DNA Isolation From Forest Soil Suitable for PCR Assays of Fungal and Plant rRNA Genes

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Abstract. This protocol for DNA isolation from forest soil samples is advantageous because it uses only one liquid transfer step and can process several samples with minimal time and equipment. The use of benzyl chloride early in the extraction protocol increases DNA yield and purity. The obtained DNA is useful for PCR amplification of nuclear and mitochondrial ribosomal related sequences from fungi and ribosomal DNA from plant chloroplasts. Isolated DNA can be used either undiluted or at low dilutions in PCR assays. A final glassmilk treatment of isolated DNA is useful to recover high molecular weight DNA fractions from agarose gel. DNA losses during glassmilk treatment can generate negative PCR results.

Key words: benzyl chloride, fungal and plant rRNA genes, glassmilk, PCR, soil DNA

Abbreviations: BC, benzyl chloride; ITS, internal transcribed spacer of nuclear ribosomal unit.

Introduction

Many studies on DNA isolation from soil bacteria without cultivation have been performed (Tsai and Olson, 1991; Young et al., 1993; Leff et al., 1995; Zhou et al., 1996; McGregor et al., 1996; Jackson et al., 1997; Miller et al., 1999; Frostegard et al., 1999). Two problems in isolating DNA from soil are effectively rupturing cells and eliminating humic acids. Factors affecting cell rupture in soil have been evaluated by combining physical and chemical treatments (Frostegard et al., 1999; Miller et al., 1999). Effective cell disruption has been achieved by shaking the sample in lysis buffers containing high concentrations of detergent, sand, or glass beads; using freeze-thawing cycles; and, with prokaryotic cells,
using lysozymes (Frostegard et al., 1999; Miller et al., 1999). To minimize contamination of DNA with humic acids, agarose gel electrophoresis (Porteous and Armstrong, 1993; Young et al., 1993; Malik et al., 1994; Moré et al., 1994; Zhou et al., 1996) and purification in silica gel and other bio-gel columns (Tsai and Olson, 1992; Moré et al., 1994; Zhou et al., 1996; Jackson et al., 1997; Kuske et al., 1998) has been used. Some of these steps might make DNA isolation expensive or impractical for processing the large number of samples usually required in ecological studies.

In contrast, knowledge of factors that affect DNA recovery from fungal mycelia or plant tissues in soil is scarce. Porteous and Armstrong (1993) amplified the fungal 18S rRNA gene using universal and fungi directed primers with DNA isolated from different soil samples. In the same way, Volossiouk et al. (1995) amplified DNA sequences from the phytopathogenic fungus, Verticillium sp. Using amplification of nuclear and mitochondrial rRNA, Kuske et al. (1998) isolated DNA from the plant pathogen, Fusarium moniliforme, in soil. Classen et al. (1996) identified the endomycorrhizal fungus, Glomus clarum, by amplifying the 18S rRNA region by means of PCR in DNA from soil of inoculated plant pot cultures. Yeates et al. (1998) amplified the eukaryotic rRNA internal transcribed spacer (ITS) but not with fungal specific primers. In basidiomycete fungi, Bahnweg et al. (1998) studied DNA recovery in experimental DNA-sterile soil mixtures useful to amplify the ITS region from the tree pathogen, Armillaria ostoyae. Ernst et al. (1996) performed detection of transgenic maize plants in soil by amplification of the pat gene, encoding phosphinotricin acetyl transferase. Recent reports describe underground plant identification by isolating roots from soil samples and amplifying the ITS region within the nuclear ribosomal unit (Linder et al., 2000) or the trnL chloroplast-associated intron (Brunner et al., 2001).

Our protocol optimizes the yield and purity of DNA isolated from forest soil samples. It is suitable for use in studying fungi and plant genes. Our aim was to design a protocol that was inexpensive, able to process several samples quickly, and capable of obtaining quality DNA for PCR studies, mainly from fungal and plant genes. We used both benzyl chloride (BC) and a treatment with glassmilk slurry to achieve these objectives. Because of its structural analogy with phenol, BC has been used in organic extraction steps in DNA isolation protocols (Zhu et al., 1993). Additionally, BC interacts with cell wall carbohydrates and lipid membranes, promoting cell disruption. BC has been used to isolate DNA from soil for the molecular detection of the basidiomycete tree pathogen, Armillaria ostoyae (Bahnweg et al., 1998). We analyzed the effect of BC on the yield and quality of DNA isolated from Quercus forest soil samples and modified a protocol to use fewer reagents and purification steps (Bahnweg et al., 1998). Different degrees of degradation and size fractions have been reported in DNA isolated from soil samples (Frostegard et al., 1999; Miller et al., 1999). It has been observed that low molecular weight DNA fractions might promote the formation of chimeric molecules in PCR assays (Pääbo et al., 1990; Liesack et al., 1991). Glassmilk treatment has been reported as an aid to purify DNA after extraction from soil (Porteous and Armstrong, 1991; Volossiouk et al., 1995). We analyzed the utility of glassmilk as a purification step and a tool to recover high molecular weight DNA fractions to determine its contribution to successful PCR assays.