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Soil pretreatment and fast cell lysis for direct polymerase chain reaction from forest soils for terminal restriction fragment length polymorphism analysis of fungal communities

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ABSTRACT

Humic substances in soil DNA samples can influence the assessment of microbial diversity and community composition. Using multiple steps during or after cell lysis adds expenses, is time-consuming, and causes DNA loss. A pretreatment of soil samples and a single step DNA extraction may improve experimental results. In order to optimize a protocol for obtaining high purity DNA from soil microbiota, five prewashing agents were compared in terms of their efficiency and effectiveness in removing soil contaminants. Residual contaminants were precipitated by adding 0.6 mL of 0.5 M $CaCl_2$. Four cell lysis methods were applied to test their compatibility with the pretreatment (prewashing + Ca^{2+} flocculation) and to ultimately identify the optimal cell lysis method for analyzing fungal communities in forest soils. The results showed that pretreatment with TNP + Triton X-100 + skim milk (100 mM Tris, 100 mM $Na_4P_2O_7$, 1% polyvinylpyrrolidone, 100 mM NaCl, 0.05% Triton X-100, 4% skim milk, pH 10.0) removed most soil humic contaminants. When the pretreatment was combined with Ca^{2+} flocculation, the purity of all soil DNA samples was further improved. DNA samples obtained by the fast glass bead-beating method ($Method_{FGB}$) had the highest purity. The resulting DNA was successfully used, without further purification steps, as a template for polymerase chain reaction targeting fungal internal transcribed spacer regions. The results obtained by terminal restriction fragment length polymorphism analysis indicated that the $Method_{FGB}$

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revealed greater fungal diversity and more distinctive community structure compared with the other methods tested. Our study provides a protocol for fungal cell lysis in soil, which is fast, convenient, and effective for analyzing fungal communities in forest soils.

Introduction

Removal of humic substances from DNA samples is a prerequisite for analyzing soil microbial communities by molecular techniques. Contaminants can be removed before, during, or after cell lysis. To obtain high-quality microbial DNA, a DNA-containing lysate may be purified by adding chemical reagents such as polyvinylpolypyrrolidone (PVPP) and polyethylene glycol or by repeated extraction with phenol–chloroform–isoamyl alcohol during and after cell lysis.[1,2] In most cases, however, further purification steps, such as electrophoresis,[3] electroelution,[4,5] or spin-column chromatography[6,7] are needed. Additional steps in DNA extraction and purification are time-consuming and expensive. More importantly, they may result in DNA loss without microbial taxon-specific predilection.[8] In other words, DNA loss during extraction and purification is likely to result in underestimation of microbial diversity and misunderstanding of microbial community structure.

Soil pretreatment before cell lysis can minimize the need for additional purification steps. Prewashing of soil with solutions such as 50 mM Tris, 20 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NaCl, and 1% PVPP (hereinafter referred to as TENP) or phosphate-buffered saline (PBS) improves DNA purity,[9–11] but trace amounts of humic substances unavoidably remain in DNA samples. Multivalent cations (Ca^{2+} and Al^{3+}) can be used to precipitate humic substances by chemical flocculation,[12–15] however, it is difficult to control the concentration of the cations, and this method can also cause DNA coprecipitation.[13,14] Therefore, neither prewashing nor chemical flocculation alone leads to the best performance.

Commercial kits are fast, simple, and effective for soil DNA extraction. The FastDNA® SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA), PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Carlsbad, CA, USA), and E.Z.N.A.® Soil DNA Kit (Omega Bio-Tek, Inc., Norcross, GA, USA) all use glass beads to rapidly lyse microbial cells. However, kits can be expensive, variable in their performance, and the recipes of the reagents in the kits remain unknown.

Although a large number of studies have compared different soil DNA extraction methods, few have assessed method-related effects on microbial diversity data. Compared with a commercial kit, a modified method (glass beads + lysozyme + proteinase K + freeze-thawing) resulted in more bacterial operational taxonomic units detected.[16] Williamson et al.[17] demonstrated that among five tested methods, a proteinase K-based method and a commercial kit both resulted in a lower bacterial Shannon–Wiener index. Meanwhile, Zhang et al.[18] found that a method using cetyltrimethylammonium bromide (CTAB)–sodium dodecyl sulfate (SDS) had a superior performance in terms of the Shannon–Wiener and Simpson indices of actinobacterial diversity. Significant differences in the resulting microbial diversity data are also observed among commercial kits. Vishnivetskaya et al.[19] tested four kits and reported that the FastDNA® SPIN Kit for Soil generated the highest Simpson value, followed by the PowerSoil® Kit and PowerLyzer® Kit, whereas use of the MetaG-Nome® DNA Isolation Kit resulted in the lowest microbial diversity.

Our goal was to improve the fast cell lysis methods used in the kits by determining the optimal prewashing agent and using Ca^{2+} flocculation to pretreat soil samples prior to cell lysis. Forest soils were used to determine the effectiveness of prewashing agents in removal of soil contaminants because these soils are typically rich in humic substances. In order to evaluate the applicability of soil pretreatment (prewashing + Ca^{2+} flocculation), three other direct cell lysis methods were assessed. Furthermore, due to their tough cell walls, fungi are generally less sensitive to cell lysis methods. Therefore, terminal restriction fragment length polymorphism (T-RFLP) analysis of fungal communities was used to compare the different cell lysis methods in terms of method-related effects on soil fungal diversity data.

Materials and methods

Soil samples

In September 2011, soil samples (0–10 cm depth) were collected from five forest types in Huoditang located on the south-facing slope of the Qinling Mountains in Shaanxi Province, China. This area is mainly covered by natural secondary forests.[20,21] Four sampled forest types were dominated by Chinese pine (*Pinus tabulaeformis*), sharptooth oak (*Quercus aliena* var. *acuteserrata*), Armand pine (*Pinus armandii*), and Wilson spruce (*Picea wilsonii*), respectively, while the fifth was a mixed forest type composed of Chinese pine and sharptooth oak. For each forest type, three plots (20 m × 20 m) were established. In each plot, 30 soil cores were collected using a soil corer (3 cm in diameter) and pooled into one composite sample. The soil samples were placed in plastic bags and transported to the laboratory on ice. After having been sieved through a 2 mm sieve, half of each sample was air dried at room temperature for analysis of soil physical and chemical parameters. This work was conducted in accordance with the Forestry Standards "Observation Methodology for Long-Term Forest Ecosystem Research" of the People's Republic of China (LY/T 1952–2011),[22,23] and the soil parameters are presented in Table 1. The other half of each sample was stored in a refrigerator at 4 °C until microbial analysis.