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SHORT COMMUNICATION

A SIMPLE AND RAPID DNA EXTRACTION METHOD FOR CYANOBACTERIA AND MONOCOTS

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ABSTRACT

The isolation of DNA from biological samples is a crucial step in the process of DNA-based molecular biological assays. In this study, we present a simple method to extract and purify genomic DNA from different sample types (standard cyanobacteria, cyanobacteria in water samples and leaves of bamboo plants) which would be suitable for any molecular analyses. In this method, cyanobacteria are lysed by three sequential freezing and heating followed by enzymatic treatment with lysozyme and proteinase K. The extraction and purification of DNA was achieved with the lysing and nuclease inactivating properties of the chaotropic agent guanidium isothiocyanate and the nucleic acid binding properties of silica particles. The DNA extraction method yielded high quality reproducible DNA. The technique described here is a rapid, robust and cost effective method suitable for the extraction of DNA from any source for routine molecular biological assays.

Key Words: Boom's method, guanidine thiocyanate, silica, Polymerase Chain Reaction (PCR)

INTRODUCTION

The isolation of DNA from biological samples is a crucial step in the process of DNA-based molecular biological assays. Whether the DNA is extracted from a plant or animal tissue or from a bacterium, the product obtained has to be pure or free from contaminants (proteins, carbohydrates) to be used in numerous applications in molecular biology including PCR, genotyping, DNA sequencing, etc.

A wide variety of protocols are found in the literature to extract and purify genomic DNA from different tissues. All protocols start with cell lysis as the first step, followed by deproteination and precipitation of DNA. The most commonly used method is the phenol /chloroform extraction, which is tedious and time-consuming (Köchl *et al*., 2005). The other extraction methods include salting out DNA extraction (Rotureau *et al*., 2005) and the guanidium isothiocyanate DNA extraction method (Kotlowski *et al*., 2004). There are many different and versatile commercial kits suitable for genomic DNA extractions from QIAamp, Puregene and Dynabeads (Cler *et al*., 2006).

The aim of this study was to develop a simple and rapid method to extract DNA from any biological tissue material which is useful for any routine molecular biological assay. The method used in this study to extract and purify genomic DNA is the Boom's method or guanidium isothiocyanate /silica DNA extraction method (Boom *et al*., 1990) with modifications. It is a cost effective, reliable and reproducible method and the isolated genomic DNA is tested with the PCR technique. The different sample types used were cyanobacteria [*Microcystis aeruginosa* (PCC 7941), *Lyngbya* (PCC 8937) obtained from Pasteur Culture Collection, water samples collected from the environment] and tender leaves of the two monocots, *Dendrocalamus giganteus* and *Bambusa atra*.

MATERIALS AND METHODS

Extraction and purification of DNA from cultured cyanobacteria

The standard strains of *M. aeruginosa* were maintained in modified BG11 medium (Rippka *et al*., 1979). Bacterial cells (BG11 inoculum) were scraped and transferred to 500 µl of 1xTE (pH= 8.0) buffer and three sequential heating (at 99 ^{0}C for 5 min) and freezing (at –5 ^{0}C for 5 min) to achieve lyses. Samples were centrifuged, and to each resulting pellet, 40 µl of TES and 20 µl of lysozyme (10mg /ml) was added, and

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incubated for 1 h at 37 ^{0}C. The samples were then treated with 10 µl of proteinase K (20 mg/ml), 30 µl of Tris HCl (20 mM, pH 8.3) and incubated at 55 ^{0}C for 1 h for further lysis. Subsequently proteinase K was inactivated by heating the samples at 95 ^{0}C for 10 min. Finally, nucleic acids were purified using diatomaceous silica and guanidine isothiocyanate (Boom *et al*., 1990).

Extraction of DNA from bacteria in water samples

Water samples (100 ml each) collected from different sources were concentrated by centrifugation. The resulting pellets were washed and subjected to the above treatment before purification by Boom's method.

