Culture-independent molecular analysis of bacterial diversity in uranium-ore/-mine waste-contaminated and non-contaminated sites from uranium mines

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Abstract Soil, water and sediment samples collected from in and around Jaduguda, Bagjata and Turamdih mines were analyzed for physicochemical parameters and cultured, and yet to be cultured microbial diversity. Culturable fraction of microbial community measured as Colony Forming Unit (CFU) on R2A medium revealed microbes between $10^4$ and $10^9$ CFU/g sample. Community DNA was extracted from all the samples; 16S rRNA gene amplified, cloned and subject to Amplified Ribosomal DNA Restriction Analysis. Clones representing each OTU were selected and sequenced. Sequence analyses revealed that non-contaminated samples were mostly represented by Acidobacteria, Bacteroidetes, Firmicutes and Proteobacteria ($\beta$-, $\gamma$-, and/or $\delta$-subdivisions) along with less frequent phyla Nitrospira, Deferribacteres, Chloroflexi. In contrast, samples obtained from highly contaminated samples showed distinct abundance of $\beta$-, $\gamma$- and $\alpha$-Proteobacteria along with Acidobacteria, Bacteroidetes and members of Firmicutes, Chloroflexi, Candidate division, Planctomycete, Cyanobacteria and Actinobacteria as minor groups. Our data represented the baseline information on bacterial community composition within non-contaminated samples which could potentially be useful for assessing the impact of metal and radionuclides contamination due to uranium mine activities.

Keywords Uranium mines · 16S rRNA gene · Bacterial community · ARDRA

Introduction

Dispersion and migration of uranium (U) and other toxic metals and radionuclides from uranium mines due to mining operations and waste piling is a serious environmental concern to all uranium producing states (Foster et al. 2008; Islam et al. 2011). Once released into the environment, fate and toxicity of these metallic contaminants are strongly regulated by abiotic and biotic components such as minerals and bacteria (Lloyd and Renshaw 2005). On the other hand, the toxic and bioavailable forms of these metals and radionuclides often affect adversely the diversity and function of autochthonous microorganisms of neighboring habitats that bequeath ecological sustains by maintaining biogeochemical cycles (Torsvik et al. 2002; Islam et al. 2011). Diversity and distribution of microbes is often site-specific, influenced by composition of geochemical matrix of microhabitats. Perturbation to this due to environmental contamination could cause change in inhabitant microbial community structure, diversity and function (Herrera et al. 2007; Desai et al. 2009). In order to gauge the impact of environmental contamination, microbial community composition and diversity are increasingly being considered as highly sensitive ecological parameters (Wang et al. 2007; Desai et al. 2009; Islam and Sar 2011b). Therefore, studies on diversity and composition of indigenous microbial communities within sites having high risk of contamination may serve as baseline information to assess the subsequent impact of contamination.

In the recent years, advances in culture-based and independent molecular approaches have elucidated
microbial diversity and function in sites contaminated with radioactive wastes or wastes generated from uranium, copper and zinc mines. These studies have revealed the presence of diverse (phylogenetically and metabolically) populations of viable and active microorganisms organized in complex communities (Radeva and Selenska-Pobell 2005; Akob et al. 2007; Rastogi et al. 2010). Particularly, the abundance of Acidithiobacillus, Pseudomonas, Acinetobacter, Nitrosonomas was observed in various U mine waste sites in Germany (Radeva and Selenska-Pobell 2005), while high abundance of Proteobacteria (Sphingomonas, Acidovorax, Acinetobacter and Ralstonia) was reported from U-contaminated radioactive waste (Akob et al. 2007). Recently, Rastogi et al. (2010) has reported abundance of Proteobacteria, Acidobacteria and Bacteriodetes in a U mine impacted site in USA. Studies conducted in our laboratory have revealed microbial communities within two U mines (Jaduguda and Banduhurang) along with potential of inhabitant bacteria in U and other metal resistance and sequestration and impact of U ore contamination on soil microbial diversity (Sar et al. 2007; Choudhary and Sar 2011; Islam et al. 2011; Islam and Sar 2011a, b). With vast genetic and metabolic diversity, these microorganisms were found to interact with metals and radionuclides directly or indirectly by redox transfer, biosorption, bioaccumulation or bioprecipitation affecting their environmental mobility and toxicity (Suzuki and Banfield 2000; Tabak et al. 2005; Nedelkova et al. 2007). Considering the significance of geomicrobiology of contaminated sites, it is therefore imperative to decipher phylogenetic diversity of indigenous microbial populations in sites having high risk of contamination or already contaminated to illuminate community resilience, their potential role in affecting metal biogeochemistry and in designing appropriate bioremediation strategies (Tabak et al. 2005; Akob et al. 2007).

