Diversity Analysis of Bacterial Community from Permafrost Soil of Mo-he in China

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Abstract The permafrost soil of Mo-he in Northeast China presents a typical cold environment colonized by psychrophilic microorganisms. This study is aimed at assessing the bacterial communities of permafrost soil of Mo-he in China by sequencing the 16S rRNA genes and Mothur analysis. PCR products with universal 16S rRNA gene primers were cloned and partially sequenced, and bacterial identification at the species was performed by comparative analysis with the GenBank/EMBL/DDBJ database. A total of 266 clones were obtained with the average length of 1,050 bp. Mothur analysis showed that the coverage value of clone library was 53.78 %, Shannon diversity (H) was 4.03, Simpson diversity value was 0.018, and 74 operational taxonomic units were generated. Through phylogenetic assignment using BLASTN by more than 97 % similarity, a total of 87 tentative taxa were identified. The majority of bacterial sequences recovered in this study belonged to the Acidobacteria, Proteobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi and Chlorobi. Among them, Acidobacteria are dominant community,

accounting for 30.1 % of total bacteria, followed by Proteobacteria which accounted for 22.2 %. This result reflected the acidic characteristics of the permafrost soil of which pH value was 6.0. Our study indicated that the permafrost soil of Mo-he in China has a high diversity of bacteria and represents a vast potential resource of novel bacteria. As far as we knew, this is the first report on bacterial diversity of permafrost soil of Mo-he in China.

Keywords Bacterial diversity · Mo-he · Permafrost soil

Our Earth is a cold planet and more than 80 % of the biosphere is permanently exposed to temperatures below 5 °C [1]. Temperature is a key factor for a given kind of organism to survive and thrive due to its influence on the organic molecules composing the living cells [2]. A wide diversity of microorganisms, including bacteria, archaea, yeasts, filamentous fungi and algae have been found in the cold environments [3, 4]. To maintain their successful propagation in the extreme cold environment, the psychrophilic microorganisms have evolved a complex range of structural and functional adaptations [1], and formed a specific microflora which reflects the geochemical characteristics of the cold ecosystem. Therefore, knowledge of the structure and diversity of the psychrophilic microorganisms community can help us to better understand their roles in the cold ecosystems. Permafrost subsoil represents a typical and special cold ecosystem because the permafrost is one of the most extreme environments on the Earth and is permanently exposed to the temperature of below zero. So far, a lot of work has been done on the diversity of microbial community in the permafrost subsoils including Arctic, Antarctic and mountain permafrost [5]. However, no information is available on the diversity of bacterial

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community from forest permafrost soil under growing trees and grasses.

Mo-he located in Daxing'anling mountains north is characterized as the most freezing place in China and is close to the Earth "cold pole" Siberia. In geography, Mo-he is of 52°10'–53°33'N latitude and 121°07'–124°20'E longitude and is famous as the "Arctic Village" of China, of which flora belongs to Holarctic Kingdom, and European-Asian forestry floristic subregion, and of which plant vegetation cover was mainly composed of bright coniferous spruce forest dominated by *Larix gmelinii* Rupr. ex Kuzen and also composed of thin forest shrubby grassland, marsh shrubby grassland and meadow grassland. This area has a frigid-temperate continental monsoon climate where the rainfall is plentiful and the summer is hot and the maximum temperature can reach above 30 °C. In winter, Mo-he area has a freezing, dry weather with the lowest temperature of –40 °C, which resulted in a 100 m thick of frozen soils. Among the permafrost soils, the shallowest is located at 20 cm below the ground.

Soil samples were collected from 30 to 40 cm depth of the permafrost soils of Mo-he county of China on July 6, 2011, the hottest season of the year, and immediately kept at 0 °C for total microbial DNA extraction. The sampling sites are located in the southwest of Mo-he, 52°38'N latitude and 121°54'E longitude, and of 548 m in altitude. The soil type is classified as brown forest soil with pH 6.0 and the water-holding capacity is 63.2 %.

