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Efficiency of DNA extraction methods on the evaluation of soil microeukaryotic diversity

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ABSTRACT

The evaluation of microbial molecular diversity has been mainly based on the extraction of total DNA from environmental samples. The indirect extraction methods, which have been used for prokaryotes, have never been used to recover soil microeukaryotic DNA. We evaluated the efficiency of an improved indirect DNA extraction protocol developed herein and the direct lysis (the sodium dodecyl sulfate (SDS)-based method and commercial DNA extraction kit) on estimating the molecular diversity of soil microbial eukaryotes. DNA quality and quantity as well as denaturing gradient gel electrophoresis (DGGE) profiles were determined using three soil samples from different stations. The indirect method detected the highest DGGE bands in spite of the low DNA yield. The commercial kit detected a lower number of DGGE bands than the indirect method. The SDS-based method produced the lowest DGGE bands and DNA purity but the highest yield. Using the indirect method, we further evaluated the effect of freezing and air-dried preservations on estimating the microeukaryotic diversity. In spite of the low DNA yield obtained from the air-dried preservation, no significant differences were found in either the number of DGGE bands or the DNA purity between two manners. Our results indicate that the improved indirect method could obtain a high purity of intracellular DNA and high efficiency in the estimation of molecular diversity of soil microbial eukaryotes.

1. Introduction

Molecular techniques such as DNA fingerprinting, clone library and metagenomics have been used to investigate soil microbial diversity and function. However, soil eukaryotic microbes, especially protozoa, lag behind other microbes despite their rather important contributions in soil carbon and nitrogen cycles [1,2]. So far, studies on microbial molecular diversity and function have been mainly based on the extraction of total DNA from environmental samples. While numerous soil DNA extraction methods have been developed in the past two decades, few paid attention to eukaryotic microbes. The methods for soil DNA recovery can be classified as direct and indirect extraction. The direct extraction (=direct lysis) is commonly used for its high DNA yield, but usually produces low DNA purity which often inhibits PCR and may result in underestimation of microbial diversity [3]. The indirect method namely the cell extraction-based method was initiated by Torsvik and Goksoyr [4]. The previous studies indicated that the indirect method could obtain a higher DNA purity and molecular diversity of soil bacteria than the direct method in spite of lower DNA yield [5,6]. However, the current indirect extraction methods concern mainly bacteria and have never been used to recover microeukaryotic DNA from soil.

The present study aimed to test an indirect DNA extraction method for microbial eukaryotes and to evaluate the effect of the indirect and the direct DNA methods (the sodium dodecyl sulfate (SDS)-based method and DNA extraction kit) on estimating the diversity of soil microbial eukaryotes. The efficiency of the three methods was tested by the DNA yield and purity and PCR products as well as the denaturing gradient gel electrophoresis (DGGE) profiles with three soil samples. Furthermore, we used the new indirect method to evaluate the effect of freezing and air-dried preservations on soil microeukaryotic diversity.

2. Materials and methods

2.1. Soil

Soil were collected from Qingdao Botanical Garden (36°03′N, 120°21′E), Gaotang County of Liaocheng (36°55′N, 116°04′E) and Yellow River Delta near Dongying (37°40′N, 118°47′E) during the fall of 2009. Under each location, 15 soil cores were randomly

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taken from an area of 10 m × 10 m using an auger. The cores were mixed, sieved with a 2 mm sized mesh, and stored at −20 °C until processed. To evaluate the effect of soil preservation manners on the molecular diversity of microbial eukaryotes, an additional soil sample was collected from Qingdao Botanical Garden, air-dried for about one month, and then stored at room temperature in a sealed plastic bag for about three months before further processing.

Soil moisture contents were determined by drying at 105 °C for 48 h. Soil pH was determined in a soil/water slurry (2 parts distilled water, 1 part soil). Particle size analyses were performed by a Cilas 940L laser particle sizer (CILAS, France). Total organic carbon was examined in Vario TOC cube (Elementar, Germany). Nitrogen content was measured by the Kjeldahl method.

2.2. The protocol of improved indirect DNA extraction

The indirect DNA extraction method is a combination of the silica sol density gradient centrifugation following [7,8] and the modified SDS-based DNA extraction method of [9]. Two kinds of silica sols (Ludox® HS 40 and Percoll®, Sigma–Aldrich) and two kinds of filters (1.2 μm microporous membrane filter and 2 μm cellulose nitrogen filter) were tested.

