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研究论文

内蒙古典型草原细菌群落结构的PCR-DGGE检测

周小奇[1],王艳芬[1,*],蔡莹[2],黄祥忠[1],郝彦宾[1],田建卿[1],柴团耀[1]

1.中国科学院研究生院生物系,北京100049
2.中国科学院微生物研究所,北京100080

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摘要 用液氮冻融法和蛋白珠法对内蒙古典型草原土壤基因组DNA进行提取，用PCR-DGGE对细菌群落结构进行分析，并对主要的条带进行测序。发现蛋白珠法比液氮冻融法更能反应出实际的微生物群落结构和组成。内蒙古典型草原土壤细菌主要有5个分支: 放线菌门（Actinobacteria），变形菌门（Proteobacteria）的α、β及γ类群，拟杆纲门（Bacteriodetes），芽单胞菌门（Gemmatimonadetes）和酸杆菌门（Acidobacteria）。与基因库中进行比较后发现有4个序列和已知的细菌种类相似达到了99%以上。

关键词 16S rDNA PCR-DGGE 液氮冻融法 蛋白珠法 内蒙古典型草原

分类号 Q78,Q938,Q948,S812

PCR-DGGE detection bacterial community structure in the Inner Mongolia steppe with two different DNA extraction methods

ZHOU Xiao-Qi[1], WANG Yan-Fen[1,*], CAI Ying[2], HUANG Xiang-Zhong[1], HAO Yan-Bin[1], TIAN Jian-Qing[1], CHAI Tuan-Yao[1]

1 Department of Biology, Graduate University of Chinese Academy of Sciences, Beijing 100049, China
2 Institute of Microbiology, Chinese Academy of Sciences, Beijing 100080, China

Abstract Soil microorganisms probably represent the greatest reservoir of biological diversity in the world and play key roles in soils through regulating organic matter decomposition and plant nutrient availability. However, due to the complexity of microorganism survival condition, only 1% -5% of the total microorganisms can be isolated by cultural method in soils. Recently, the advances of molecular biological techniques, e. g., PCR DGGE (denaturing gradient gel electrophoresis) can provide information regarding soil bacterial community structure through the extraction of microbial DNA from soil, and bacterial community profiles can be generated through the PCR amplification of 16S rRNA genes. During this process, extract efficiency of soil genomic DNA is the most important step. At present, the most widely used methods to extract soil genomic DNA are frozen-thawing method and bead beating method that each has its unique property and advantage. Nevertheless, little research has been conducted to compare of the genomic DNA when dealing with different types of soil using these two methods, especially when soil is rich in humus.

The objective of the work are to evaluate these two methods themselves in soil genomic DNA extract efficiency when dealing with high humus soil of Inner Mongolia steppe based on the PCR-DGGE analysis of bacterial community structure and to determine bacterial community through cloning and sequencing of the bands in the DGGE patterns.

According to of the results of PCR-DGGE pattern using the bacterial primers 338F and 758R, we found that bead beating method is better than frozen thawing method in genomic DNA extract efficiency. Twenty-one bands in the DGGE pattern were selected, cloned and sequenced. Based on similarity matching, all the sequences formed five major clusters: *Actinobacteria*; α-, β-, γ-

, *Proteobacteria*; *Bacteriodetes*; *Gemmatimonadetes* and *Acidobacteria*. Of the 21 clones ob tained from DGGE patterns, YC4 exhibited 99.7% similarity to *Pseudomonas* sp. (DQ33915 3); YC5, YC18 and YC19 exhibited 99.9% similarity to *Gram-positive bacterium* (AB00851 0), *Virgisporangium ochraceum* (AB006162), *Micromonospora chalcea* (X92613), respectiv ely.

Key words 16S rDNA _ PCR-DGGE _ Frozen-Thawing method _ Bead-Beating method _ Inner Mongolia steppe

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通讯作者 王艳芬 yfwang@gucas.ac.cn