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Metagenomic investigation of bacterial diversity of hot spring soil from Manikaran, Himachal Pradesh, India

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ABSTRACT

Sequencing and analysis of 16S rRNA genes holds immense potential in identifying novel and uncharacterized bacterial phyla from any ecological habitats. Present study was designed to delineate and understand the bacterial diversity from hot spring soil of Manikaran, Himachal Pradesh, India (32°02′N, 77°21′E; elevation 1760 m). The average temperatures of thermal springs in Manikaran vary from 50 to 90 °C. PCR amplification of 16S rRNA genes resulted in DNA bands ranging from 500 to 1500 bp. Subsequent molecular manipulations, sequencing and bioinformatics analysis of 16S rRNA genes confirmed that ~61% of the recovered clones were affiliated to uncultured bacteria. Phylum *Actinobacteria* represent ~8% of the total sequences. Other minor groups that constituted 1–2% of the total sequences showed sequence similarity with the uncultured Acidobacteria (Gram-positive), Cyanobacteria (Gram-negative) Alpha Proteobacterium (Gram negative), Uncultured Sphingomonas, Uncultured Arthrobacter sp. Ucultured Rihizobiales and other uncultured sp., uncultured Aliihoeflea sp. Along with the uncultured Marmoricola Sp. Paracoccus Mersussis strain. Nocardiolles, alpha proteobacterium, uncultured acidobacteria, uncultured Bdellovibrio sp. Nitriliruptor Alakaliphilus strain, Streptomyces, uncultured cynobacteria, Amylolaptosis Kentuckyensis. Construction and analysis of a phylogeny tree demonstrates that the recovered clones of 16S rRNA can be clustered into nine major groups.

1. Introduction

Bacterial diversity in an environment is dependent upon various ecological conditions [1]. Metagenomics is the study of microbial communities structure and function, and the metabolic pathways directly from environmental sample, prior culturing [2]. From literature, it becomes evident that over 99.8% of the microbes in few environments remain uncultured [3]. Therefore it is important to assess not only the species diversity of microbial communities, but also to examine how microbial structures of those communities vary over time and space [4]. Hot spring of Manikaran is situated at an altitude of 1760 m along the side of Parvati valley, Northeast of Kullu District of Himachal Pradesh, India. Investigation of thermal waters spring from Manikaran in Parbati valley has revealed that the thermal waters springs here contains NaCl, and calcium carbonate [5]. A thermal water flow rate measured by the Geological Survey of India has shown that it varies from 200 l/m to more than 1000 l/m [6]. The major hot springs in the world are found in Honduras, Canada, Chile, Hungary, Iceland, Israel, Japan, New Zealand and United States, Malaysia, India [7]. India also referred as one of the most tectonically active areas in the world, and according to geological surveys; it harbor ~340 hot-water springs. The average temperature of these hot spring ranges from 30 to 96 °C, which are classified into six geothermal provinces [8]. Bacterial diversity in hot spring is affected by factor like temperature, chemistry of the underlying rocks, pH, concentrations of various dissolved sulphides and inorganic carbons etc. [9–11]. Hot spring presents an archetype of extreme environments [12,13]. Culture independent investigation of 16S rRNA genes from these ecosystems can provide valuable insights about the comprehensive microbial diversity associated with these ecosystems, and may also help in recovery of the novel biocatalysts and other bioactive molecules, as reported previously [14–18].

Herein, we report bacterial phylogeny structure of soil collected from hot spring of Manikaran, India. To meet our aim, we collected soil samples aseptically in autoclaved Oak ridge tubes, from hot spring soil, after digging the soil surface to 2–3 inches. The average water temperature during sampling was 50 °C, and the pH was around 8. Soil DNA was extracted and PCR amplification of 16S rRNA genes was carried out. Amplified DNA fragment ranging from 500 to 1500 bp were

