Rapid Extraction and Purification of Environmental DNA for Molecular Cloning Applications and Molecular Diversity Studies

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Abstract

A rapid method for the extraction and purification of DNA from environmental samples for molecular cloning applications was developed. The indigenous cells from plant debris, organic materials, sediments, and soils were lysed directly by using DAS-I[Z] solution and the nucleic acids were precipitated with isopropanol. A simple purification step using DAS-II[Z] solution without binding matrix produced highly pure, colorless and undegraded DNA with molecular weight of more than 20 kb. The superiority of this method was tested for wide applications in molecular cloning, i.e., construction of genomic library by using Lambda DASH[(R)]II Vector and Gigapack[(R)]III XL, plasmid library, cloning of gene encoding protease, and molecular microbial diversity analysis. An additional advantage of this method is that only 0.1 g of sample is required, if analysis of many samples in short time should be done. To extract large amounts of environmental DNA for molecular cloning lasts only 30 min and to purify it less than 1 h.

Index Entries: Environmental DNA; extraction; purification; molecular diversity; molecular cloning; biotechnological potential.

1. Introduction

Soil, sediment, and other environmental samples, particularly those from unique and unusual ecosystems are important sources of useful enzymes or other related biotechnological products. Until recently most of these bio-products were isolated or produced from microorganisms through cultivation-screening-mutation procedures. Unfortunately, only a very small proportion of the total microbial community can be isolated. Conventional methods that rely on the cultivation of microorganisms on laboratory media isolate only an estimated 0.001–0.1 % of the total population of bacteria in natural habitats *(1,2)*.

Our study on the black water ecosystem, which is one of the most unique ecosystems on Kalimantan island, Indonesia, also gave a similar result. About 100 million–10 billion of viable bacteria/mL sediment are observed, but only 0.001–1 percent of the total members of bacteria are cultured *(3)*, which means that more than 99% of genetic information and biotechnological potential of those cannot be studied or used by conventional methods.

The development of methods to extract and purify DNA from environmental samples will be very important to search the untapped biotechnological potential of such a rare and extreme environment. Many authors have developed methods for isolating DNA from soil, as well as sediments *(4–12)* but most of these methods produce degraded DNA in a molecular weight range between 1 kb–20 kb, which is not suitable for molecular cloning purposes and the construction of genomic libraries.

The purification of crude DNA from environmental samples is the other critical step in direct extraction of DNA. Phenolic compounds, humic

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