DNA was extracted from 1.0 g of permafrost soil samples using Power Soil DNA Isolation kit (Mo Bio Labs) following the manufacturers' protocols. The 16S rRNA genes of bacterium in the permafrost soils were amplified by the PCR with the universal primers: 27f and 1492r [6]. One hundred ng of genome DNA was used as template for a 50 µl PCR amplification reaction system. To construct the 16S rRNA gene library, the purified PCR products were ligated to pMD18-T cloning vector before transformation into chemically competent cells of *E. coli* strain DH5α (Invitrogen).

266 positive clones were selected and sequenced. When the sequence similarity was above 97 %, one species was determined. The phylogenetic tree of the 16 rRNA gene sequences was generated using ClustalX 2.09 and MEGA 4.1 by neighbour-joining method. Mothur soft analysis was used to evaluate the diversity of clone libraries where S_{Chao1} , S_{ACE} , Shannon diversity, Simpson diversity were selected. To reveal the ratio of microbial species represented by operational taxonomic units (OTUs) in gene library to the total microbial species of the sampling, the 16S rRNA gene library was evaluated by Coverage C theory and rarefaction curve method with the formula: $C = 1 - n1/N$, where N = number of total clones, $n1$ = number of clone of which OTU appeared only once.

Fig. 1 Rarefaction curve of the bacterial 16S rRNA clone library of Mo-he permafrost

At a cut off value of 0.03, the values of S_{Chao1} and S_{ACE} were 222.50 and 361.72, respectively. The values of Shannon diversity (H) and Simpson diversity were 4.03 and 0.018, respectively, and 266 clones were classed into 74 OTUs, suggesting that the permafrost soil of Mo-he had a high diversity of bacterial communities. Rarefaction curve analysis showed that the coverage (C) value of the clone library was 53.78 % (Fig. 1).

The phylogenetic tree showed that the bacteria from permafrost were generally classed into 14 groups of the bacterial domain including Acidobacteria, Proteobacteria, Verrucomicrobia, Bacteroidetes, Chloroflexi, Chlorobi and other bacteria. Acidobacteriar represented 15 OTUs, Proteobacteria covered 15 OTUs, of which 4 are Alphaproteobacteria, 2 Deltaproteobacteria, 3 Gammaproteobacteria including Methylococcales and 6 are Betaproteobacteria including Gallionella, Rhodocyclaceae, Methylobacillus, Oxalobacter. The rest groups included Verrucomicrobia covering 5 OTUs, Bacteroidetes covering 6 OUTs, Chlorobi, Chloroflexi and uncultured candidate division.

In the permafrost soils of Mo-he, Acidobacteria are the dominant community among the soil bacteria. Several bacterial diversity studies have been done on the permafrost soils in the world including the Siberian tundra Soil [7] and the permafrost-affected soils in Qinghai-Tibet plateau [8]. In Siberian tundra soils, Actinobacteria and Proteobacteria are dominant communities, and in the permafrost-affected soils in Qinghai-Tibet plateau, Actinobacteria and γ -proteobacteria were the predominant phyla. However, in the permafrost soils of Mo-he, it is Acidobacteria and Proteobacteria that dominate the soil bacterial communities.

Acidobacteria forms a new an independent phylum of bacteria, and belonged to acidophile bacteria [9] and are widely distributed in the various natural environments [10] and accounted for 5–46 % of total microbial communities in the soil environments. In this study, the abundance of Acidobacteria in the permafrost soil was about 30 %, which agreed with the previous research results. Proteobacteria

including α -Proteobacteria and β -Proteobacteria belongs to methane oxidizing bacteria. It is well known that methane oxidizing bacteria have industrial potentials. Our study indicated that Proteobacteria are the second largest bacterial communities in the permafrost soil of Mo-he, suggesting that the permafrost soil of Mo-he are of high values for screening and utilizing the methane oxidizing bacteria.

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