) of a particular band was recorded to generate a binary matrix, which was imported into the NTSYS-pc software version 2.02 k for cluster analysis (Rohlf, 1990). Genetic similarities among all 5 representative strains and the type strain ATCC 13880 were calculated according to a simple matching coefficient by using the SIMQUAL similarity program (Sneath and Sokal, 1973). The similarity coefficients were used to perform a combined cluster analysis with the 7 RAPD primers by using the SAHN program employing the unweighted pair group method with arithmetic average (UPGMA).

Analyses of physiological and biochemical characteristics

The representative *S. marcescens* isolates and the type strain ATCC 13880 were tested for 60 phenotypic characteristics (Grimont and Grimont, 1992). All the strains were evaluated for morphological characteristics, Gram staining, growth under anaerobic conditions, motility, catalase, oxidase, production of urease, gelatinase, lipase, DNase, and other biochemical properties. Thirty-five carbohydrate utilisation tests were performed using KB009 HiCarbohydrate^{TM} kit (HiMedia).

Determination of MIC for uranium

The MIC for uranium was determined for all the 5 *S. marcescens* isolates and the type strain ATCC 13880. Analytical grade salts of uranyl nitrate $[UO_2(NO_3)_2{\cdot}6H_2O]$ were used to prepare stock solutions. These salts were filter sterilized through a 0.22 micron nitrocellulose membrane filter (Millipore, India). Mid-log phase cells (of equivalent cell density) were inoculated on Mueller-Hinton agar (Hi Media)

plates supplemented with uranium concentrations ranging from 0 to 5 mM. The pH of the medium was adjusted to 7.0 prior to inoculation. Growth was recorded after 72 h of incubation at 32°C (Yilmaz, 2003). The lowest concentration of uranium that completely inhibited the visible growth of the test strains on the plates was considered as the MIC.

Uranyl-binding and viability studies

The 5 representative *S. marcescens* isolates along with the type strain ATCC 13880 were grown using the M9 minimal medium (Davis *et al.*, 1986). Mid-exponential phase cells were harvested by centrifugation (7,000 rpm for 3 min), washed with sterile deionised water and used for uranyl adsorption studies. Ten milliliters of sterile uranyl nitrate solutions (containing 100 μM, 200 μM, 500 μM, 1 mM, and 2 mM U) were allowed to equilibrate for 30 min under aerobic conditions. All the *Serratia* isolates and the type strain (OD_{600} 2) were resuspended in the uranium solutions separately and incubated at 32°C under continuous shaking for 24 h. The cell density did not change measurably during such incubations. Timed samples (100 μl) were withdrawn and centrifuged at 13,000 rpm for 3 min. The supernatants containing residual uranium were acidified with 0.01 N HCl to prevent precipitation. The uranium-loaded cell pellets were washed with distilled water to remove loosely bound uranium and digested at room temperature with 0.2% HCl (Acharya *et al.*, 2009). The mineralised samples were then estimated for uranium by using the arsenazo III method (Savvin, 1961). Spontaneous chemical precipitation of uranium was checked in the absence of the test organism. Each treatment comprised 3 replicates.

The viability tests to evaluate the tolerance of these isolates to acidic pH (pH 3.5) and uranium (100 μM and 2 mM) were done as described by Suzuki and Banfield (2004). Three aliquots of 1 ml mid-log phase cells of equivalent OD (OD_{600} 2) were washed with MilliQ water (pH 7.0) and each aliquot was assayed for viability as follows: (a) serial 10-fold dilutions were made and plated immediately on LB agar, (b) transferred to MilliQ water (pH 3.5) without uranium, incubated for 1 h at 32°C and plated (serial 10-fold dilutions) on LB agar and (c) inoculated in uranyl nitrate solutions (100 μM and 2 mM separately) at pH 3.5 and incubated for 1 h at 32°C (to ensure minimum loss in cell density due to nutrient limitation; our studies showed that the uranyl adsorption reached equilibrium by 1 h) and serial 10-fold dilutions were plated on LB agar. All the plates were incubated for 24 h and enumeration was done for 3 replicates.

