Metal Tolerant *Bacillus* and *Pseudomonas* from Uranium Rich Soils of Meghalaya

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Abstract

Domiasiat in West Khasi Hills district of Meghalaya was identified as an area with huge deposits of Uranium by India's Atomic Minerals Division in 1984. Domiasiat (25° 30' N; 91° 30' E) has been characterized as the largest, richest, near-surface and low-cost sandstone-type uranium deposit discovered in India. The ores are spread over a 10-square-kilometer area in deposits varying from 8 to 47 meters from the surface. Three metal tolerant Bacillus and Pseudomonas were isolated from the proposed mining sites of Domiasiat. The bacteria were tested for their tolerance against five different heavy metals viz. Lead as Lead Nitrate [$Pb(NO_3)_2$], Chromium as Potassium Chromate [K_2CrO_4], Copper as Copper Sulphate [$CuSO_4.5H_2O$], Zinc as Zinc Sulphate [$ZnSo_4.7H_2O$] and Cadmium as Cadmium Nitrate [$Cd(NO_3)_2.5H_2O$]. The bacteria were studied for their metal tolerance and antibiotic resistance using various metals and antibiotics. The bacteria exhibited high MIC values for metals and also considerably larger spectrum of antibiotic resistance. The order of toxicity of the metals to the bacteria varied with the isolates, Cadmium being the most toxic metal. These metal tolerant isolates adapted to uranium rich environment can be further explored for their activities such as biosorption, bioprecipitation, extracellular sequestration, transport mechanisms, and/or chelation of heavy metals including Uranium which may open vistas for their utilisation as bioremediation agents.

Keywords: Metal tolerant, *Bacillus, Pseudomonas*, Uranium Deposit, Meghalaya

Introduction

Metal-resistant bacteria are nothing bizarre, instead it is reported that almost every group of bacteria possess the potential of resisting metals.[35] And this ability in bacteria has not aroused very recently but even at 3 to 4 billion years ago bacteria had to have resistance mechanisms in order to survive the high concentration of metals in volcanic environment.[36] A number of metal resistant bacteria has been isolated from various naturally occurring and anthropogenically created extreme habitats such as a metal decantation tank of a zinc factory,[30] a heavy metal site, [28] material of waste heaps from a former uranium mining area,[1] serpentinite derived soils,[19,23] at acid mine drainage (AMD) site from former uranium mining site.[14]

In present work, metal-resistant *Bacillus* and *Pseudomonas* strains are isolated from the soils around the proposed uranium mining sites in Domiasiat area of West Khasi Hill district in Meghalaya. Exploration of the Upper Cretaceous Mahadek sediments by the Atomic Minerals Directorate for Exploration and Research (Atomic Mineral Division, Govt of India) over the last four decades has established Domiasiat as one of the country's largest sandstone-type uranium deposits, the second one being Wahkyn from the same area.[37] The Domiasiat deposit was estimated to be around 9,500 tonnes. Domiasiat (25° 30' N 91° 30' E), has been characterized as the largest, richest, near-surface and low- cost sandstone-type uranium deposit discovered in India. The ores are spread over a 10-square-kilometer area in deposits varying from 8 to 47 meters from the surface in the uranium deposit areas.[2]

Microbial composition of such heavy metal and radionuclide contaminated extreme environments has been extensively studied during the last decade and it was demonstrated that they are inhabited by a large variety of bacteria. [9,12,24,25,6,8,10,31,32,33,34] Investigations from heavy metal and radionuclide contaminated environment have reported that constituent microbes play a very significant role in bioaccumulation of uranium,[11,22] in biotransformation of it,[7,10,18] and for bioremediation of uranium-contaminated sites by *in situ* biostimulation.[16,17,21]

Bacillus and *Pseudomonas* strains are also reported from the uranium mine water of Jaduguda, India.[13, 29]

The adaptation to heavy metal rich environments is resulting in microorganisms which show activities for biosorption, bioprecipitation, extracellular sequestration, transport mechanisms, and/or chelation. Such resistance mechanisms are the basis for the use of microorganisms in bioremediation approaches.[15] The metal-resistant bacteria well-adapted to such a heavy metal rich environment, as of Domiasiat, hence could probably support remediation and could be of great interest for strategies on environmental conservation.

