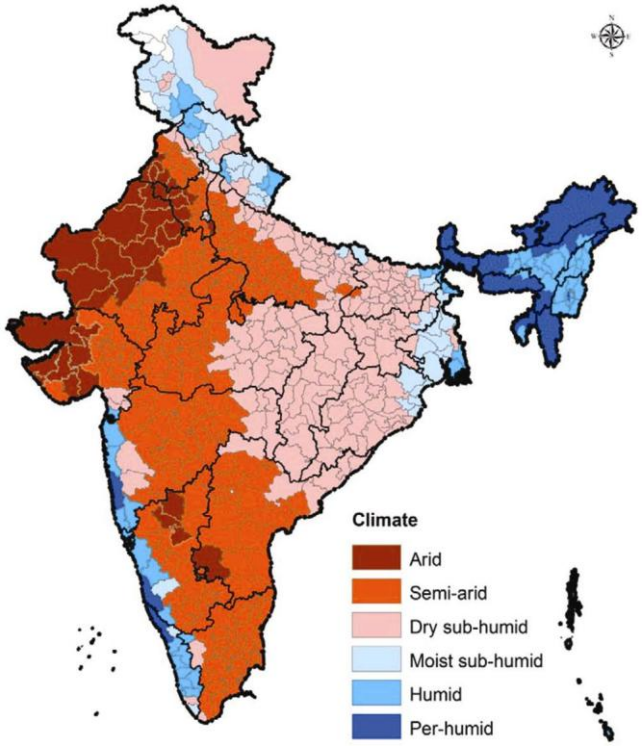

Figure 3. Climatic classification at district level (1971–2005).

The climatic classification needs to be revisited at least once in 30 years; may be more frequently in future since more warming trends have been projected for future. Such an exercise may not reveal a substantial change in the overall area under different climates, even then may reveal spatial shifts of climatic zones, which has bigger implications for crop planning, water resources assessment and launching of special schemes on drought and floods including disaster management. Based on the revised classification, there is an urgent need to revisit the present DPAP and DDP districts in the country, previously reviewed by MoRD[6] in 1994 under the chairmanship of C. H. Hunumantha Rao.

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Soil bacterial metagenomic analysis from uranium ore deposit of Domiasiat in Northeast India

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Total bacterial community analyses were performed for uranium ore deposit soil samples of Domiasiat utilizing cultivation-independent approach. Screening based on amplified ribosomal DNA restriction analysis (ARDRA) using *Msp*I and *Hae*III was performed to analyse 150 clones which generated 59 distinct ribotypes from the clone library. Representative 96 clone partial 16S rRNA gene were phylogenetically related to 10 different bacterial groups. *Proteobacteria* and *Acidobacteria* were the most abundant bacterial group while 7% of the clones represented novel bacterial lineages. The bacterial diversity obtained by the culture-independent approach presented a larger diver-

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sity of bacteria as compared to the conditioned cultivation method. The study also provides baseline metagenomic information to assess subsequent impact of environment perturbation consequent to uranium mining at the studied site.

Keywords: ARDRA, clone library, cultivation-independent, Domiasiat, ribotypes.

URANIUM (U) ore deposit in Domiasiat is hosted by the Kylleng and Rangam blocks with peneconcordant, tabular sandstone-type U mineralization lying in Northeast India. It contains 9.22 million tonnes of ore reserves of average 0.1% U_3O_8 (triuranium octoxide) ore grade covering 10 km^2 area in and around Domiasiat and Phlangdiloin[1]. The present study revealed baseline data for the bacterial community structure harboured in the soils containing uranium utilizing culture-independent approach. This study was undertaken to supplement our previous data on the bacterial community diversity using culture-dependent approach[2] that advanced our understanding of the interaction and function of the complex microbial community within their niches[3–6]. There are numerous metagenomic studies on the microbial communities of radionuclide contaminated environments[3,7–14] and a few on the microbial communities of the U ore deposits[6,15]. Earlier studies on the culturable bacteria isolated from the Domiasiat uranium ore deposit focused on their diversity, bioremediation potential and horizontal gene transfer among the indigenous isolates[2,16,17]. In the present investigation, metagenomic study of the above-mentioned uranium ore deposit involved construction of 16S rRNA gene-based clone library and screening by amplified ribosomal DNA restriction analysis (ARDRA). Representatives from the ARDRA groups were utilized to generate partial 16S rRNA gene sequences to analyse the soil bacterial community. The uncultured bacterial diversity, thus obtained, was surprisingly much varied and higher than the cultured diversity of the site.