Results

Isolation and molecular characterization of uranium-tolerant *S. marcescens* strains

The cultivation-based methods employed for isolation of aerobic chemoheterotrophic bacteria from the subsurface soils of the uranium ore-bearing sites revealed the occurrence of diverse prokaryotic species and led to recovery of nearly 200 isolates. On the other hand, the enrichment-based cultivation methods targeted at uranium-tolerant bacterial populations yielded 57 isolates. Molecular characterization with 16S rRNA gene sequences of these isolates revealed their phylogenetic affiliation with 13 genera, ranging from *Serratia*, *Pseudomonas*, *Burkholderia*, *Stenotrophomonas*, *Acinetobacter*, and *Klebsiella* to *Citrobacter*, *Chryseobacterium*, *Paenibacillus*, *Enterobacter*, *Staphylococcus*, *Bacillus*, and *Arthrobacter.* As many as 20 (35%) of these isolates (comprising both pigmented and non-pigmented) were found to be closely related to *S. marcescens* (≥98% maximum identity) when the BLAST program was performed against the nucleotide database of NCBI. Of these 20 isolates, 5 representative isolates (1 red-pigmented and 4 non-pigmented) exhibiting superior uranium tolerance

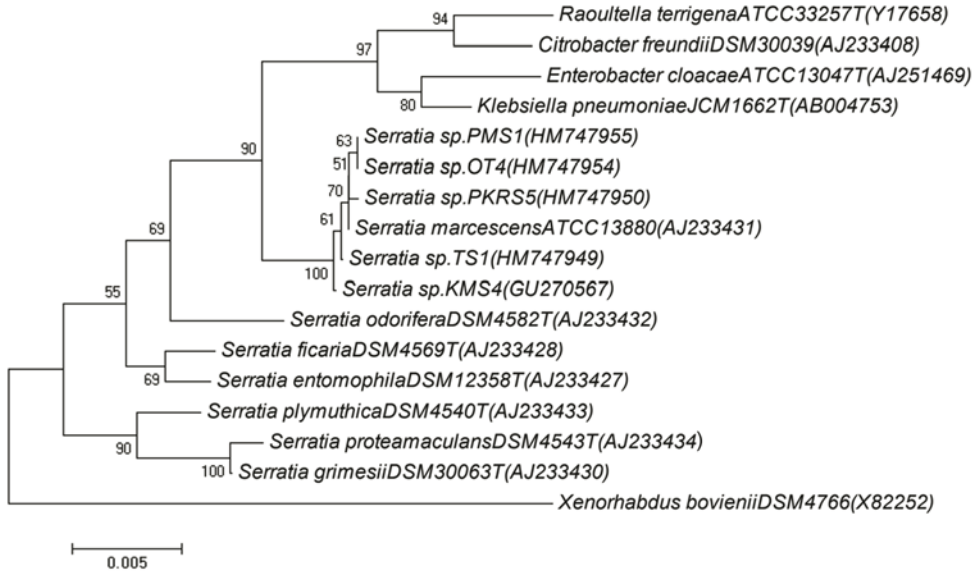

Fig. 1. Neighbor-joining tree based on 16S rRNA (more than 1,400 bases) gene sequences depicting the phylogenetic relationships between the representative *Serratia* isolates and the related species obtained from the database of type strains with validly published prokaryotic names at the EzTaxon server. *X. bovienii* DSM 4766 was taken as the outgroup organism. The scale bar corresponds to the expected number of changes per nucleotide position.