Material and Methods

Sample Collection and Isolation of Metal-Resistant Bacteria: Soil samples from three proposed uranium mining sites in the Domiasiat area from West Khasi Hills district of Meghalaya were collected in sterile plastic bags and transported on ice to the laboratory. Samples were diluted in sterile distilled water and plated on tryptone soya agar (M1615, HiMedia Laboratories Pvt Limited) plates supplemented with analytical grade of 1mM $Pb(NO_3)_2$, K_2CrO_4, $CuSO_4.5H_2O$ and $ZnSo_4.7H_2O$ one metal at a

time or as heavy metal mixture. Plates were incubated at 37°C for 48 hours to screen metal-resistant colonies. In this preliminary screening, colonies showing resistance to Pb, Cr, Cu and Zn were selected for further study. Assumed Bacillus and Pseudomonas cultures specified by the colony characteristic were selected for further characterization studies and for determination of Minimum Inhibitory Concentration of heavy metals and Antibiotic susceptibility.

Characterization Studies

The selected metal-resistant bacterial cultures were streaked onto fresh nutrient agar plates to verify their purity. The separated pure colonies of the metal-resistant bacteria are then used for their microscopic and biochemical studies.

Colony Characters: The colony characteristics were studied based on their shape, size, elevation, margin and colour.

Microscopic Studies: Gram staining of the isolated strains was done and cell shape was noted based on microscopic studies using Inverted Light Microscope (Leica DM 1000, Leica Microsystems, USA)

Biochemical Studies: Various Biochemical tests like Motility, Oxidase , Catalase , Starch hydrolysis, Lipid hydrolysis, Nitrate reduction, Gelatin liquefaction, HL Oxidative, HL Fermentative, TSI Agar, Urease, Indole, MR-VP and Simmon's Citrate test were performed to characterize the isolated bacterial strains .

By performing above characterization steps the bacterial strains were able to be identified to only genus level. Further characterization using Sugar utilization tests and 16 S rDNA analyses is on the process to establish it to species level.

Determination of Minimal Inhibitory Concentrations (MICs): Analytical Grade salts of Lead Nitrate [$Pb(NO_3)_2$], Potassium Chromate [K_2CrO_4], Copper Sulphate [$CuSO_4.5H_2O$], Zinc Sulphate [$ZnSo_4.7H_2O$] and Cadmium Nitrate [$Cd(NO_3)_2.5H_2O$] were used to prepare 0.1 M stock solution and sterilized by autoclaving at 121°C, 15 psi for 20 minutes. Mueller-Hinton (MH) Agar No. 2 (M1084, HiMedia Laboratories Pvt Limited) plates were supplemented with different concentration of heavy metals adjusted to pH 7.0 and then inoculated. Mid-log phase cultures grown in 5ml of Mueller Hinton Broth (M391-500G, HiMedia Laboratories Pvt Limited) were used to analyse metal-resistance. Cells were streaked on MH agar plates containing concentrations of metal salts at the interval of 0.5 milimolar (mM) as 0 (Conrol), 0.5, 1.0, 1.5, 2.0, 2.5 and so on. Growth was recorded after three days of incubation at 37°C. The Minimum Inhibitory Concentration (MIC) was determined to be the lowest concentration of metal in which growth was completely inhibited. Escherichia coli 118 (MTCC, Chandigarh, India) was used as the control. Strains were considered resistant if MIC values exceeded that of the control organism for minimum one of the metal.

Antibiotic Susceptibility Test: The bacterial strains were tested for its sensitivity to 13 different antibiotics. The reaction to antibiotics was examined by the disk diffusion method.[3] The bacteria were grown in MH broth at 37 °C for 24 h. 100 µl inoculums is taken from the culture and plated on MH agar plates. The antibiotic disks were placed on freshly prepared lawns of the isolate on MH agar plates and incubated at 37°C for 24 h. The diameter of the inhibition zones was measured, and the bacterium was classified as resistant (R), intermediate (I) and susceptible (S), following the standard antibiotic disc sensitivity testing method [7] to the following antibiotics: Ampicillin 10µg, Aztreonam 30µg, Amikacin 30µg, Chloramphenicol 10µg, Gentamicin 10µg, Erythromycin 10µg, Kanamycin 30µg, Streptomycin 25µg, Tetracycline 10µg.

Results and Discussion

Isolation of Heavy Metal-Resistant Bacillus and Pseudomonas Species: Fifty colonies were screened from initial (1mM) level of heavy metal supplemented MH agar plates. After secondary screening, based on the distinct colony characteristics, three *Bacillus* and three *Pseudomonas* metal-resistant bacterial strains were screened from the proposed uranium mining sites soil samples.