Extraction of DNA from pre - treated samples

To 900 µl of L_6 lysis buffer (5 M guanidine isothiocyanate, 1% Triton X-100, 50 mM Tris HCl (pH = 6.4) and 20 mM EDTA was added 100 µl of pre-treated sample followed by 20 µl silica suspension (200 mg/ml). The samples were vortexed and allowed to mix in a rotary shaker (RT/ 10 min/ 100 rpm) and then centrifuged in an eppendorf microfuge (12000 g, 15 s). The supernatant was discarded and the pellet was washed twice with 500 µl of L_2 washing buffer [5 M guanidinium isothiocyanate, 50 mM Tris HCl, (pH= 6.4)], followed by two washes with 750 µL of ethanol (70%) and once with 1.0 ml acetone. The supernatant was discarded and the open vial was dried at 56 ^{0}C in a water bath for 10 min. The pellet was re suspended in 0.1TE (pH= 8.0) (60 µl) and incubated for 10 min at 56 ^{0}C, followed by centrifugation for 2 min at 12000 g. The supernatant was removed to a new tube. For PCR analysis, 10 µl of this supernatant was used in duplicate. Purity and the quantification of DNA were carried out using a UV-Vis spectrophotometer (UV 2450).

Extraction and purification of DNA from *Dendrocalamus giganteus* and *Bambusa atra*

To extract DNA from the two monocot species, 0.25 g each from the leaf base was used. The plant material was ground using sterile motor and a pestle with TE (pH= 8.0). The pellet obtained was transferred to 500 µl of 1xTE buffer and three sequential heating (at 99 ^{0}C for 5 min) and freezing (at –5 ^{0}C for 5 min) to achieve lyses. Samples were centrifuged, and to each resulting pellet, 40 µl of TES and 20 µl of lysozyme (10 mg /ml) was added, and incubated for 1 h at 37 ^{0}C. Finally genomic DNA was purified by the Boom's method as described below. Purity and the quantification of DNA were carried out using a UV-Vis spectrophotometer (UV 2450).

DNA amplification for detection of cyanobacteria

DNA amplification was performed for the 16S rRNA gene using the modified protocol of Nübel *et al*., (1979) and cyanobacterial specific primers, forward primer Cya359F (5'-GGGGAATYTTCCGCAATGGG-3') and the reverse primer Cya781Ra (5'- GACTACT GGGGTATCTAATCCCATT-3'), or the reverse primer Cya781Rb (5'-GACTACA GGGGTATCTAATCCCTTT-3') (Nübel *et al*., 1979).

Each reaction contained 0.6 µM of each primer, 0.1 mM of each deoxynucleoside triphosphate, 5 µl of 10x PCR buffer (100 mM Tris–HCl [pH= 9.0], 15 mM $MgCl_2$, 500 mM KCl, 1% [v/v] Triton X -100), 0.5 U of Super *Taq* DNA polymerase (HT Biotechnology Ltd., Cambridge, UK) and template DNA. The PCR assay was carried out in a Perkin-Elmer/ Cetus DNA Thermal Cycler in a final volume of 50 µl with 50 µl of mineral oil.

The following thermal cycling parameters were used: 5 min initial denaturation at 94 ^{0}C, followed by 35 cycles of denaturation for 1 min at 94 ^{0}C, annealing for 1 min at 60 ^{0}C and elongation for 1 min at 72 ^{0}C, followed by a final extension at 72 ^{0}C for 15 min. The resulting PCR products were electrophoresed in 1.5% agarose gels containing 10 µg/mL ethidium bromide and documented through a Polaroid instant camera.

DNA amplification for *mcyE* gene detection

DNA amplification was performed for the *mcyE* gene using the modified protocol of Vaitomaa *et al*., 2003. The general microcystin synthetase gene E forward primer Mcy E-F2 (5'-GAAATTTGTGTAGAAGGTGC-3') and the gene specific reverse primer for *Microcystis* MicmcyE-R8 (5'-CAATGGGAGCATAACGA G-3') (Vaitomaa *et al*., 2003) were used for the PCR.

Reaction mixtures contained 0.4 µM of each primer, 0.1 mM of each deoxynucleoside triphosphate, 5 µl of 10x PCR buffer (100 mM Tris–HCl [pH= 9.0], 15 mM $MgCl_2$, 500 mM KCl, 1% [v/v] Triton X -100), 0.5 U of *Taq* DNA polymerase (HT Biotechnology Ltd., Cambridge, UK) and template DNA. Amplifications were carried out in 50 µl volumes in a Perkin-Elmer/ Cetus DNA Thermal Cycler with 5 min initial denaturation at 94 ^{0}C,

35 incubation cycles each consisting of 1 min at 94 ^{0}C, 1 min at 59 ^{0}C, and 1min at 72 ^{0}C followed by a 15 min extension at 72 ^{0}C. The resulting PCR products were electrophoresed in 1.5% agarose gels containing 10 µg/ml ethidium bromide and documented through a Polaroid instant camera.

