Shotgun metagenomic sequencing based microbial diversity assessment of Lasundra hot spring, India

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This is the first report on the metagenomic approach for unveiling the microbial diversity of Lasundra hot spring, Gujarat State, India. High-throughput sequencing of community DNA was performed on an Ion Torrent PGM platform. Metagenome consisted of 606,867 sequences represent 98,567,305 bps size with an average length of 162 bps and 46% G + C content. Metagenome sequence information is available at EBI under EBI Metagenomic database with accession no. ERP009313. MG-RAST assisted community analysis revealed that 99.21% sequences were bacterial origin, 0.43% was fit to eukaryotes and 0.11% belongs to archaea. A total of 29 bacterial, 20 eukaryotic and 4 archaeal phyla were detected. Abundant genera were Bacillus (86.7%), Geobacillus (2.4%), Paenibacillus (1.0%), Clostridium (0.7%) and Listeria (0.5%), that represent 91.52% in metagenome. In functional analysis, Cluster of Orthologous Group (COG) based annotation revealed that 45.4% was metabolism connected and 19.6% falls in poorly characterized group. Subsystem based annotation approach suggests that the 14.0% was carbohydrates, 7.0% was protein metabolism and 3.0% genes for various stress responses together with the versatile presence of commercially useful traits.

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central reservoir was 52 °C and the nearby reservoirs have a temperature varying from 42 °C to 49 °C. Each reservoir was about 12 ft in depth. The base of the reservoir contains stones and algal growth along with soil sediments.

In the present investigation, the total community DNA was isolated from water samples of the spring using a HiPurA™ Water DNA Isolation kit (MB547; Hi-Media, Mumbai, India). Shotgun metagenomic sequencing was performed using the 318 Chip and 300-bp chemistry Ion Torrent PGM platform as per the manufacturer’s instructions. Analysis and annotation of output data were carried out by MG-RAST V 3.5 online server using default parameter [8]. The output file contained a total of 606,867 sequences comprised of 98,567,305 bps with 46% G + C content. The community analysis revealed predominance of domain bacteria (99.21%), followed by Eukaryota (0.43%) and archaea

Fig. 1. Microbial community structure in Lasundra hot spring metagenome.

Fig. 2. Functional structure based on subsystem annotation of Lasundra hot spring metagenome.
A total of 33 prokaryotic phyla including 29 bacterial and 4 archaeal phyla along with 20 eukaryotic phyla were detected. Abundant prokaryotic phyla were Firmicutes (95.5%) followed by Proteobacteria (2.0%), Actinobacteria (0.8%), Bacteroidetes (0.1%), Cyanobacteria (0.1%) and Euryarchaeota (0.09%). At the family level, Bacillaceae (90.1%) followed by Paenibacillaceae (1.3%), Clostridiaceae (0.8%), Listeriaceae (0.5%) and Staphylococcaceae (0.5%) were major dominating bacterial families. Most leading genera were Bacillus (86.7%), Geobacillus (2.4%), Paenibacillus (1.0%), Clostridium (0.7%) and Listeria (0.5%) (Fig. 1).

Besides the community analysis, functional analysis was performed with MG-RAST. A total of 388,662 sequences have passed quality control and out of them 76.9% produced 183,408 predicted protein coding regions. From this 183,408 predicted protein features, 66.7% of features assigned an annotation using MSNR database whereas, 33.3% of features have no significant similarities to the protein database. A total of 104,110 features were assigned to functional categories with COG approach and interestingly 45.4% was metabolism connected and 19.6% falls in poorly characterized group that is higher than the earlier metagenome study of saline desert [9] indicating the possibilities of gaining novel gene from the metagenome. Similarly, in subsystem based annotation approach 14.0% was carbohydrates, 7.0% was protein metabolism and 3.0% genes were belonged to stress responses i.e. oxidative stress, periplasmic stress, osmotic stress, heat shock, cold shock, acid stress, and detoxification (Fig. 2). Furthermore, the detection of genes encoded for secondary metabolism and metabolism of aromatic compounds indicates that the hot spring is prosperous with commercially valuable enzymes (Fig. 2). Lipase producing Bacillus licheniformis and Bacillus subtilis were reported from this hot spring earlier, which support our study [10]. The detection of the photosynthetic bacteria, heterotrophs and autotrophs in the hot spring metagenome suggested the nutritive interaction among the microorganism. To the best of our knowledge, this is the first study that describes the complete profiling of microbial diversity from the Lasundra hot spring using the next generation sequencing approach. Metagenomic sequence analysis may provide significant clues in understanding the taxonomic structure and functional potential of Lasundra hot spring with the possibility of getting novel genes and microbes for research and commercial application.

**Nucleotide sequence accession number**

Metagenome sequence data are available on EMBL Metagenomics under the accession no. http://www.ebi.ac.uk/ena/data/view/ERP009313.

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