Uranium mines at Jaduguda, Bagjata, and Turamdih are all located in highly mineral-rich areas of East Singhbhum district, Jharkhand, India. Jaduguda mine is the oldest U mine in India operating since 1968, while the other two mines are commissioned relatively recently (2002–2003) (Gupta and Sarangi 2005). Present work was undertaken to ascertain diversity and structure of bacterial communities within sites in and around these U mines as a means to obtain the baseline data on microbial diversity. Sites contaminated with U-ores/-mine wastes as well as sites located away from such contaminants (ores and wastes; especially, agriculture fields, streams, etc. located outside the mine areas) were considered. Samples collected from all these sites were analyzed for their physicochemical properties (pH, conductivity, total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP) and heavy metal content). Culturable bacterial counts were recorded and finally the compositions of microbial communities were determined at molecular level with the determination of diversity indices and identification of dominant ribotypes.

Materials and methods

Collection of samples

Soil, water and sediment samples were collected from various locations of proposed and existing uranium mines of at Jaduguda (N 22°39′, E 86°20′), Bagjata (N 22°28′, E 86°29′), and Turamdih (N 22°43′, E 86°11′). Samples B209, B210, B211, B214, B218 and T112, T219, T221, T222 were collected from agriculture field; pond and small streams located outside the boundary of Bagjata and Turamdih mines, respectively, and are designated as non-contaminated samples. Samples CW1, CW2 and CW3 were obtained from sites within the mines and contaminated with mine wastes and ores and are designated as contaminated samples. All samples were collected aseptically and stored immediately in ice till further analysis.

Geomicrobiological analysis

Physiochemical parameters including pH, conductivity/salinity of the samples were measured by an Orion star series meter (Thermo Electron Corporation) following the procedure as described elsewhere (Islam and Sar 2011a). Heavy metals and actinide elements were estimated by ICP-MS (Varian) and/or AAS (Perkin-Elmer). Culturable microbial populations were enumerated by CFU counts using R2A medium. Measured quantities of samples were dispensed in sterile saline (0.9%), shake (1 h), diluted and plated in triplicate on R2A medium. The composition of R2A medium was (g/lit): yeast extract, 0.5; peptone, 0.5; casamino acid, 0.5; dextrose, 0.5; soluble starch, 0.5; sodium pyruvate, 0.3; dipotassium phosphate, 0.3; magnesium sulphate, 0.05; (Fredrickson et al. 2004). Plates were incubated at 30 °C in dark and colonies were counted after 1 week.

Extraction of community metagenomes, PCR amplification of 16S rRNA genes, cloning and clone library analysis

Community metagenome from each sample was extracted using Power soil DNA kit (MO BIO laboratories, Inc.). 16S rRNA genes from each extracted metagenomes were PCR amplified using universal and bacteria-specific primers (27F and 1492R, respectively) (Islam et al. 2011). Amplified products were purified using gel-purification kit (Qiagen), cloned into pGEM-T easy vector (Promega) and transformed...
into *E. coli* JM109 following manufacturer’s instructions. For each sample, positive clones were selected for clone library construction. Detailed description of methodology was same as described previously (Islam and Sar 2011a).

Amplified ribosomal DNA restriction analysis (ARDRA)

From each library 50–100 clones were used for reamplification of cloned 16S rRNA gene by colony PCR with vectorspecific primers. Colony PCR products were purified and digested with restriction enzymes in separate reactions. Portion (10 µl) of the amplification products were digested at 37 °C for 3 h with the restriction endonuclease *Rsa*I and *Hae*III. Digestion products (10 µl) were run on 2.5% agarose gel for 3 h and band patterns were compared visually.

