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Isolation of High Molecular Weight DNA from Forest Topsoil for Metagenomic Analysis

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Abstract. In this study, we employed a modified method to extract DNA from forest topsoil that was suitable for construction of large insert soil metagenomic library. The DNA extraction method used produced considerable DNA yield with DNA fragments ranging from 48 kb up to 290 kb. The recovery of soil DNA suitable for PCR and metagenomic library construction is difficult because soil DNA is often co-purified with polyphenolics and contaminants that interfere with the downstream applications. PCR amplification of 16S and fungal 18S SSU rRNA genes from the extracted soil DNA suggesting that the DNA isolated using this modified method contained low concentration of PCR inhibitory substances and had sufficient purity for PCR without the need of further purification. Sequence analysis of PCR amplicons revealed this extraction method can efficiently capture a wide range of microorganisms including the hard-to-lyse Gram-positive bacteria and fungi. We have also successfully constructed a metagenomic fosmid library with insert size of between 23.1 kb – 40 kb. This metagenomic library will serve as basis for screening of novel biocatalysts from the soil metagenome.

Keywords. Metagenomic, High molecular weight (HMW) DNA, Metagenomic library construction

INTRODUCTION

The microbial diversity of soil is extremely complex. It has been estimated that there are approximately 2.6 X 10^{29} prokaryotes residing in soil (Whitman *et al.*, 1998). Traditional methods to identify and characterize indigenous soil bacteria are based on direct cultivation and isolation of microorganisms that involve morphological examination through the microscope (Torsvik *et al.*, 1990). However, most of the soil microorganisms need special handling in order to grow them in laboratory conditions and up to 99.9% remain uncultured (Hugenholtz and Pace, 1996; Lorenz and Schleper, 2002).

In order to circumvent limitations of the culture method, DNA-based approaches, have been adopted to explore the entire microbial community (Nordgard *et al.*, 2005). In theory, metagenomic DNA recovered from a sample represents the total DNA of all indigenous soil microorganisms (Handelsman *et al.*, 1998; Rondon et al., 1999). Metagenomic coupled with PCR techniques and bioinformatics, is a powerful tool to study the phylogeny and taxonomy of particular genes in a particular community (Torsvik and Ovreas, 2002), including numerous unculturable microbes (Theron and Cloete, 2000). Cloning of metagenomic DNA in an appropriate vector and then transforming the resulting recombinant molecules into a surrogate host enables us to access to the gene pool of unculturable microorganisms (Rondon *et al.*, 2000).

Recovery of metagenomic DNA that is suitable for PCR and metagenomic library construction remains a challenge (Daniel, 2005) because coextracted polyphenolics substances in isolated soil DNA will interfere with downstream applications (Tsai and Olson, 1992). Isolation of high molecular weight (HMW) metagenomic DNA is crucial for metagenomic library construction in order to improve the possibilities of retaining the gene clusters involved in biosynthetic pathways in clones (Bertrand *et al.*, 2005). Furthermore, extreme efficiencies of DNA extraction are needed to assure representative of the entire metagenome (Handelsman *et al.*, 1998; Martin-Laurent *et al.*, 2001).

In this study, we employed a modified DNA extraction method from Yeates and colleagues (1998) which was suitable for construction of large insert soil metagenomic libraries.

Abbreviations: High molecular weight (HMW), Polyketide synthase (PKS), ketosynthase (KS_α)

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