Characterization of the Isolated Bacterial Strains: Colony characteristics, gram staining, microscopic study of cell shape and various biochemical tests is shown in Table 1 (a) and (b). Based on the Characterization studies performed here, using Bergey's manual of systemic bacteriology, the isolates could be closely established to the genus level. The characterization steps were too limited to relate the strains to species level. Characterization based on sugar utilization and 16 S rDNA analyses is on the progress.

Resistance to Metals

Minimum Inhibitory Concentrations (MICs) of various metals for the selected metal-resistant *Bacillus* and *Pseudomonas* are presented in Table 2. The order of toxicity of the metals to bacterial strains varied with the strain concerned. In general, among the studied metals, Cd was found to be the most toxic metal.

However, determination of MIC varies with the type of media used. Due to different conditions of diffusion, complexation and availability of metals, higher MIC is reported in solid media then in liquid.[27, 38] Same factors affect the varied MIC observations in rich and minimal media.

In present study, the resistant bacteria selected have shown MIC higher than the control organism *E. coli* 118 (MTCC, Chandigarh, India) for at least one of the selected metals.

Antibiotic Resistance

Antibiotic resistance of the bacterial strain is depicted in Table 3. Strain KMSII-1 is the most resistant strain showing resistance to Ampicillin 10µg, Aztreonam 30 µg, Gentamicin 10µg and Streptomycin 25µg while strain PMS-1 is least resistant showing resistance only to Aztreonam 30 µg. It was observed that strains showing resistance to heavy metals are also usually resistant to antibiotics.[20] Resistance to metallic salts as well as antibiotics is either chromosomally controlled[26] or plasmid mediated.[5,26]

Conclusion

Metal-resistant *Bacillus* and *Pseudomonas* strains were isolated from uranium rich deposits, morphological and biochemically characterizations were performed to establish the bacteria to genus level. The confirmation of the bacteria to species level using sugar utilization and molecular characterization is in progress. Minimum Inhibitory Concentrations (MICs) for five of the heavy metals were checked, metals like Pb, Zn & Cu were considered which are associated with acidic rock as that of uranium and metal like Cd & Cr were also checked which shows less affinity with uranium. The toxicity of metals varied with the strain concerned and in general Cd remained the most toxic of all. Among the antibiotics Amikacin 30µg, Chloramphenicol 10µg and Erythromycin 10µg showed highest sensitivity. The metal-resistant *Bacillus* and *Pseudomonas* will be further screened for their metal accumulating potential so as to ascertain that they are not only resistant to heavy metals, but can also bind considerable amounts of heavy metals from the growth medium which will suggest that the bacterial strains may also be able to remove metals from different sources of pollution and hence can play an active role in bioremediation approaches.

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Table 1 (a): Morphological and Biochemical Characterization of Isolates Suspected to be *Bacillus*.

Characteristics	Bacterial Isolates		
	KMSII-1	**KMSII-3**	**PMS-3**
Colony Shape	Irregular	Irregular	Irregular
Colony Elevation	Raised	Raised	Raised
Colony Size (mm)	6.0-10.0	6.0-10.0	5.5-10.0
Colony Margin	Entire	Erose	Erose
Colony Colour	White	White	White
Cell Shape	Rod	Rod	Rod
Endospore formation	+	+	+
Gram Staining	+	+	+
Motility	+	+	+
Cytochrome Oxidase test	+	+	+
Catalase test	+	+	+
Starch hydrolysis	+	+	+
Lipid hydrolysis	+	+	+
Nitrate reduction	+	+	+
Gelatin liquefaction	+	+	+
HL Oxidative	A (W)	A (W)	A (W)
HL Fermentative	A (W)	A (W)	A (W)
TSI Agar Test (Bud/Slant)	A/K	A/K	A/K
Gas production	-	-	-
H_2S production	-	-	-
Urease test	-	-	-
Indole test	-	-	-
Mthyl Red test	+	+	+
Voges Proskauer	-	-	-
Simmon's Citrate	+	-	-
Closely Related Genus	*Bacillus*	*Bacillus*	*Bacillus*

Abbreviations used:
H: Hugh Leifson; TSI: Triple Sugar Iron; +: Positive Result; -: Negative Result; A: Acidic; K: Alkaline; (W): Weak.

Table 1 (b): Morphological and Biochemical Characterization of Isolates Suspected to be *Pseudomonas*.