Phylogenetic analysis 16S rRNA gene

All positive 16S rRNA gene clones were grouped according to their ARDRA pattern and each group was referred as an operational taxonomic unit (OTU). About first 600–700 nucleotides of representative clones from each dominant OTU were sequenced. All sequences were examined as potential chimeras using the CHIMERA_CHECK program at the Ribosomal Database Project II (RDP-II) with default settings. Both BLAST program of NCBI database and Hierarchy Browser from Ribosomal Database Project II (RDP-II) were used to find out nearly identical sequences for the 16S rRNA gene sequences determined. Phylogenetic trees were constructed using the Neighbor-joining method with Jukes-Cantor distance correction in MEGA software version 4 using selected sequences (>500 nucleotides and positive polarity) (Tamura et al. 2007).

Statistical analysis

Based on ARDRA profiles, Shannon’s equitability and diversity indices were calculated for each sample (Shannon 1948).

Nucleotide sequence accession numbers

Nucleotide sequences obtained in the present study were deposited in GenBank database under the accession numbers HM469536-HM469615 and HQ909283-HQ909337.

Results and discussion

Physicochemical and microbiological analyses

All samples were analyzed for their physicochemical and microbiological properties (Figs. 1, 2). As evident from the

![Fig. 1 Geomicrobial properties of the samples](image1)

![Fig. 2 Analysis of heavy metals and radionuclides (U and Th) content within the samples](image2)
concentrations of trace elements estimated in non-anthropogenic soils (Burt et al. 2003). Level of Th was found to be within a relatively narrow range of 3–17 mg/kg across all the samples tested. Concentrations of metals like Co, Ni and Zn were considerably higher in contaminated samples (over the non-contaminated counterparts) exceeding the values reported for non-anthropogenic soils or matches well with those reported for anthropogenically contaminated soils (Burt et al. 2003). Uranium, though present in several non-contaminated samples as well, was present at elevated level in two contaminated samples (CW1 and CW2) (Fig. 2). Noticeably, in all three non-contaminated samples simultaneous presence of three or more metals (including U) at higher concentrations was observed. Presence of U and other metals in non-contaminated samples could be explained considering the fact that this whole region is highly mineral-rich and natural weathering may easily enrich the soil and other parts with metals (Sarangi and Singh 2006).

16S rRNA gene clone library analysis and bacterial diversity indices

For analyzing bacterial diversity nearly 50 or 100 clones were used to construct individual clone library for each sample. Based on ARDRA profiles, statistical analysis was followed to determine the bacterial diversity within these samples. Interestingly, all three contaminated samples showed relatively higher Shannon diversity indices (H) but the equitability value (\( E_{\text{H}} \)) varied considerably among the samples [B209 (2.88, 0.71), B210 (3.0, 0.93), B211 (2.77, 0.89), B214 (2.48, 0.63), B218 (2.99, 0.94), T112 (3.22, 0.95), T219 (3.52, 0.97), T221 (2.6, 0.70), T222 (2.9, 0.95), CW1 (3.65, 0.92), CW2 (3.51, 0.89) and CW3 (3.69, 0.92)]. Based on diversity result, we inferred that high nutrient content of the samples possibly make the heavy metal contamination as less stressful event as the inhabitant bacterial members might be nutritionally well supported to withstand metal toxicity. Nevertheless, near neutral pH may allow formation of relatively insoluble metal complexes for most cations thereby reducing their availability as well.

