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## 大豆根际未培养与培养细菌群落结构差异比较研究

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摘要: 根际土壤微生物群落结构是根际微生态系统中的重要组成部分,与根际养分有效性、植物生长发育及抗病性等关系密切,不同植物间、同一植物的不同基因型之间根际微生物群落结构差异较大。利用从两种基因型大豆根际土壤中直接提取和从平板培养菌落提取的微生物DNA为模板,采用细菌通用引物GC-357f和517r进行PCR扩增,对PCR产物的DGGE图谱进行聚类和主成分分析。结果表明,不同基因型大豆根际土壤未培养的细菌群落结构差异不大,而在土壤浸提液和NA培养基上形成的可培养的细菌群落结构受培养基种类和接种浓度( $10^{-2}$ 和 $10^{-3}$ )影响较小,但受不同大豆基因型影响而产生了差异。对DGGE条带进行分析表明,大豆根际未培养的细菌群落物种丰富度(S)和多样性指数(H)明显高于可培养细菌,说明培养过程是一个再选择的过程,在这个过程中一些微生物的信号得到放大,而大量的微生物信息缺失。对主要DGGE条带测序显示,大豆根际有三大类细菌:拟杆菌门(Bacteroidetes)、变形杆菌门(Proteobacteria)和放线菌门(Actinobacteria)。变形杆菌门(Proteobacteria)中的 $\gamma$ -Proteobacteria、 $\alpha$ Proteobacteria和放线菌门(Actinobacteria),在未培养和培养细菌中都表现为优势种群。与培养细菌相比,变形杆菌门中的 $\beta$ -Proteobacteria和拟杆菌门(Bacteroidetes)细菌在可培养细菌中占优势,而在未培养细菌中丰度较低。结果证明,大豆根际细菌经培养后已使原有的群落结构发生改变,影响对原位细菌群落结构的认识。

Abstract: Microbial community in rhizosphere is an important component in rhizosphere ecosystems, which is closely related to the nutrient availability, plant growth and disease resistance. There are great differentiations in rhizosphere microbial community among plant species or even genotypes. In this study, using the microbe DNA extracted from the rhizosphere of two soybean genotypes (culture-independent) and from colony incubated on two culture media (culture-dependent) as the template, the bacterial 16S rRNA fragments were amplified with primer set GC-357f and 517r, and the PCR products were separated by DGGE, the band pattern was analyzed by Cluster and PCA analysis. The results showed that bacterial communities in the two soybean rhizosphere were similar, but the communities which were obtained from culture plate differed with genotype, and not influenced by culture medium as soil extract medium and NA medium as well as inoculation density. DGGE results showed that the bacterial species diversity index(H) and abundance (S) of culture-independent method were higher than those of culture-dependent, which indicated that the culture-dependent method is an re-choice process. In this process some microbes information was enhanced while many of microbes information were lost. Sequence results showed that the bacteria of Bacteroidetes, Proteobacteria and Actinobacteria commonly existed in the soybean rhizosphere, and the  $\gamma$ -Proteobacteria,  $\alpha$ -Proteobacteria and Actinobacteria were all the dominate bacteria. The bacteria of  $\beta$ -Proteobacteria and Bacteroidetes did not dominate in the soybean rhizosphere, but

in the culture plate. This study clearly identified that the bacterial community structure changed after culture, compared with uncultured, which may impede the research of bacterial community in natural condition.

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