

Diversity of culturable and unculturable bacteria in soil samples from Hoh Xil, China

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Abstract:

Hoh Xil, where is situated at the Yushu Tibetan Autonomous Region in southwest Qinghai, is one of the most primitive and well-preserved natural environment in the world. The microbial diversity in this area is largely unknown till date. In the present study an attempt has been made to explore the microbial diversity using both of cultivation-dependent and independent approaches. In the culture-dependent experiment, twenty-two different media and three pretreatment methods were used to isolate general, halophilic and alkalophilic bacteria, in 25 soil samples from Hoh Xil. As a result, 78 strains have been isolated, among which 48 isolates were identified to belong to 13 genera of actinobacteria and other 30 strains represent 4 genera of non-actinobacteria bacteria. Based on 16S rRNA gene sequences analysis, the strains CPCC100076 and 100153 were recognized as two novel taxa. 19 isolates were obtained from the soil sample IMB08-049, which exhibited the best biodiversity among all the samples. 16S rRNA gene sequence analysis showed that these 19 strains belonged to 8 different genera, including Phyllobacterium, Variovorax, Pseudomonas, Streptomyces, Microlunatus, Kribbella, Promicromonospora and Bacillus. Culture-independent method was also employed to investigate microbial community structure in this sample. Total genomic-DNA were extracted and purified directly from the soil sample and used as template for polymerase chain reaction (PCR) amplification with universal bacterial primer sets. Biodiversity was assessed by amplified ribosomal DNA restriction analysis (ARDRA), DNA sequencing and phylogenetic analysis. 19 operational taxonomic units (OTUs) were clustered in 3 the following phyla including Proteobacteria (91%), Actinobacteria(7%) and Firmicutes(2%). Comparing the results from culture-dependent and culture-independent, we found at least 11 genera including some dominant species were not isolated using the above culture-dependent method. Such result suggested that new isolation method should be developed to discover more bacteria exciting.

Key words: Biodiversity, culture-dependent, culture-independent, 16S rRNA gene library