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生物技术—研究报告

普洱茶发酵样品细菌和真菌DNA同时提取方法研究

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摘要:

本研究的目的是建立提取普洱茶发酵样品中微生物DNA的方法。首先比较了3种发酵样品中菌体收集方法，其次改进了omega小量植物DNA提取试剂盒的提取方法，以DNA的纯度、提取率和细菌16S rDNA、真菌?-微管蛋白基因PCR扩增为指标，评价提取DNA质量。实验发现茶样（5 g）加Tween-NaCl缓冲液（50 mL），静置30 min，超声波振荡10 min，离心（10 min，12000 r/min）的菌体收集方法和在omega小量植物DNA提取试剂盒中引入液氮研磨、溶菌酶和破壁酶联合裂解细胞的方法提取的DNA纯度好，可以分别扩增出细菌16SrDNA和真菌?-微管蛋白基因。建立了普洱茶发酵样品细菌和真菌DNA同时提取方法，为开展普洱茶固态发酵过程微生物多样性的分子生物学研究奠定了基础。

关键词: 同时提取

Simultaneous Isolation of Bacterial and Fungal DNA from Pu-erh tea Fermentation Samples

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Abstract:

The aim was to develop a method to isolate microbial DNA from pu-erh fermentated samples. Three assays for extraction of microorganisms from pu-erh fermentation samples were compared, the DNA isolation methods were improved on the basis of HP Plant DNA Kit (omega), and the DNA quality were measured by the quantity (OD260), purity (OD260/280), and the possibility for PCR amplification of both bacterial 16SrDNA and fungi ?-tubulin genes. The results of ultraviolet spectrophotometer analysis, PCR amplification, and agarose gel electrophoresis showed that microbes were accumulated by steeping fermented tea sample (5 g) in Tween-NaCl buffer (50 mL), washing with a sonicator for 10 min, and then centrifugation (10 min, 12000 r/min); DNA was extracted employed with cell lyses by grinding with liquid nitrogen, hydrolysis with lysomyme and lyticase, and then purification using the HP Plant DNA Kit (omega). Both bacterial 16S rDNA and fungi ?-tubulin genes could be amplified using the extracted DNA as templates. A simultaneous isolation of bacterial and fungal DNA from pu-erh tea fermentation samples was developed, and this provides a basement for the molecular biology research on the microbial communities and dynamics during pu-erh tea fermentation.

Keywords: simultaneous isolation

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