Sequence analysis and affiliation of major ribotypes

Compositions of bacterial communities within the samples were ascertained by analyzing 16S rRNA gene sequences of major ribotypes from each library (Figs. 3a, b and 4). Our analysis revealed that samples from non-contaminated sites were mostly represented by Acidobacteria, Bacteroidetes, Firmicutes and Proteobacteria (\( \beta-\), \( \gamma-\) and/or \( \delta-\)subdivisions) along with less frequent Nitrospira, Deferrribacteres, Chloroflexi in one or few samples. Among the samples collected from Bagjata, bacterial communities in B209 (agriculture field) and B211 (river sediment) showed representatives of Bacteroidetes, Acidobacteria and Firmicutes. Presence of \( \beta-\) and \( \delta-\)Proteobacteria in B211 was noticeable. Sample B210 (pond sediment) comprised Bacteroidetes, Gemmmatimonadetes and Candidate division along with \( \gamma-\)Proteobacteria. Although members of \( \beta-\) and \( \gamma-\)Proteobacteria were found in B 214 (garden soil) and B218 (river sediment), the absence of all three major phyla like Acidobacteria, Bacteroidetes and Firmicutes was notable. Additionally, phyla Chloroflexi and Deferrribacteres were present in high percentage in B214, while Cyanobacteria and Chloroflexi were present in B218. All the samples from Turamdih showed the presence of Acidobacteria as a major group along with Bacteroidetes/ Firmicutes/Cyanobacteria or Gemmmatimonadetes in one or more samples. Members of Proteobacteria were not so abundant in these samples. Particularly, \( \gamma-\)Proteobacteria was not found, although the presence of either \( \beta-\) or \( \delta-\)Proteobacteria was detected in few samples.

Samples obtained from highly contaminated locations of Jaduguda mine showed distinct abundance of \( \beta-\)Proteobacteria, along with \( \gamma-\) and \( \alpha-\)Proteobacteria, Acidobacteria, Bacteroidetes and Firmicutes. Members of Actinobacteria, Chloroflexi, Planctomycetes, Cyanobacteria and Gemmmatimonadetes were detected as relatively minor groups. Sample CW1 showed diverse assemblage of bacterial populations well represented by Proteobacteria (\( \beta- \rangle \gamma- \rangle \delta- \rangle \alpha-\)subdivisions) > Acidobacteria > Gemmmatimonadetes > unclassified bacteria > Bacteroidetes. The observed community structure within this sample corroborates well with its relatively higher organic carbon, nitrogen and phosphorous content and CFU counts suggesting that in spite of higher metal contamination bacterial flora can flourish very well possibly by developing appropriate homeostatic mechanisms (Akob et al. 2008).

Phylogenetic analysis of microbial groups

Sequences representing ARDRA OTUs were analyzed to ascertain their phylogenetic lineage with similar taxa/groups from diverse ecological habitats and with environmentally relevant metabolic functions (Table 1; Figs. 5, 6, and 7).

**Acidobacteria**

Similarity search in NCBI and RDP databases indicated that all the Acidobacterial sequences had strong identity with uncultured members of this phylum. Particularly, sequences from non-contaminated samples showed a strong similarity with uncultured Acidobacteria from undisturbed mixed grass prairie and rice field soils. In
contrast, *Acidobacteria* sequences retrieved from contaminated samples showed high similarity with uncultured clones mostly obtained from hydrocarbon-contaminated soil and sediment, soil adjacent to silage storage bunker and Altamira cave, etc. In order to determine the distribution of *Acidobacteria* sequences retrieved from ten clone
**Fig. 4** Relative abundance of bacterial phyla detected within the samples

![Graph showing relative abundance of bacterial phyla.](image)

