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研究报告

一种高效可直接用于PCR分析的土壤总微生物DNA抽提方法

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摘要 以CTAB-溶菌酶-蛋白酶K-冻融裂解法直接抽提土壤总微生物的基因组DNA，利用G8000沉淀和纯化DNA. 结果表明，该方法是一种简便、有效可直接应用于PCR分析的土壤总微生物基因组DNA的抽提方法. 采用含聚乙烯吡咯烷酮(PVP)的缓冲液预洗，添加$CaCl_2$和BSA，可以去除腐殖酸；用PEG8000沉淀DNA，可以提高DNA质量；采用冻融法破碎细胞，CTAB、溶菌酶和蛋白质酶K共同作用以裂解细胞，可以保证获得大片段的DNA，提高DNA产率. 用该方法抽提的七子花林下土壤总微生物DNA产率为9.22 μg·g^{-1}，A260/A280为1.65，可适用于 PCR扩增及扩增rDNA限制酶切分析(ARDRA)技术，适宜的模板DNA浓度为0.67 ng·μl^{-1}. 快速、有效、可直接用于PCR分析的土壤总微生物DNA提取方法的建立，为大规模的土壤微生物分子生态学研究提供了可能.

关键词 土壤总微生物 DNA 抽提 PCR扩增

分类号

A highly effective extraction method for PCR analysis of soil microbial DNA

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Abstract

In this paper, soil microbial DNA was extracted by cetyltrimethyl ammonium bromide (CTAB)-lysosome-protease K-freezing thaw lysing, and precipitated and purified with PEG 8000. The results showed that this method was simple and effective for the PCR analysis of soil microbial DNA. To effectively remove humid acid, test soil was pre-washed by polyvinylpyrrolidone (PVP) buffer and added with $CaCl_2$ and bovine serum albumin (BSA). The precipitation with PEG8000 could obtain high quality DNA, and the lysing with (CTAB)-lysosome-protease K-freezing thaw could get large fragment DNA. Using this method, the yield of soil microbial DNA under *Heptacodium miconioides* forest was 9.22 μg·g^{-1}, with A_{260}/A_{280} being 1.65. The extracted DNA was proved to be successfully used for further PCR amplification and amplified ribosomal DNA restriction analysis (ARDRA). The optimal template DNA concentration in PCR amplification was 0.67 ng·μl^{-1}. The establishment of this simple, rapid and effective method made it possible to study the molecular ecology of soil microbes in a large scale.

Key words Soil microbe DNA Extraction PCR amplification

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