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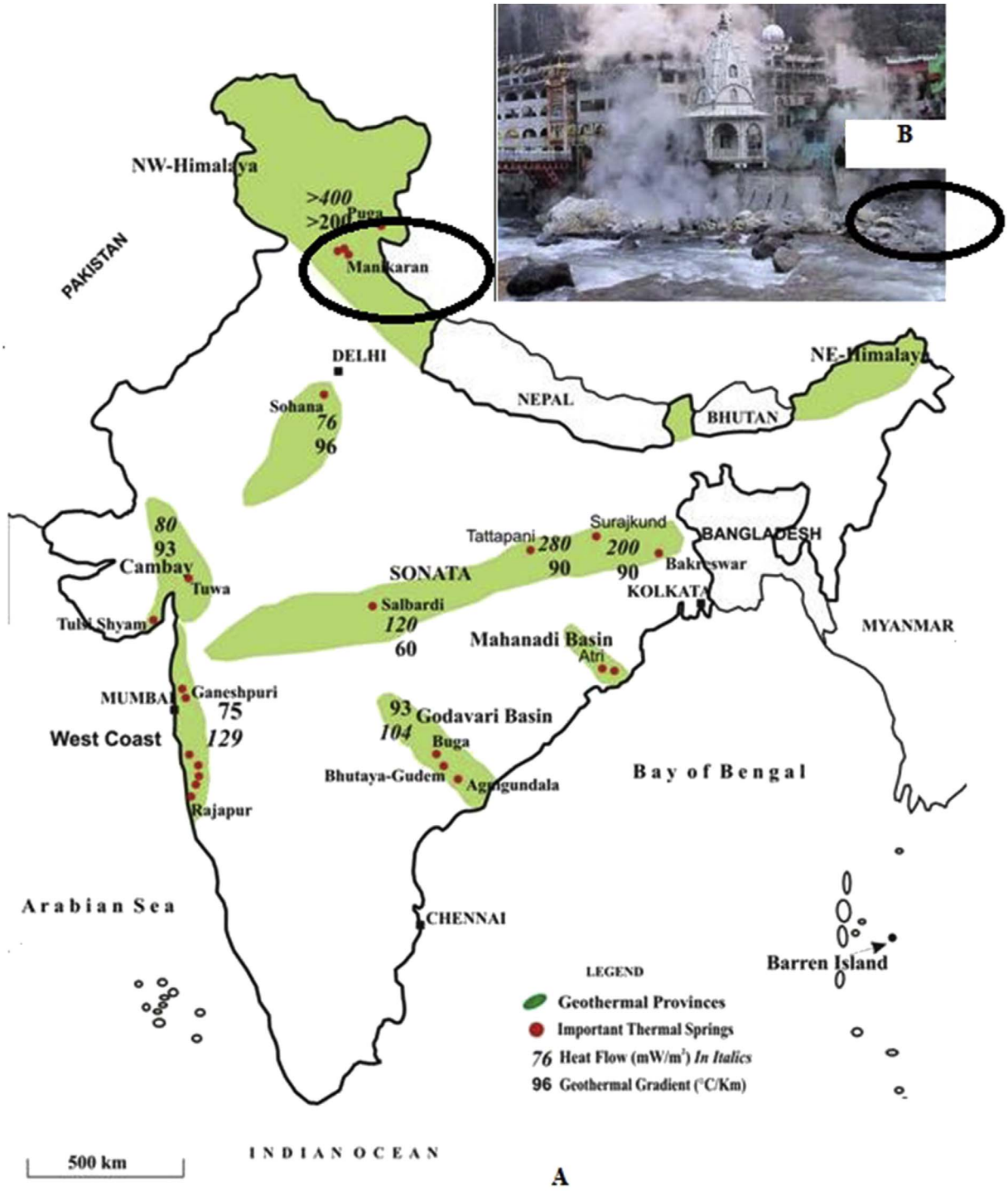

Fig. 1. Geographical location of Himachal Pradesh, Manikaran, India (a) Map (b) site of soil collection.

ligated to pGEMT-easy vecor, followed by construction and analysis of 16S rRNA library.

2. Materials and methods

2.1. Collection of soil samples

Soil samples were collected in triplicates in an autoclaved oak ridge centrifuge tube from hot spring soil of Manikaran area of Himachal Pradesh, India (Fig. 1a and b) (32°02′N, 77°21′E; elevation 1760 m). The soil surface was dig to 2–3 inches, while collecting the soil. The average temperature during the collection of soil was recorded to be 50 °C, the pH of the soil was calculated to be 8. The samples were transported aseptically to the lab in cold conditions, and stored in −80 °C till further processing.

2.2. DNA extraction, PCR amplification and molecular manipulations

Metagenomic DNA from three soil sample was extracted employing MoBio kit as per the manufacturer's instructions. The DNA was pooled from three samples and checked for its purity and concentration in a spectrophotometer (GE ultra spec 7000). The purified DNA was used as