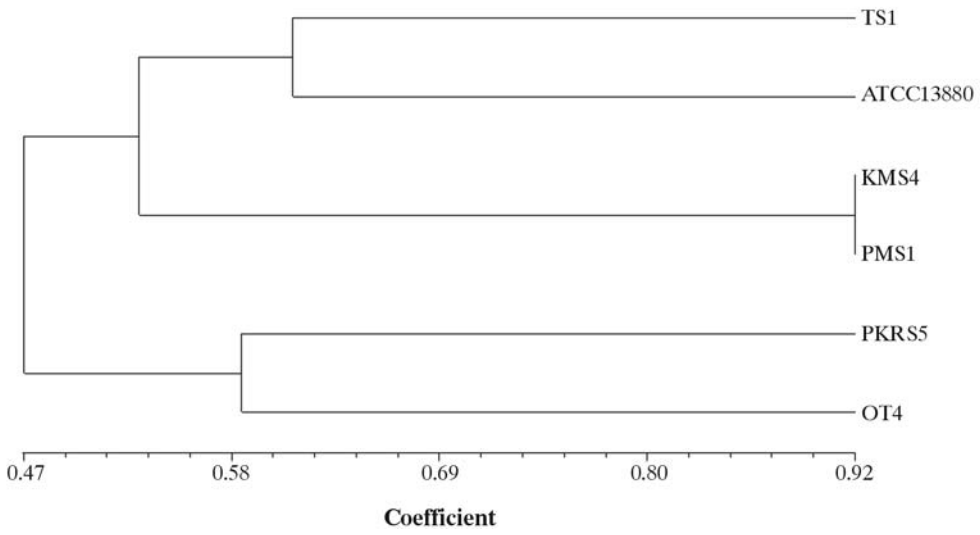

Fig. 2. Combined dendrogram for representative *S. marcescens* isolates and type strain *S. marcescens* ATCC 13880 generated by combining the RAPD-PCR profiles obtained with the primers RBa1, RBa2, RBa3, RBa4, RBa7, RBa8, and RBa9. Simple match correlation coefficient was used to generate the UPGMA cluster.

were selected for further analyses comprising phylogenetic, physiological, biochemical, MIC and bioadsorption studies.

16S rRNA gene sequences more than 1,400 base pairs in length for the 5 representative isolates were used in the BLAST program and run against the database of type strains at the EzTaxon server to identify the nearest phylogenetic neighbours. The nearest homology (>99.4% similarity) was with 16S rRNA gene sequences of *S. marcescens* subsp. *marcescens* Bizio 1823 [ATCC 13880, DSM 30121(T)]. Sequences with high similarity scores were retrieved and a phylogenetic tree was created using MEGA (version 4). Other members of the family *Enterobacteriaceae* that displayed close sequence similarity were also included to study their relatedness with the representative isolates. Five major clusters were obtained; the isolates TS1, PKRS5, KMS4, PMS9, and OT4 clustered together with *S. marcescens* ATCC 13880 with good bootstrap support (Fig. 1).

RAPD analyses

The RAPD profiles revealed polymorphism among the 5 representative isolates and the type strain ATCC 13880. Of the 10 RAPD primers used, primers RBa1, RBa2, RBa3, RBa4, RBa7, RBa8, and RBa9 were chosen for further analyses since they yielded reproducible, polymorphic bands. A dendrogram based on the combined similarity matrix generated with the 7 RAPD primers using UPGMA analysis indicated that the 5 isolates and 1 type strain ATCC 13880 formed 2 major clusters (Fig. 2). The similarity coefficients ranged from 0.47 to 0.92. The RAPD fingerprinting technique revealed a variable genetic profile for each representative isolate and

Table 1. Variable biochemical characteristics of the representative *S. marcescens* strains and the type strain ATCC 13880