Domiasiat (25°30′N, 91°30′E), located 130 km southwest of Shillong (capital city of Meghalaya) in Northeast India possesses rich uranium deposits forming a part of the Cretaceous Mahadek[1,18]. The 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. Soil samples were collected from a depth of 15 to 30 cm in sterile plastic bags and transported on ice to the laboratory. Genomic DNA was extracted from soil samples using HiPurA™ Soil DNA Kit (HiMedia, India) according to manufacturer's instructions. The 16S rRNA gene sequences were amplified from the total soil DNA using the general bacterial 16S rRNA gene primers 27F and 1492R[2].

The amplified 16S rRNA gene products (~ 1500 bp) were purified using QIAquick Gel Extraction Spin Kit (QIAGEN, Germany) and were cloned into the pGEM-T® Easy vector (Promega, USA) and transformed into *E. coli* JM109 (competent cell provided with the kit) following manufacturer's instructions and plated into IPTG-X-gal plates. The clone library designated as RMSRJ with the clone insert of full length 16S rRNA gene fragment was constructed from the total DNA of the soil sample of U ore bearing site. Colony polymerase chain reaction (PCR) was performed to amplify 16S rRNA gene fragments of the clones directly from the fresh cell suspension using the vector-specific primers SP6 and T7 (universal sequencing primer present in pGEM-T vectors). The colony PCR-amplified products were first checked for the presence of the targeted gene in 1.2% (w/v) agarose gel and after confirmation, the amplified product (5 µl) from each clone was digested separately with two commonly used restriction endonucleases, 1 unit of *Hae*III and 1 unit of *Msp*I for 4 h at 37°C according to manufacturer's instructions (Fermentas, Canada) in a 20 µl reaction mixture. Reaction was stopped by thermal inactivation at 65°C for 20 min. The digested products were resolved on a 3% (w/v) agarose gel electrophoresis at 80 V with ethidium bromide staining. Restriction patterns were analysed visually using transilluminator (UVITEC, UK) to group the clones based on similar banding patterns. Screening of the clone library was performed by studying the ARDRA patterns. The clones were considered members of the same ribotype or operational taxonomic unit (OTU) when their ARDRA patterns were identical for both the restriction enzymes.

One or more representative clone(s) from each dominant OTU was selected for determination of partial sequences of the 16S rRNA gene fragments using the primers 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5′-TACCAGGGTATCTAATCC-3′) at Macrogen Inc., Korea. The partial region of 16S rRNA gene used in this study comprised the hypervariable region V4 which ranges from 589 to 650 bp of the 16S rRNA gene. Basic Local Alignment Search Tool (BLAST)[19] was used to compare the sequence data of the uncultured bacterial DNA obtained from the U ore bearing site of Domiasiat with the 16S rRNA gene sequences available in GenBank database of NCBI and Ribosomal Database Project (RDP) (available online at http://rdp.cme.msu.edu/). Molecular Evolutionary Genetics Analysis software (MEGA version 4) was used for phylogenetic analyses[20]. The sequences retrieved for the nearest phylogenetic neighbours from GenBank were aligned with the sequences of uncultured bacteria using ClustalW inbuilt with MEGA4. *Acidilobus saccharovorans* (AY350586) was used as the outgroup organism. Neighbour-joining method was employed to construct the phylogenetic tree with 1000 bootstrap replications to assess nodal support in the tree.

The nucleotide sequences analysed in the present study were deposited in the NCBI GenBank under the accession numbers: JN600358–JN600439 and JN618165–JN618178.