Characteristics	Bacterial Isolates		
	KMSI-2	KMSI-R1	PMS-1
Colony Shape	Circular	Circular	Circular
Colony Elevation	Convex	Convex	Convex
Colony Size (mm)	0.75-1.25	0.75-1.25	0.5-1.0
Colony Margin	Entire	Entire	Entire
Colony Colour	Creamish White	Creamish White	Dirty white
Cell Shape	Rod	Rod	Rod
Gram Staining	-	-	-
Motility	+	+	+
Cytochrome Oxidase test	+	+	+
Catalase test	+	+	+
Starch hydrolysis	+	-	-
Lipid hydrolysis	+	+	+
Nitrate reduction	+	+	-
Gelatin liquefaction	+	-	-
HL Oxidative	A	K	A (W)
HL Fermentative	-	-	-
TSI Agar Test (Bud/Slant)	K/K	K/K	K/K
Gas production	-	-	-
H_2S production	-	-	-
Urease test	-	+	-
Indole test	-	-	-
Mthyl Red test	-	-	+
Voges Proskauer	-	-	+
Simmon's Citrate	+	+	+
Growth in King's A	+	+	+
Growth in King's B	+	+	+
Closely Related Genus	*Pseudomonas*	*Pseudomonas*	*Pseudomonas*

Abbreviations used:
HL: Hugh Leifson; TSI: Triple Sugar Iron; +: Positive Result; -: Negative Result; A: Acidic; K: Alkaline; (W): Weak.

Table 2. Minimum Inhibitory Concentrations (MICs) of the Selected Metals Against the Bacterial Isolates.

Bacterial Strains	Metals				
	Lead Nitrate [$Pb(NO_3)_2$]	Potassium Chromate [K_2CrO_4]	Copper Sulphate [$CuSO_4.5H_2O$]	Zinc Sulphate [$ZnSo_4.7H_2O$]	Cadmium Nitrate [$Cd(NO_3)_2.5H_2O$]
KMSII-1	6.0 mM	6.5 mM	4.5 mM	7.0 mM	0.5 mM
KMSII-3	6.5 mM	7.0 mM	4.0 mM	7.0 mM	1.5 mM
PMS-3	5.5 mM	3.5 mM	4.0 mM	6.5 mM	1.5 mM
KMSI-2	5.0 mM	7.0 mM	4.0 mM	6.0 mM	0.5 mM
KMSI-R1	4.5 mM	3.5 mM	3.5 mM	3.5 mM	1.5 mM
PMS-1	6.5 mM	6.0 mM	6.0 mM	7.0 mM	2.0 mM
E. coli 118 (Control)	5.0 mM	4.0 mM	3.5 mM	3.0 mM	0.5 mM

Abbreviations used:
mM: Milli molar

Table 3. Antibiotic Sensitivity Profiles of the Selected Metal-Tolerant Bacterial Species.

Antibiotics	Bacterial Isolates					
	KMSII-1	KMSII-3	PMS-3	KMSI-2	KMSI-R1	PMS-1
Ampicillin 10µg	R	R	R	R	R	S
Aztreonam 30µg	R	R	R	R	S	R
Amikacin 30µg	I	S	S	S	I	S
Chloramphenicol 10µg	S	I	S	S	S	S
Gentamicin 10µg	R	S	S	S	I	S
Erythromycin 10µg	I	S	S	S	S	S
Kanamycin 30µg	I	I	I	S	S	S
Streptomycin 25µg	R	S	S	S	R	S
Tetracycline 10µg	I	I	S	I	S	S

