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Evaluation of Different Methods for Total DNA Extraction from Sago Pith Residue

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ABSTRACT

Direct extraction of DNA from the environment has become major importance for molecular analyses for the study of microbial communities in soil and other decomposing agrowaste. The presence of humic substances in Sago pith residue not only results in low DNA quality but also can lead to PCR amplification inhibition which may hinder most molecular studies. Many of the published protocols have been found to be unsuitable to obtained high amount of yield and low of humic acids contamination. This study presents the evaluation of three different methods for extracting total DNA from Sago pith residue. The methods evaluated were enzymatic lysis, glass bead homogenization and freeze-thaw treatment. Each method were evaluated by 260/280 nm absorbance ratio for protein contamination, 260/230 nm absorbance ratio for other contaminants and PCR amplification for molecular work suitability. Among the three methods, freeze-thaw treatment provided the highest yield of DNA, 5.06±0.01 µg/g of Sago pith residue. Nevertheless, all three methods resulted in poor DNA quality which could be used for PCR amplification. Additional steps of agarose electrophoresis and silica column purification were found to be effective for increasing the quality of the extracted DNA and were validated by positive PCR amplifications.

INTRODUCTION

Most microorganisms from environmental samples are difficult to be cultured. Only a small proportion of soil microorganisms are culturable on standard media [1]. For this reason, there are obstacles in understanding microbial ecology and diversity [2]. Isolation of bacterial nucleic acids from natural environments has become a useful tool to identify bacteria that cannot be cultured [3,4], to determine species of selected bacteria or genes under indigenous conditions [5], and to reveal genotypic diversity and its change in microbial ecosystems [6]. Environmental samples such as soils and composting agrowaste present some of the most difficult challenges to the development of suitable extraction and purification procedures. Most DNA extraction methods produced low DNA yield. Direct extraction of total DNA always results in co-extraction of other organic components, mainly humic acids or other organic substances, which negatively interfere with DNA transforming and detecting processes [1,4,5]. It has been reported that those substances inhibit restriction endonucleases [6,7] and Taq polymerase, the key enzyme of the polymerase chain reaction (PCR), and decrease efficiencies in DNA-DNA hybridizations [8]. This study were set to evaluate several DNA extraction methods in order to develop an effective DNA extraction method for extraction with and without further purification for production of higher DNA yields and less humic acid contaminations for PCR amplification.

MATERIALS AND METHODS

Sampling

Sago pith residue samples were collected from Ladang Dalat, Sago plantation in Mukah region. Samples were obtained between 5-10 cm in depth of Sago pith residue. Samples were maintained at 4 °C until use.

DNA extraction using enzymatic lysis

Extraction buffer (20 ml of 100 mM Tris-HCl [pH 8.0], 100 mM sodium EDTA [pH 8.0], 1.5 M NaCl) was mixed with 10 g (wet weight) of residue. 0.5 mg of proteinase K were added and