**Table 1** Details of 16S rRNA gene sequences retrieved in this study and used for phylogenetic analysis

| Sl no. | Accession number | Clone id | Phylum | Present in |
|--------|------------------|----------|--------|------------|
| 1      | HM469555         | T112-61C1| Acidobacterium | Fig. 5 |
| 2      | HM469561         | B209-3B2 | Bacteroidetes | Fig. 7 |
| 3      | HM469565         | B209-18B5| Nitrospira | Fig. 7 |
| 4      | HM469566         | B209-3B6 | Bacteroidetes | Fig. 7 |
| 5      | HM469560         | B209-8B1 | Firmicutes | Fig. 7 |
| 6      | HM469567         | B209-33B8| Acidobacteria | Fig. 5 |
| 7      | HM469568         | B209-35B9| Acidobacteria | Fig. 5 |
| 8      | HM469570         | B210-6S4 | Acidobacteria | Fig. 5 |
| 9      | HM469571         | B210-7S5 | Acidobacteria | Fig. 5 |
| 10     | HM469573         | B210-14S1| γ-Proteobacteria | Fig. 6 |
| 11     | HM469574         | B210-44S6| Acidobacteria | Fig. 5 |
| 12     | HM469575         | B210-1S8 | Acidobacteria | Fig. 5 |
| 13     | HM469578         | B211-9R5 | δ-Proteobacteria | Fig. 6 |
| 14     | HM469579         | B211-39R2| Bacteroidetes | Fig. 7 |
| 15     | HM469580         | B211-27R7| Firmicutes | Fig. 7 |
| 16     | HM469581         | B211-10R1| β-Proteobacteria | Fig. 6 |
| 17     | HM469584         | B214-22N1| Chloroflexi | Fig. 7 |
| 18     | HM469585         | B214-3N2 | β-Proteobacteria | Fig. 6 |
| 19     | HM469586         | B214-5N3 | Chloroflexi | Fig. 7 |
| 20     | HM469591         | B218-31E1| Cyanobacteria | Fig. 7 |
| 21     | HM469594         | B218-6E4 | Chloroflexi | Fig. 7 |
| 22     | HM469597         | B218-10 | δ-Proteobacteria | Fig. 6 |
| 23     | HM469592         | B218-15E2| γ-Proteobacteria | Fig. 6 |
| 24     | HM469593         | B218-2E3 | β-Proteobacteria | Fig. 6 |
| 25     | HM469596         | B218-35E7| β-Proteobacteria | Fig. 6 |
| 26     | HM469598         | T219-15D1| Cyanobacteria | Fig. 7 |
| 27     | HM469600         | T219-36D3| Bacteroidetes | Fig. 7 |
| 28     | HM469601         | T219-04D8| Bacteroidetes | Fig. 7 |
| 29     | HM469602         | T219-27D5| Acidobacteria | Fig. 5 |
| 30     | HM469603         | T219-31D4| Cyanobacteria | Fig. 7 |
| 31     | HM469604         | T219-03D7| Cyanobacteria | Fig. 7 |
libraries into different subgroups, a Neighbor-joining tree was constructed using representative Acidobacteria sequences of different subgroups along with our sequences (Fig. 5). Representative sequences for various subgroups were obtained from previously published works of Sait et al. 2006 (subgroup 1) and Hugenholtz et al. 1998 (subgroup 2, 3, 4, 5, 6, 7 and 8). Hugenholtz et al. (1998) mentioned that Acidobacteria subdivisions 1, 3, 4, and 6 are well represented by environmental clone sequences with only five cultured representatives. In this regard, our analysis indicated that members of subgroup 1, 3, 4 and 5 were present within all eight libraries. Members that belong to other subgroups (2, 6, 7 and 8) were not detected in the present libraries. Phylogenetic tree further revealed that the

