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ORIGINAL ARTICLE

Comparisons of direct extraction methods of microbial DNA from different paddy soils

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Abstract Molecular analyses for the study of soil microbial communities often depend on the direct extraction of DNA from soils. The present work compares the effectiveness of three different methods of extracting microbial DNA from seven different paddy soils. Comparison among different DNA extraction methods against different paddy soil samples revealed a marked variation in DNA yields from 3.18–20.17 μg DNA/g of dry soil. However, irrespective of the soil samples and extraction methods the DNA fragment size was >10 kb. Among the methods evaluated, method-C (chemical–enzymatic–mechanical) had better cell lysis efficiency and DNA yield. After purification of crude DNA by Purification Kit, A_{260}/A_{230} and A_{260}/A_{280} ratios of the DNA obtained by method-C reached up to 2.27 and 1.89, respectively, sustaining the efficacy of this technique in removing humic acid, protein and other contaminants. Results of the comprehensive evaluation of DNA extraction methods suggest that method-C is superior to other two methods (chemical–enzymatic and chemical–mechanical), and was the best choice for extraction of total DNA from soil samples. Since soil type and microbial community characteristics influence DNA recovery, this study provides

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guidance for choosing appropriate extraction and purification methods according to experimental goals.

1. Introduction

Microbial communities play a critical role in maintaining soil productivity by regulating the cycling, retention and release of major nutrients in soil (Torsvik and Øvreås, 2002; Islam et al., 2011). But till to date, up to 99% of the microbes present in soil are neither cultivable nor accessible for basic biotechnological research (Knietsch et al., 2003; Lakay et al., 2007). Conventional approaches currently being used appear to be inaccurate, and the results obtained hardly indicate comprehensive profile of soil microbial diversity in situ (Luo et al., 2003). On the other hand, molecular techniques such as PCR amplification of 16S rRNA genes or other genes of ecological significance yield relatively less biased information about microbial communities than traditional culturing approaches. Therefore, molecular analyses of microbial communities in complex environmental samples such as soil warrant efficient unbiased DNA extraction procedures.

Numerous techniques have been developed for direct extraction and purification of total community DNA from different environmental samples (Bürgmann et al., 2001; Roose-Amsaleg et al., 2001; Luna et al., 2006). Among them, the most commonly applied approach involves the in situ lysis of cells (Roose-Amsaleg et al., 2001) through chemical and/or enzymatic and/or mechanical lysis (Robe et al., 2003; Luna et al., 2006). Though these methods generally provide the highest DNA yields within acceptable processing times by complete in situ lysis of all microorganisms, each method has its own disadvantages (Robe et al., 2003). The lysis efficiency in any nucleic acid extraction procedure is critical in determining its success, such that an accurate representation of the microbial community can be achieved (Robe et al., 2003; de Lipthay et al., 2004).

The purity of the DNA from soil is often found unsatisfactory, particularly in soils rich in humic compounds (Courtois et al., 2001) such as bulk soil from paddy fields. Because of its physico-chemical similarity with nucleic acids, humic substances are usually co-extracted during extraction of DNA from soils and this can interfere with DNA detection, measurement and purification (Zhou et al., 1996). This contamination can inhibit the activity of *Taq* DNA polymerase during PCR amplification of genes (Luo et al., 2003).

Paddy soils represent one of the principal agricultural systems in Korea. Fertile soil provides essential nutrients for crop growth, and then supports a diverse and active microbial community. Knowledge of the microbial community structure in different paddy soils can advance our understanding of soil processes and microbial functions in rice-based cropping system (Islam et al., 2009). Though many methods for community DNA extraction from soil samples have already been described, none of these have been shown to be robust enough to be accepted by the scientific community as a standard protocol. Moreover, most of the methods involve re-purifying process, which are not only time consuming and costly but also subject to DNA loss. In the present study, we compared and evaluated three different methods for extraction of microbial community DNA from seven different paddy soils through analyzing simplicity, purity, and yields of DNA.

2. Materials and methods

2.1. Sample collection

The soil samples were collected from seven different paddy fields located at the National Institute of Agricultural Science and Technology, Suwon city, Republic of Korea in October 2008. The sampling was done by collecting soils from nine randomly selected points within each field at 0–20 cm depth using a 1.45 cm diameter soil core. Samples from each field were then combined to form one composite sample and stored at 4 °C during experimental period. The properties of bulk soil texture are described in Table 1.

2.2. Extraction of soil DNA

To extract total microbial community DNA from paddy soils, we applied three different methods; method-A (chemical–enzymatic lysis), method-B (chemical–mechanical lysis), and method-C (chemical–enzymatic–mechanical lysis). The basic–differences among the three extraction methods are shown in Table 2.

In method-A, DNA was extracted by the protocol of Zhou et al. (1996) with a little modification. Briefly, 5 g of soil samples were mixed with 13.5 mL of DNA extraction buffer (100 mM Tris–HCl, pH 8.0; 100 mM sodium EDTA, pH 8.0; 100 mM sodium phosphate, pH 8.0; 1.5 M NaCl; 1% CTAB [Hexadecylmethylammonium bromide]) and 100 μL Proteinase-K (10 mg/mL) in a Oakridge tube by horizontal shaking at 225 rpm under 37 °C for 30 min. 1.5 mL of 20% sodium dodecyl sulfate (SDS) was added to the sample mixture, which was then incubated for 2 h at 65 °C in a water bath with gentle end-over-end inversions every 15–20 min. After centrifugation at 6000 rpm for 10 min under room temperature the supernatants were collected, and the pellets were transferred into a 50 mL centrifuge tube. The pellets remaining were then extracted two more times by adding 4.5 mL of the extraction buffer and 0.5 mL of 20% SDS, vortexed for 10 s, followed by incubation at 65 °C for 10 min, and centrifugation as described earlier.

For Method-B, Kuske's (1997) extraction protocol was followed with slight modifications. Ten milliliters of TENS buffer (50 mM Tris, pH 8.0; 20 mM disodium EDTA; 0.1 M NaCl; 1% [w/v] SDS) was added to 5 g of soil samples and vortexed. The samples were incubated in a water bath at 70 °C for 1 h, and centrifuged at 6000 rpm for 10 min to collect the supernatant. The soil pellet was then washed with 5 mL of TEN buffer (TENS buffer without SDS), and the supernatant was collected upon centrifugation. Thereafter, the soil pellet was re-suspended in 7.5 mL of TEN buffer and exposed to three sets of thermal shocks by immersion of the tubes at −20 °C for