Abbreviations used:
R: Resistant; I: Intermidiate; S: Sensitive

References

1. Amoroso, M.J., Schubert, D., Mitscherlich, P., Schumann, P. and Kothe, E. Evidence for high affinity nickel transporter genes in heavy metal resistant Streptomyces spec. *Journal of Basic Microbiology*, **40**, 295-301**(2000)**.
2. Anonymous. WISE News Communique No.459, 4 Oct 1996, India -- 30,000 to be displaced for Domiasiat uranium mine **(1996)**.
3. Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. Antibiotic susceptibility testing by a standardized single disc method. *Annual Journal Clinical Pathology*, **45,** 493–496**(1966)**.
4. Bhowmik, S. (2003) Tribes dig in to fight uranium. BBC news. Source : http://news.bbc.co.uk/1/hi/world/south_asia/3000991.stm
5. Collard, J.M., Corbisier, P., Diels, L., Dong, Q., Jeanthon, C., Mergeay, M., Taghavi, S., Lelie, D., Wilmotte, A. and Wuertz, S. Plasmid for heavy metal resistance in *Alcaligens eutrophus* CH34: Mechanisms and applications. *FEMS Microbiologyn Review* , **14:** 405-414 **(1995)**.
6. Crozier, R.H., Agapow, P.M. and Pedersen, K. Towards complete biodiversity assessment: an evaluation of the subterranean bacterial communities in the Oklo region of the sole surviving natural nuclear reactor. *FEMS Microbiology Ecology* **28**, 325-334 **(1999)**.
7. DiSpirito, A.A. and Tuovinen, O.H. Uranous ion oxidation and carbon dioxide fixation by *Thiobacillus ferrooxidans*. *Archives of Microbiology*, **133**, 28–32 **(1982).**
8. Ehrlich, H.L. Geomicrobiology: its significance for geology. *Earth-Science Reviews* **45**, 45–60 **(1998)**.
9. Fields, M.W., Yan, T., Rhee, S.K., Carroll, S.L., Jardine, P.M., Watson, D.B., Criddle, C.S. and Zhou, J. Impacts on microbial communities and cultivable isolates from groundwater contaminated with high levels of nitric acid-uranium waste. *FEMS Microbiology Ecology* **53,** 417-428 **(2005).**
10. Francis, A.J. Biotransformation of uranium and other actinides in radioactive wastes. *Journal of Alloys and Compounds* **271,** 78–84 **(1998)**.
11. Francis, A.J., Gillow, J.B., Dodge, C.J., Harris, R., Beveridge, T.J. and Papenguth, H.W. Uranium association with halophilic and non-halophilic bacteria and archaea. *Radiochimica Acta* **92**, 481–488 **(2004)**.
12. Fredrickson, J.K., Zachara, J.M., Balkwill, D.L., Kennedy, D., Li, S.W., Kostandarithes, H.M., Daly, M.J., Romine, M.F. and Brockman, F.J. Geomicrobiology of high-level nuclear waste-contaminated vadose sediments at the Hanford Site, Washington State. *Applied and Environmental Microbiology* **70**, 4230-4241 **(2004)**.
13. Gahan CS, Samal SK, Pradhan N, Kar RN, Shukla LB, Pandey VM and Misra VN Isolation and characterization of Bacterial strains available in Uranium mine water from Jaduguda. In Proc. of International conference on Emerging Trends in Mineral Processing & Extractive metallurgy, ICME, Eds. V. N. Misra, S. C. Das and T. Subbiah, Allied publishers, pp. 124-128 **(2005)**.
14. Haferburg, G., Reinicke, M., Merten, D., Büchel, G. and Kothe, E. Microbes adapted to acid mine drainage as source for strains active in retention of aluminum or uranium. *Journal of Geochemical Exploration* **92**, 196–204 **(2007)**.
15. Haferburg, G. and Kothe, E. Microbes and metals: interactions in the environment. *Journal of Basic Microbiology* **47**, 453–467 **(2007)**.
16. Holmes, D.E., Finneran, K.T., O'Neil, R.A. and Lovley, D.R. Enrichment of members of the family Geobacteraceae associated with stimulation of dissimilatory metal reduction in uranium contaminated aquifer sediments. *Applied and Environmental Microbiology* **68**, 2300–2306 **(2002)**.
17. Istok, J.D., Senko, J.M., Krumholz, L.R., Watson, D., Bogle, M.A., Peacock, A., Chang, Y.J. and White, D.C. In situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. *Environmental Science and Technology* **38**, 468–475 **(2004)**.
18. Lovley, D.R., Phillips, E.J.P., Gorby, Y.A. and Landa, E.R. Microbial reduction of uranium. *Nature* **350**, 413–416 **(1991)**.
19. Mengoni, A., Barzanti, R., Gonnelli C., Gabbrielli, R. and Bazzicalupo, M. Characterization of nickel-resistant bacteria isolated from serpentine soil. *Environmental Microbiology* **3**, 691-698 **(2001)**.