Test	TS1	PKRS5	KMS4	PMS1	OT4	ATCC 13880
Pigment	Red	−	−	−	−	Red
Xylose	−	−	−	+	−	−
Maltose	+	+	+	−	+	+
Fructose	+	−	+	−	+	−
Dextrose	+	−	−	−	+	+
Trehalose	+	−	−	−	+	−
Melibiose	−	−	−	−	+	−
Sucrose	−	−	−	+	+	−
L-Arabinose	−	−	−	−	+	−
Mannose	+	−	+	+	+	+
Inositol	−	+	+	+	+	−
Sorbitol	+	+	−	+	+	+
Mannitol	+	−	+	+	+	−
Ribose	+	−	−	+	+	−
Xylitol	−	+	+	+	+	+
ONPG	+	+	−	−	−	+

the type strain ATCC 13880. With DNA polymorphism studies, 2 major clusters were formed with a variation of approximately 50%. The first major cluster comprised KMS4 and PMS1 with high similarity of 92% and type strain ATCC 13880 and red-pigmented TS1 with approximately 63% similarity, while the second major cluster contained PKRS5 and OT4 with approximately 59% similarity.

Physiological and biochemical characteristics

Five *S. marcescens* isolates and the type strain *S. marcescens* ATCC 13880 selected for physiological and biochemical analyses were Gram-negative rods, fermentative, facultatively anaerobic, motile, oxidase-negative, catalase-positive, and did not possess endospores. They produced lipase, gelatinase, nitrate reductase, and DNase. All isolates showed positive results for citrate utilisation, malonate utilisation, Voges-Proskauer test, and esculin hydrolysis and negative results for methyl red test, indole production, hydrogen sulphide production, urease, amylase, and potato-like odour. All the isolates, including the type strain, produced acid from adonitol, salicin, mannose, maltose, galactose, and glycerol and none of them produced acid from cellobiose, lactose, raffinose, inulin, sodium gluconate, glucosamine, dulcitol, rhamnose, melezitose, α-methyl-D-glucoside, α-methyl-D-mannoside, D-arabinose, and sorbose. Of the 60 characteristics studied, 44 similarities were observed among the representative isolates and the type strain ATCC 13880. The sixteen variable characteristics observed included the lack of red pigment, β-galactosidase activity and carbohydrate utilisation (Table 1).

Uranium tolerance, bioadsorption, and viability studies

The MIC of uranium for representative *S. marcescens* isolates suggested significant tolerance to uranium. TS1 and OT4 showed an MIC of 4.0 mM, while PKRS5, KMS4, and PMS1 exhibited an MIC of 3.5 mM as compared to type strain ATCC 13880, which showed an MIC of 2.0 mM. In addition, all these isolates displayed high tolerance towards heavy metals such as cadmium, zinc, copper, and lead (data not shown). These results were coherent with the findings of other investigators indicating the increased occurrence of metal-tolerant bacteria with the increase of heavy metal/radionuclide concentration in metal-contaminated sites (Angle *et al*., 1993; Roane and Kellogg, 1996).

One of the established mechanisms demonstrated by the metal-tolerant microbes is bioadsorption, through which they inhibit the toxic metal concentrations entering into their cytoplasm. Since these natural isolates could tolerate toxic concentrations of soluble U(VI), they were assayed for their uranyl adsorption properties. The uranyl-binding profiles of all *Serratia* isolates were evaluated with respect to incubation time (5 min to 24 h) as well as initial uranium concentrations (100 μM to 2 mM). Uranyl binding by all these isolates was found to be very rapid and monophasic, amounting to 90-92% uranium removal from the uranium solutions (100-500 μM) at pH 3.5 within 5-10 min of exposure reaching an equilibrium by 1 h. This capacity for uranium scavenging was retained by all the isolates for 24 h. Uranium adsorption was highest (>90%) at low concentration (100-500 μM) (Fig. 3). The efficiency of uranium adsorption decreased with increasing uranium concentrations. At higher concentrations (>1 mM), the adsorption capacity was found to be lower (60-65%) for all the isolates, which indicated that this concentration (2 mM) might have led to saturation of binding sites on the cell surfaces. The isolate TS1 seemed to be the most promising isolate for uranium sequestration. The type strain used could hardly bind 15-20% uranium from the test solutions (100 μM to 2 mM) at pH 3.5, indicating the superiority of our natural isolates for uranium adsorption. Uranium remained stable under our