| Sl no. | Accession number | Clone id      | Phylum         | Present in |
|-------|-----------------|---------------|----------------|------------|
| 32    | HM469610        | T221-14M5     | Acidobacteria  | Fig. 5     |
| 33    | HM469612        | T222-8G2      | Acidobacteria  | Fig. 5     |
| 34    | HM469613        | T222-5G3      | Acidobacteria  | Fig. 5     |
| 35    | HM469614        | T222-18G5     | Acidobacteria  | Fig. 5     |
| 36    | HM469538        | CW1-8A4       | β-Proteobacteria| Fig. 6     |
| 37    | HM469540        | CW1-60A8      | Acidobacteria  | Fig. 5     |
| 38    | HM469536        | CW1-14A2      | Gemmatimonadetes| Fig. 7    |
| 39    | HM469543        | CW1-3A11      | Acidobacteria  | Fig. 5     |
| 40    | HM469550        | CW1-77A19     | Acidobacteria  | Fig. 5     |
| 41    | HM469541        | CW1-36A9      | β-Proteobacteria| Fig. 6     |
| 42    | HM469545        | CW1-22A13     | Acidobacteria  | Fig. 5     |
| 43    | HM469548        | CW1-56A17     | γ-Proteobacteria| Fig. 6     |
| 44    | HM469551        | CW1-78A20     | β-Proteobacteria| Fig. 6     |
| 45    | HM469549        | CW1-57A18     | Bacteroidetes  | Fig. 7     |
| 46    | HQ909289        | CW2-36        | Cyanobacteria  | Fig. 7     |
| 47    | HQ909290        | CW2-44        | β-Proteobacteria| Fig. 6     |
| 48    | HQ909293        | CW2-77        | α-Proteobacteria| Fig. 6     |
| 49    | HQ909288        | CW2-35        | Cyanobacteria  | Fig. 7     |
| 50    | HQ909294        | CW2-96        | Cyanobacteria  | Fig. 7     |
| 51    | HQ909295        | CW2-97        | Actinobacteria | Fig. 7     |
| 52    | HQ909296        | CW2-128       | γ-Proteobacteria| Fig. 6     |
| 53    | HQ909324        | CW3-166       | β-Proteobacteria| Fig. 6     |
| 54    | HQ909306        | CW3-12        | β-Proteobacteria| Fig. 6     |
| 55    | HQ909307        | CW3-16        | β-Proteobacteria| Fig. 6     |
| 56    | HQ909309        | CW3-25        | β-Proteobacteria| Fig. 6     |
| 57    | HQ909326        | CW3-145       | Acidobacteria  | Fig. 5     |
| 58    | HQ909316        | CW3-130       | α-Proteobacteria| Fig. 6     |
| 59    | HQ909319        | CW3-144       | Firmicutes     | Fig. 7     |
| 60    | HQ909317        | CW3-133       | α-Proteobacteria| Fig. 6     |
| 61    | HQ909318        | CW3-136       | β-Proteobacteria| Fig. 6     |
| 62    | HQ909327        | CW3-20        | α-Proteobacteria| Fig. 6     |
| 63    | HQ909330        | CW3-167       | β-Proteobacteria| Fig. 6     |
| 64    | HQ909311        | CW3-138       | Firmicutes     | Fig. 7     |
| 65    | HQ909308        | CW3-88        | β-Proteobacteria| Fig. 6     |
| 66    | HQ909312        | CW3-33        | α-Proteobacteria| Fig. 6     |
| 67    | HQ909314        | CW3-52        | β-Proteobacteria| Fig. 6     |
| 68    | HQ909320        | CW3-147       | α-Proteobacteria| Fig. 6     |
| 69    | HQ909322        | CW3-161       | β-Proteobacteria| Fig. 6     |
| 70    | HQ909332        | CW3-142       | Acidobacterium | Fig. 5     |
| 71    | HQ909337        | CW3-45        | γ-Proteobacteria| Fig. 6     |
| 72    | HQ909335        | CW3-11        | α-Proteobacteria| Fig. 6     |
most dominant Acidobacteria clones from libraries B209, B210, T219, T221 and TD222 were present within subgroup 1. Within this subgroup, seven sequences representing the major ribotypes from the above libraries were included. Sequence of the clone B210-44 although shared identity with an uncultured bacterium, showed a close phylogenetic lineage with culturable representative Acidobacterium capsulatum with 90% bootstrap value. The next cluster that accommodated maximum numbers of ribotypes showed their affiliation to subgroup 4. In this subgroup, four Acidobacteria clones from the contaminated site CW1, and three from non-contaminated sites (T112, B209 and B210) were included. Subgroup 5 was composed of four dominant Acidobacteria clones; two retrieved from contaminated library CW3 and other two from non-contaminated library T112. Subgroup 3 was represented by members from the library T222. Abundance of Acidobacteria in uranium-contaminated samples was reported by Barns et al. 2007. The ubiquity and abundance of Acidobacteria in soils and their ability to withstand polluted and extreme environments suggest that they serve functions important in the environment and are potentially quite varied (Ward et al. 2009).