20. Nikolaev, Y.A. Extracellular factors of Bacterial adaptation to unfavourable environmental conditions. *Applied Biochemistry and Microbiology*, **40(4):** 327-336 **(2004)**.
21. North, N.N., Dollhopf, S.L., Petrie, L., Istok, J.D., Balkwill, D.L. and Kostka, J.E. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. *Applied and Environmental Microbiology* **70**, 4911-4920 **(2004)**.
22. Ohnuki, T., Yoshida, T., Ozaki, T., Samadfam, M., Kozai, N., Yubuta, K., Mitsugashira, T., Kasama, T. and Francis, A.J. Interactions of uranium with bacteria and kaolinite clay. *Chemical Geology* **220**, 237–243**(2005)**.
23. Park, J.E., Young, K.E., Schlegel, H.G., Rhie, H.G. and Lee, H.S. Conjugative plasmid mediated inducible nickel resistance in *Hafnia alvei* 5-5. *International Microbiology* **6**, 57-64**(2003)**.
24. Pedersen, K., Arlinger, J., Ekendahl, S. and Hallbeck, L. 16S rRNA gene diversity of attached and unattached bacteria in boreholes along the access tunnel to the Äspö hard rock laboratory, Sweden. *FEMS Microbiology Ecology* **19**, 249-262 **(1996)**.
25. Pedersen, K. Microbial life in deep granitic rock. *FEMS Microbiology Reviews* **20**, 399–414 **(1997)**.
26. Peitzsch, N., Eberz, G. and Nies, D.H. *Alcaligens eutrophus* as a bacterial chromate sensor. *Applied Environmental and Microbiology*, **64(2):** 453-458 **(1998)**.
27. Raja, C.E., Anbazhagan, K. and Selvam, G. S. Isolation and characterization of a metal-resistant Pseudomonas aeruginosa strain. *World Journal of Microbiology & Biotechnology* **22**: 577–585 **(2006)**.
28. Roane, T. M. and Kellogg, S. T. Characterization of bacterial communities in heavy metal contaminated soils. *Canadian Journal of Microbiology* **42**, 593-603 **(1996)**.
29. Samal SK, Pradhan N, Kar RN, Shukla LB and Misra VN. Metal ions and antibiotic resistance pattern of bacteria isolated from mine water samples. In Proc. of International conference on Emerging Trends in Mineral Processing & Extractive metallurgy, ICME, Eds. V. N. Misra, S. C. Das and T. Subbiah, Allied publishers, pp. 187-196 **(2005)**.
30. Schmidt, T., Stoppel, R.D. and Schlegel, H.G. High-level nickel resistance in *Alcaligenes xylosoxydans* 31A and *Alcaligenes eutrophus* KTO2. *Applied and Environmental Microbiology* **57**, 3301-3309 **(1991)**.
31. Selenska-Pobell, S., Kampf, G., Flemming, K., Radeva, G. and Satchanska, G. Bacterial diversity in soil samples from two uranium mining waste piles as determined by rep-APD, RISA and the 16S rDNA retrieval. *Antonie van Leeuwenhoek* **79**, 149-161 **(2001)**.
32. Selenska-Pobell, S., Flemming, K., Tzvetkova, T., Raff, J., Schnorpfeil, M. and Geißler, A. Bacterial communities in uranium mining waste piles and their interaction with heavy metals. In *Uranium in the Aquatic Environment* (eds Merkel BJ, Planer-Friedrich B, Wolkersdorfer C). Springer, Berlin, Germany, pp. 455-464 **(2002)**.
33. Selenska-Pobell, S. Diversity and activity of bacteria in uranium waste piles. In *Interactions of Microorganisms with Radionuclides* (eds Keith-Roach MJ, Livens FR) Elsevier Sciences Ltd, Oxford, UK, pp. 225-254**(2002)**.
34. Selenska-Pobell, S. Uranium contaminated environments as a reservoir of unusual bacteria prospective for bioremediation and nanotechnology. Perspectives in *Environmental Microbiology*, Elsevier Sciences Ltd, Oxford, UK. **(2006)**
35. Silver, S. and Misra, T.K. Bacteria transformation of and resistance to heavy metals. *Basic Life Science* **28**, 23-46 **(1984)**.
36. Silver, S., Misra, T.K. and Laddaga, R.A. Bacterial resistance to toxic heavy metals. In *Metal Ions and Bacteria* (eds Beveridge, T.J. and Doyle, R.J.) John Wiley and Sons Ltd., New York, pp. 121-139 **(1989)**.
37. Srivastava, S.K., Kothari, P.K., Majumdar, A. and Umamaheswar, K. Occurrence of sandstone-type uranium mineralization in the Umthongkut area, Mahadek Basin, West Khasi Hills District, Meghalaya, India. *Current Science* **94(12)**, 1573-1575 **(2008)**.
38. Yilmaz, E.I. Metal tolerance and biosorption capacity of *Bacillus circulans* strain EB1. *Research in Microbiology* **154**, 409–415 **(2003)**.

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