EVALUATION AND OPTIMIZATION OF EXTRACTION AND PURIFICATION METHODS FOR OBTAINING PCR – AMPLIFIABLE DNA FROM MANGROVE SOIL FOR STUDIES IN MICROBIAL ECOLOGY

ABSTRACT

Recovery of microbial DNA from environmental samples such as mangrove soil is a significant challenge. In this study, combinations of DNA extraction and purification methods were compared based on DNA yield, humic acid and protein contamination and PCR amplifiability.

Several approaches were analysed to obtain optimum DNA yield. The effects of different treatments of DNA extraction such as extraction buffer, chemical and enzymatic cell lysis (sodium dodecyl sulfate, lysozyme and proteinase K), mechanical cell lysis (rotex, vortex and sonicate) and removal of cell fragment were compared and evaluated. A method for the extraction of microbial DNA from mangrove soil was developed during this study and it is called the Soil DNA Direct Lysis Method. The advantages of this method are that it is simple, robust, rapid and may be used to analyse many samples simultaneously.

Several reagents were used to reduce protein and humic acid contaminations. Such reagents include sodium chloride, ammonium acetate, potassium acetate and sodium acetate for removal of proteins contaminants and polyvinylpolypyrolidone and cylmethylammonium bromide for removal of humic acid. Nucleic acid precipitation using ethanol, isopropanol or polyetheleneglycol were also compared and evaluated. A method for the purification of microbial DNA

from mangrove soil was developed during this study and it is called the Soil DNA Purification Method. The advantages of this method are that it is rapid cleanup of DNA, removing inhibitors to produce PCR-ready DNA.

PCRs were successful in amplifying 16S rRNA gene fragments using the extracted and purified microbial DNA from mangrove soil.

PENILAIAN DAN PENGOPTIMAAN KAEDAH PENGEKSTRAKAN DAN PENULINAN DNA BAGI MENDAPATKAN AMPLIFIKASI PCR DARIPADA TANAH PAYA BAKAU UNTUK KAJIAN EKOLOGI MIKROB

ABSTRAK

Mendapatkan DNA mikrob daripada persekitaran seperti tanah paya adalah sesuatu yang sangat mencabar. Dalam kajian ini, gabungan kaedah pengekstrakan dan penulinan DNA telah dibandingkan berdasarkan kepada hasil DNA, pencemaran asid humik, protein dan amplifikasi PCR.

Beberapa langkah telah diambil untuk menghasilkan DNA yang optima. Ini termasuklah menganalisa kesan perbezaan rawatan seperti rawatan kimia dan enzim (sodium dodecyl sulfate, lysozyme dan proteinase K), rawatan mekanikal (rotex, vortex dan sonicate) dan pengasingan fragmen sel. Semua keadaan ini dibandingkan dan dinilai.

Satu kaedah baru telah dibuat untuk pengekstakan DNA mikrob daripada tanah dan ia dikenali sebagai Kaedah Lisis DNA Tanah Secara Terus. Kebaikan kaedah ini ialah ianya mudah, cepat dan banyak sampel tanah boleh dianalisa dalam masa yang sama.

Beberapa bahan kimia digunakan untuk mengurangkan pencemaran protein dan asid humik dalam ekstrak DNA. Bahan ini adalah seperti natrium klorida, ammonium asitat, potassium asitat and sodium asitat digunakan untuk mengurangkan pencemaran protein. Manakala bahan kimia seperti polyvinylpolypyrolidone dan cylmethylammonium bromide digunakan untuk

mengurangkan pencemaran asid humik. Perbandingan dan penilaian juga dilakukan ke atas pengikatan asid nukleik seperti ethanol, isopropanol dan polyetheleneglicol. Satu kaedah baru dibuat untuk penulinan DNA mikrob daripada tanah dan ia dikenali sebagai Kaedah Penulinan DNA Tanah. Kebaikan kaedah ini ialah ia cepat membersihkan dan menulinkan DNA daripada bahan perencat dan DNA ini sedia untuk amplifikasi PCR.

Semua PCR berjaya mengamplifikasi fragmen gen 16S rDNA dalam ekstrak DNA darpada tanah paya bakau menggunakan kaedah penulinan Yeates *et al.* (1997) dan Kaedah Penulinan DNA Tanah.