(1) Put 5 g of soil sample in 15 ml distilled water and horizontally shake the mixture at 225 rpm for 10 min.
(2) Fast inject 3 ml subsample of soil–water mixture into 9 ml Ludox HS 40 (and Percoll, respectively) solution in a 15 ml conical centrifuge tube.
(3) Carefully add about 2 ml distilled water on the top of the sample-silica sol mixture and centrifuge at 4300*g* (Sigma centrifuge) for 15 min in a swing-arm rotor.
(4) Pipette the extracted organisms between the water column and the diluted silica sol, then concentrate on a 25 mm cellulose membrane (tested with 2 μm cellulose nitrogen filter and 1.2 μm microporous membrane filter, respectively) using low vacuum suction. Cut the filters with a clean scissors into small strips.
(5) Extract the DNA from the filters containing microorganisms using the modified SDS-based DNA extraction method of [9]. The main procedure includes lysis with a high-salt extraction buffer, extended heating (2–3 h) of the soil suspension in the presence of SDS and proteinase K, removal of protein and nucleic acid precipitation and purification.

2.3. Additional extraction protocols tested

To compare the indirect extraction method with the traditional SDS-based method and commercial DNA extraction kit, a 1 g of each soil sample was processed with the modified SDS-based DNA extraction method [9], and another 1 g of each soil sample was processed with the Ultraclean soil DNA isolation kit (MoBio Laboratories, USA).

2.4. Analyses of DNA yield, purity and size

DNA yield and purity were evaluated by NanoDrop™ 1000 spectrophotometer (Thermo scientific, USA), the means and standard deviations were calculated on triplicate subsamples. The size of DNA was analyzed on a 0.7% agarose gel with λDNA/HindIII maker (Dongsheng Biotech, China).

2.5. PCR amplification

The eukaryotic 18S rRNA gene was PCR amplified using primers Euk1A (5′-CTGGTTGATCCTGCCAG-3′) and Euk516r (5′-ACCAGACTT GCCCTCC-3′) with a GC-clamp (5′-CGCCCGGGGCGCGCCCCGGGCGG GGCGGGGGCACGGGGGG-3′), as suggested by [10]. The PCR amplification mixture contained: 0.3 μmol L^{-1} of each primer, 12.5 μl 2 × PCR TaqMIX (100 mmol L^{-1} KCl; 20 mmol L^{-1} Tris–HCl; 3 mmol L^{-1} $MgCl_2$; 400 μmol L^{-1} dNTP mix; 0.1 U μl^{-1} Taq DNA polymerase) (Dongsheng Biotech, China), template DNA and deionized water in a final reaction volume of 25 μl. Soil DNA was amplified using GeneAmp® PCR System 9700 (PE Applied Biosystems, USA) with the following program: 94 °C for 130 s; 35 cycles consisting of 94 °C for 30 s, 56 °C for 45 s and 72 °C for 130 s; followed by a final extension cycle of 72 °C for 10 min.

2.6. Denaturing gradient gel electrophoresis

DGGE was performed with the DCode universal mutation detection system (Bio-Rad laboratories, USA). About 60 μl PCR products were loaded on 6% polyacrylamide gels prepared with denaturing gradient ranging from 20% to 50% (100% denaturant defined as 7 mol L^{-1} urea and 40% deionised formamide) in 1× TAE buffer. Electrophoresis was run 100 V for 16 h at 60 °C. Gels were stained with Gene Finder (Bio-v, China) for 30 min, visualized with a visible light transilluminator and analyzed with the Quantity One software (Bio-Rad laboratories, USA).

2.7. Statistical analyses

Statistical analyses were performed using SAS version 8.0 (SAS institute, USA). The purity of DNA extracted by different methods was analyzed by the absolute value obtained from subtracting the A260/A280 from 1.8 [11]. Two-way ANVOA was used to detect statistical differences in the DNA yield, DNA purity and DGGE bands among different methods and soil types. Duncan analysis was used for multiple comparisons. The cluster analysis base on the Dice similarity coefficient and unweighted pair-group method using arithmetic averages (UPGMA) was performed using PRIMER 5 (Plymouth Marine Laboratory, UK).

3. Results

3.1. Soil properties

The physical and chemical properties of the three soil samples used in the test were described in Table 1. The soils from Dongying, Liaocheng and Qingdao were classed as silty clay loam, silty loam and loam, respectively, with pH ranging from 5.7 to 8.5. The soil from Qingdao had the highest total organic carbon (TOC) and nitrogen contents.

3.2. Effect of different methods on the yield, purity and size of DNA and PCR products

DNA was extracted from three types of soil with six treatments (Table 2). The SDS-based method resulted in the highest DNA yield 10–200 times more amounts than the others. The DNA extraction kit produced 2–4 times more DNA yield than the indirection extraction method, which yielded no statistical differences between the four treatments. The yield of extracted DNA varied significantly not only for different methods ($P < 0.0001$) but also for soil types ($P < 0.05$). Significant differences were also detected in the purity of extracted DNA among different methods. Two-way ANOVA analysis showed that the DNA purity varied significantly for both the extraction methods ($P < 0.0001$) and soil types ($P < 0.01$). The SDS-based method and the treatment with the Percoll and 2 μm filter were more contaminated with proteins and thus produced poorer value of A260/A280 than the others. Electrophoresis of extracted DNA obtained by different methods on 0.7%