Proteobacteria

Predominance of phylum Proteobacteria was observed mainly in contaminated samples, although their presence was detected within non-contaminated samples as well. Among the Proteobacteria, members of the class β-Proteobacteria was found to represent the abundant ribotypes in contaminated as well as non-contaminated samples. As evident from Fig. 5, within the contaminated samples nine ribotypes of CW3 library (42% coverage), five ribotypes (29% coverage) from CW2 library and two ribotypes (11% coverage) of library CW1 were affiliated to β-Proteobacteria. Several major ribotypes from non-contaminated samples (B18, B211 and B214) were belonging to β-Proteobacteria. As evident from the tree topology, three clones (CW3-63, CW3-161 and CW3-52) from CW3 formed a separate monophyletic clade showing their relatedness with thiolsulfate oxidizing autotrophic bacterium Thiobacillus thioparus (ATCC 8158). In another clade clone B218-35 (covering 9% of the library) showed affiliation with N2-fixing bacterium Herbaspirillum sp. MS-F-86 (FJ460080) isolated from Ninghai harbor, China. In the same clade clone, CW3-136 and CW3-166 of library CW3 showed strong phylogenetic
affiliation and lineage (100% bootstrap support) with nickel-resistant Massilia timonae (AY512824). Clone CW3-12 showed a strong affiliation with uncultured β-Proteobacteria (EF492892) isolated from soil around iron-manganese nodule from China. Other clones in the same library formed a single clade showing their affiliation with hydrocarbon degrader Propionibiviro loricola (AJ307983). Clone CW2-44 and CW3-25 showed affiliation with perchlorate-reducing bacterium Dechloromonas sp. LT-1 (AY124797) and denitrifying Azonexus fungiphilus (AF011350), respectively. Clone B214-3, the most abundant ribotype (covering 22%) of the library B218 formed a monophyletic node showing close relatedness with ‘nanometric’ bacterium Ramlibacter tataouinensis (AF144383) isolated from meteorite-impacted subdesert soil in Tunisia, France. The other node was comprised six clones. Clones B218-167 and B211-10 of this cluster showed a strong affiliation with uncultured β-Proteobacteria (AY921995). Clone CW3-167 showed affiliation with cholesterol-oxidizing, denitrifying bacterium Sterolibactrum denitrificans (AJ306683). Along with clones CW2-75 and CW2-162 having relatedness with Burkholderiales bacterium YT0099, the later clones showed a strong lineage with members of Burkholderiaceae capable of redox transformation of metals to satisfy metabolic requirements. Sequence of clone CW3-88 formed a separate clade showing its distinctness from other members but strong phylogenetic lineage (99% bootstrap value) with uncultured Alcaligenaceae (AM936094) isolated from hydrocarbon contaminated soil. Members of the...
class \( \gamma \)-Proteobacteria were relatively more abundant in contaminated samples. Sequence of clone B218-15 was highly related to an uncultured member of \( \gamma \)-Proteobacteria. Two clones (CW2-3 and CW2-128 covering 21% of CW2 library), showed a strong phylogenetic affiliation with arsenic-resistant soil bacterium (AF308875). Sequence of clone B210-14 marked its affiliation with uncultured Xanthomonadace bacterium, while two clones CW1-36 and CW3-45 showed their lineage to polychlorinated dioxin dechlorinating bacterium. Members of the class \( \alpha \)-Proteobacteria were represented by ribotypes from contaminated samples CW2 and CW3, covering 25 or 27% in respective libraries. Within this class, sequences of clones CW2-52 and CW2-77 showed a strong relatedness with \( N_2 \)-fixing bacteria Azospirillum amazonense. Sequences of clones CW3-33 and CW1-36 showed phylogenetic affiliation with uncultured bacterium isolated from floor dust. Together with sequence of CW2-29, both these two clades were found to be related sharing the same node, thus indicating a common phylogenetic origin. The remaining clade

Fig. 7 Neighbor-joining tree constructed based on 16S rRNA gene sequences of phyla Bacteroidetes, Firmicutes, Actinobacteria, Gemmatimonadetes and Cyanobacteria detected in the present study along with similar sequences retrieved from NCBI and RDP databases.