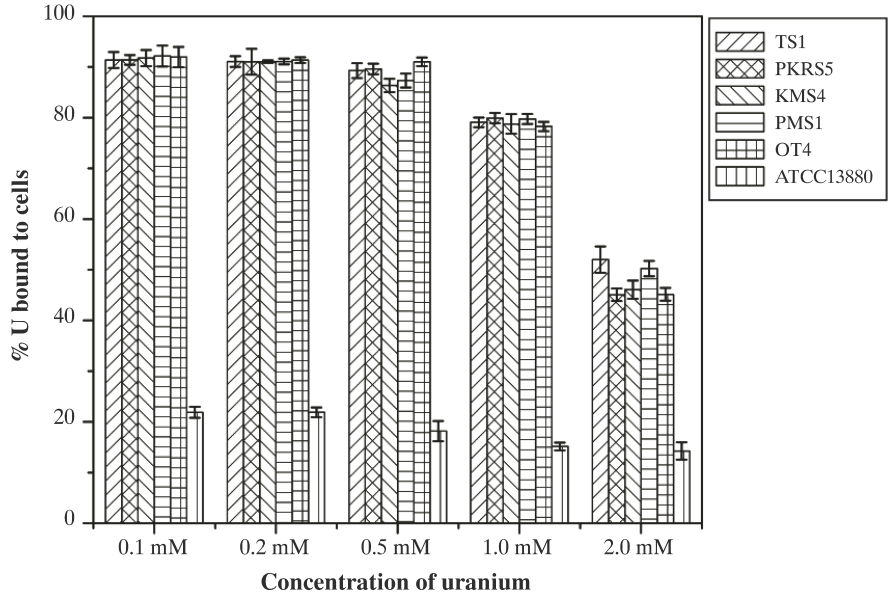

Fig. 3. Uranium binding by representative *S. marcescens* isolates and type strain *S. marcescens* ATCC 13880. Equivalent cells (OD_{600} 2) were exposed to 100 μM-2 mM uranyl nitrate solutions and were assayed for bound uranium. Uranium was estimated in the cell pellets following 24 h incubation. Error bars denote standard deviation of triplicate experiments.

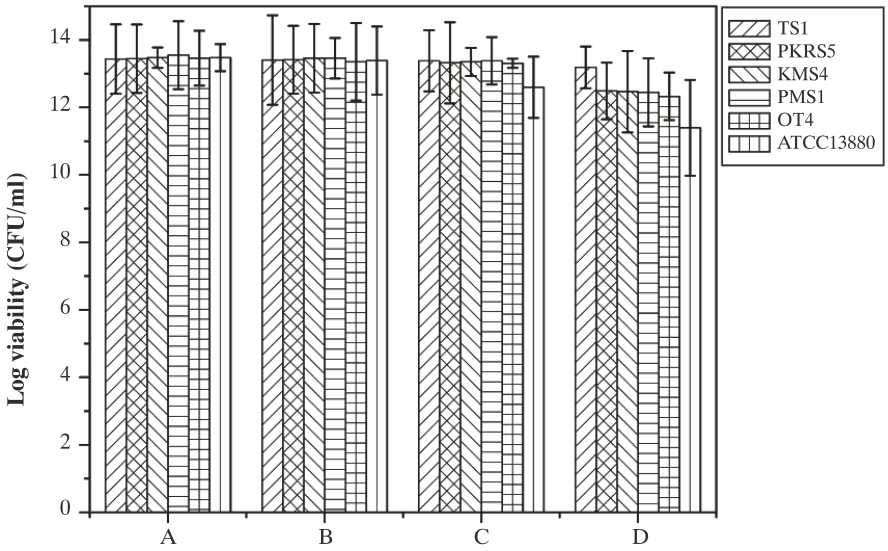

Fig. 4. Viable cell counts (log CFU/ml) determined after washing and incubating for 1 h (A) at pH 7.0, (B) at pH 3.5, (C) with 100 μM uranyl nitrate at pH 3.5 and (D) with 2 mM uranyl nitrate at pH 3.5. Error bars denote standard deviation of triplicate experiments.