The 16S rRNA genes of randomly selected clones were amplified and the restriction patterns generated by the enzymes *Msp*I and *Hae*III were recorded. ARDRA patterns revealed a total of 59 distinct OTUs from 150 analysed clones. The partial 16S rRNA gene sequences (~ 300 bp) of the representative 96 clones were used to analyse the diversity of uncultured bacteria from the subsurface soil of U ore deposit. Phylogenetic analyses were based on the 16S rRNA gene sequence of one or more representative clones from each major ribotype. Phylogenetic clustering with the nearest neighbours from the NCBI GenBank clones revealed that the clones obtained from the library RMSRJ belonged to phyla *Acidobacteria*, *Actinobacteria*, *Firmicutes*, *Verrucomicrobia*, *Chloroflexi* and *Chlorobi* and to four classes within the *Proteobacteria* phylum (Figure S1*a–e*; see supplementary material online). Out of the representative 96 clones sequences analysed, 7 sequences did not form clusters with its nearest phylogenetic neighbours with good bootstrap support and were seen as outgroup in the respective tree and hence were placed as unclassified group (Figure 1). These unclassified sequences included one each from *Actinobacteria*, *Gammaproteobacteria* and *Deltaproteobacteria* and two from *Alphaproteobacteria* and *Firmicutes*. The most abundant group of sequences among the analysed clones which comprised 44% clones of the RMSRJ library were affiliated to *Proteobacteria* group (Figure 1). Among the *Proteobacteria*, maximum representation was of *Alphaproteobacteria* (16%), followed by *Betaproteobacteria* (15%), *Gammaproteobacteria* (9%) and *Deltaproteobacteria* (4%). The proteobacterial group was followed in abundance by *Acidobacteria* (28% of the library clones), *Firmicutes* (11% of the clones) and *Actinobacteria* (5% of the clones). Other groups which were represented in comparatively lower abundance were *Chloroflexi* (2%), *Verrucomicrobia* (2%) and *Chlorobi* (1%). Bacterial diversity obtained by the culture-independent approach portrayed a larger diversity of bacteria as compared to the conditioned cultivation method which was described in our previous study[2]. In our earlier study, enrichment-based culture-dependent technique from 10 soil samples across three designated sites[2] recovered bacterial diversity spreading to five different groups, viz. *Firmicutes*, *Gammaroteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Betaproteobacteria* isolated under metal-enriched condition. In the present study, culture-independent approach from these U-containing soil samples led to a recovery of 10 different bacterial groups and 7% of the clones represented novel bacterial lineages. Random selection of 150 clones utilizing two restriction enzymes led to generation of 59 unique ARDRA patterns (OTUs). More number of restriction enzymes would have resulted in higher number of OTUs and hence a single OTU did not represent a single species. To overcome the problem of the underrated bacterial diversity of U ore site, multiple representatives of single OTU were selected for sequencing and further analysis. In total, 96 sequences analysis were performed to determine the phylogenetic clustering of the clone library RMSRJ represented by 59 OTUs. Among the diverse bacterial groups obtained from the studied samples, *Proteobacteria* was observed to be the most abundant among which *Alphaproteobacteria* was found to be predominant group. *Acidobacteria* however happened to be the most abundant group if four proteobacterial groups were considered separately. Similar findings with predominant *Alphaproteobacteria* and *Acidobacteria* were reported from soil samples collected from different depths of the U mining waste pile, Haberland, Germany applying the 16S rRNA gene retrieval with different primer sets[11,21,22]. The present findings corroborate the findings of U mine tailings having *Acidobacteria* as the predominant phyla in terms of the most numerical clones[14]. Soil samples belonging to geographically distinct sites had high U presence and hence observation of 7% previously uncharacterized bacterial lineage is acceptable corroborating with the earlier study on cultivated bacteria[2]. Environments such as the present study site, influence microbial diversity by regulating bioavailability of metal ions, and/or by enhancing the microbial potential to withstand metal toxicity[23]. Culture-independent 16S rRNA gene-based molecular analysis of microbial diversity presented a clear distinction from the culture-dependent study[2]. The present study revealed that *Proteobacteria* and *Acidobacteria* were the most abundant phyla while it was untraced in the earlier reported enrichment-based cultivation study. It is known that standard cultivation techniques fails to account for more than 99% of microbial diversity[23–25]. Our present study using metagenomic approach supplements the previous study on baseline bacterial community of the site and it pro-

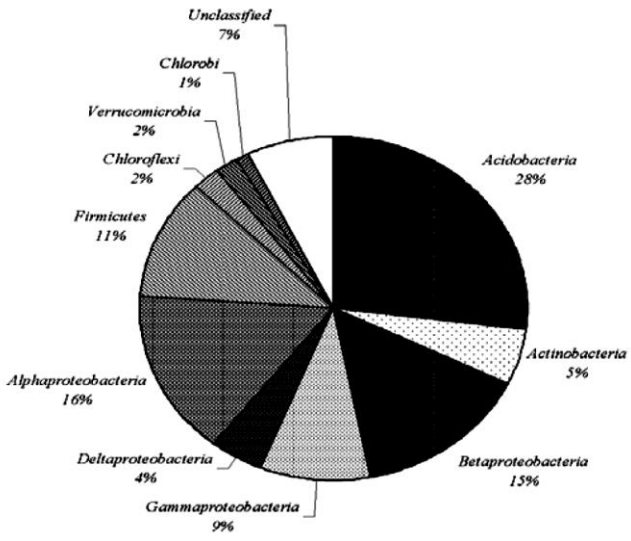

Figure 1. Relative abundance in percentage of bacterial groups detected from the clone library RMSRJ.

vides an additional dataset on bacterial community structure to assess subsequent impact of environment perturbation consequent to uranium mining at the U ore deposit of Domiasiat. Screening of the genes that allow the survival of these uncultivable bacteria in such extreme environment will be explored in future. This would advance our understanding on their adaptation behaviour giving insights into the various mechanisms harboured by these bacteria to alleviate heavy metal and uranium toxicity. Functional screening of 7% previously unclassified bacteria might lead to finding of novel enzymes/biomolecules which can be utilized for bioremediation of uranium and heavy metal contaminated waste.

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