Numbers at nodes indicate percent bootstrap values above 80 supported by 1,000 replicates. Bar indicates Jukes-Cantor evolutionary distance.
within this class was composed of five clones. Clone CW3-130 and CW3-147 (covering 7% of the CW3 library) showed affiliation with mixed grass bacterium where as clone CW2-100 showed affiliation with Microvirga sp. (EU529844) isolated from soil of cucumber farming greenhouse. Sequence of clone CW2-140 showed a strong affiliation with Parvibaculum lavamentivorans (AY387398) bacterium capable of alkylbenzenesulfonate surfactant (LAS) biodegradation. Sequences of three other clones CW3-133, CW3-20 and CW3-11, although strongly related to each other showed phylogenetic affiliation with uncultured bacterium isolated from a poplar tree microcosm. Class δ-Proteobacteria was represented by two clones (B218-10 and B211-9). Sequence of clone B218-10 was the most abundant ribotype in library B218 that showed affiliation with Desulfovibrio alkalitolerans (AY649785) isolated from a heating water of Denmark. Dominancy of Proteobacteria in contaminated sites is well reported (Rastogi et al. 2010; Ellis et al. 2003). Lineages belonging to Proteobacteria are well known to survive in oligotrophic environments, capable of metal reduction and resistance and play very important roles in uranium immobilization in contaminated sediments or groundwater.

Bacteroidetes, Firmicutes, Actinobacteria, Gemmatimonadetes and Cyanobacteria

Members of these phyla were more abundant in non-contaminated samples (Fig. 6). Phylum Bacteroidetes was represented by five ribotypes. Sequence of clone B209-3 showed a strong phylogenetic lineage (100% bootstrap value) with Flavobacterium sp. (EU194891) while clone (B211-39) showed a strong affiliation with uncultured bacterium isolated from golden mine tailings of Nederland. Clone CW2-129 showed affiliation with Sediminibacterium sp. (FM179320) where as clone CW1-57 showed affiliation with uncultured bacterium isolated from a waste-gas biofilter. Presence of the phylum Bacteroidetes in heavy metal and uranium-contaminated soils and sediments were reported previously by Ellis et al. 2003; Reardon et al. 2004; Brodie et al. 2006 and Akob et al. 2008.

Phylum Cyanobacteria was represented by eight clones. Among the four clones from non-contaminated sites, three from library T219 showed lineage with Oscillatoria duplexsecta (AM398647) isolated from a thermal mud of Euganean thermal springs (Italy). Clone CW2-104 from sample CW2 also showed affiliation with Oscillatoria acuminata (AB039014). Other three clones (CW2-96, CW2-34 and CW2-35) from contaminated sample CW2, covering 11% of the clone library, and showed affiliation with uncultured Chlorophyta chloroplast 16S rRNA gene (AB374385). Cyanobacteria were the ubiquitous group present in most soil habitats and possessing high tolerance to environmental stress (Chong et al. 2010). Our results indicated that it may be present both in contaminated and non-contaminated samples. It also can act as a biosorbent of heavy metals in sewage water (El-Enany and Issa 2000). Phylum Firmicutes was represented by four clones. Clone B211-27 showed a strong phylogenetic affiliation (100% bootstrap value) with Firmicutes bacterium isolated from Pere Marquette River sediment. Clone B219-8, CW3-144 and CW3-138 showed a strong phylogenetic affiliation (100% bootstrap value) with Manganese (II)-oxidizing Bacillus sp. Akob et al. (2008) showed the presence of Firmicutes in radionuclide-contaminated subsurface sediments during the early part of the ethanol microcosm incubation suggesting that these groups may have actively involved in nitrate reduction and subsequent uranium reduction both important for the immobilization of U(VI) in contaminated sediments. Merroun et al. (2005) showed that Bacillus sp., can be recovered from uranium mining waste pile and can make complexion with uranium by either whole cells or S-Layer sheets. Phylum Chloroflexi was mainly represented by ribotypes from sample B214. Sequences of clone B214-22 and B214-6 formed a distinct monophyletic clade along with Anaerolinea thermolimosa (AB109437) isolated from thermophilic granular sludge. Phylum Actinobacteria, Nitrospira and Gemmatimonadetes were all represented by single ribotype. In the phylum Actinobacteria, clone CW2-97 was found to be phylogenetically affiliated (with 100% bootstrap value) with Cellulomonadaceae str. KB8 isolated from paddy rice. Clone B209-18 showed a strong phylogenetic affiliation with nitrite oxidizing Nitrospira moscoviensis. In the phylum Gemmatimonadetes, clone CW1-14 showed affiliation with uncultured bacterium isolated from oil palm empty fruit bunches compost heap.

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