experimental conditions and did not precipitate as seen in the abiotic controls. Uranyl adsorption was not evaluated beyond 24 h, as there was a decrease in cell density due to nutrient limitation. However, none of these isolates could remove any uranium from test solutions at pH>7. Above pH 7, the uranyl ions form stable and soluble complexes with carbonates and phosphates (Fortin *et al*., 2007). The uranium ore-bearing sites chosen for our studies consist of acidic soils contaminated with heavy metals and radionuclides. To be useful for bioadsorption, the cells must be viable at pH 3.5, where most of the heavy metals and uranium exist in their cationic species (UO_2^{2+}), which are known to be highly soluble and more toxic than their precipitated or hydrolysed species. The viability studies in this investigation clearly indicated that all the *S. marcescens* isolates exhibited tolerance to acidic pH (pH 3.5) and toxic concentrations of uranium U(VI) (100 μM to 2 mM) (Fig. 4). Although the type strain remained viable at acidic pH, it exhibited a 100-fold and 1000-fold loss in viability on exposure to 100 μM and 2 mM U, respectively, at pH 3.5. The characteristics of uranyl binding at acidic pH and tolerance to toxic concentrations of heavy metals and uranium exhibited by these natural isolates correlates with the *in situ* environment of the studied areas.

Discussion

Isolation and characterization of uranium-tolerant, aerobic, *S. marcescens* isolates from the subsurface soils of uranium-rich deposits of Domiasiat in North East India were attempted in this investigation. All these isolates were gram negative and mesophilic, growing most rapidly at 32°C. The analyses of the metal- and uranium-contaminated soils showed a wide variety of prokaryotic species. A sub-group of *S. marcescens* was targeted for our investigation on the basis of its superior metal/uranium-resistant phenotype and wide occurrence. These isolates revealed significant tolerance for uranium (3.5-4.0 mM). The MIC of U(VI) for *E. coli* has been reported to be 2 mM, while a multimetal-resistant natural isolate of *Pseudomonas putida* showed an MIC of 1.7 mM (Nies, 1999). It has been shown that a Gram-negative, aerobic, chemoheterotroph like *Rahnella* sp. isolated from acidic subsurface radionuclide and metal-contaminated soils lost its viability on exposure to 200-μM uranium (Martinez *et al*., 2006). The Gram-negative, aerobic, natural isolates of *S. marcescens* used in our studies adsorbed 21-22 mg/L and 285-335 mg/L when challenged with 23.8 mg/L and 476 mg/L uranyl nitrate solutions, respectively, at pH 3.5 while retaining their viability. Microorganisms capable of maintaining metabolic activity in presence of oxygen have been shown to play an important role in adsorption and precipitation of toxic radionuclides such as uranium (Barkay and Schaefer, 2001), which was observed in our studies also. A report on multimetal-resistant strains belonging to *Bacillus*, *Pseudomonas*, and *Serratia* isolated from another uranium ore-bearing site in India, namely, Jaduguda, containing 0.03-0.06% U_3O_8 (Ramana *et al*., 2001) revealed their potential for sequestering heavy metals such as Ni, Co, Cu, and Cd, but their uranium-tolerant/binding properties were not evaluated (Choudhary and Sar, 2009). To our knowledge, this is the first study that examines the uranium-resistant (and heavy metal-resistant) phenotypes of natural isolates from uranium-rich deposits of Domiasiat in North East India and highlights their uranium adsorption capacities from aqueous solutions. These natural isolates, owing to their superior bioadsorption properties, appear to be ideal candidates for *in situ* clean up of heavy metal- and uranium-contaminated sites.

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