DNA amplification by RAPD

DNA amplification was carried out by the Random Amplified Polymorphic DNA (RAPD) method (Ramanayake *et al*., 2007). The oligonucleotide primers OPI20 (5'-GTGCGGATGGTCGCAGAGAT-3') and F9 (5'-CCAAGCTTCC-3') were used to amplify genomic DNA by PCR.

Reaction mixtures contained 5 pM of primer, 125 µM of each of deoxynucleoside triphosphate, 1.25 U of *Taq* DNA polymerase in 10x PCR buffer, 2.5 mM $MgCl_2$ (Promega Corporation, Madison, Wisconsin, USA) and template DNA. Amplifications were carried out in 25 µl volumes in a Perkin-Elmer/ Cetus DNA Thermal Cycler with the first cycle at 94 ^{0}C 4 min, 36 ^{0}C for 1 min and 72 ^{0}C for 2 min followed by 44 cycles at 94 ^{0}C for 1 min, 36 ^{0}C for 1 min and 72 ^{0}C for 2 min. The amplified products were subjected to electrophoresis in 1.5% agarose gels containing 10 µg/ml ethidium bromide and documented through a Polaroid instant camera.

RESULTS

Isolation of DNA is essential for molecular studies in any tissue whether it is a bacterium, plant or animal source. The technique described here is a rapid, robust and cost effective method suitable for the extraction of DNA from any source for routine molecular biology assay. This DNA extraction method yielded high quality DNA from different types of samples (standard cyanobactria, cyanobacteria, water samples and leaves from bamboo plants) which were suitable for molecular analyses. The cost of materials is approximately $ 10 per sample (excluding labour).

The method employed for genomic DNA extraction resulted in reproducible high quality DNA. The A_{260} /A_{280} ratio was between 1.7 and 1.9. DNA samples submitted to PCR reactions from the *M. aeruginosa* (PCC 7941), *Lyngbya* (PCC 8937) and the water samples collected from the environment for the 16S rRNA gene, yielded the fragment of about 450 bp, using the cyanobacterial specific oligonucleotide primers of Cya359F forward, Cya781 Ra and Cya781 Rb reverse (Fig. 1). All DNA samples submitted to PCR reactions from the water samples from the environment and from the cultured *M. aeruginosa* (PCC 7941) for the *mcyE* gene, yielded the fragment of about 250 bp, using the microcystin synthetase gene E forward primer (mcyE –F2) and genus specific reverse primer for *Microcystis* (MicmycE-R8) (Fig. 2).

The same protocol was employed to extract and purify genomic DNA from the two bamboo plants *D. giganteus* and *B. atra* except for the addition of proteinase K. The A_{260}/A_{280} ratio for the extracted DNA was between 1.6 and 1.9. The average DNA yield of *B. atra* was 6.4 mg/g (wt) and of *D. giganteus* 7.02 mg/g (wt). Figure 3 shows the banding pattern observed for the PCR with the two RAPD primers OPI 20 and OPF 9 for *D. giganteus*.

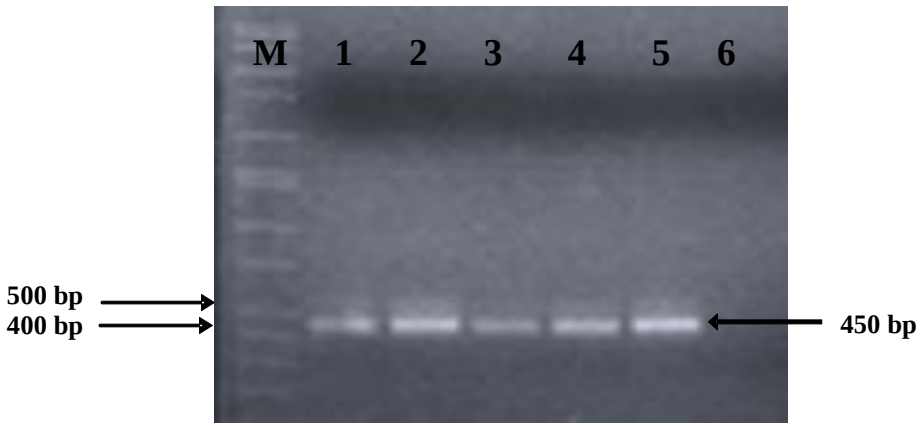

Figure 1. Detection of cyanobacterial strains by amplification of a region of the 16S rRNA gene. Lane M: DNA marker; Lane1: PCR amplified gene fragments from *M. aeruginosa* (PCC 7941); Lane 2: *Lyngbya* (PCC 8937); Lanes 3, 4 and 5: Environmental water samples; Lane 6: Negative control.

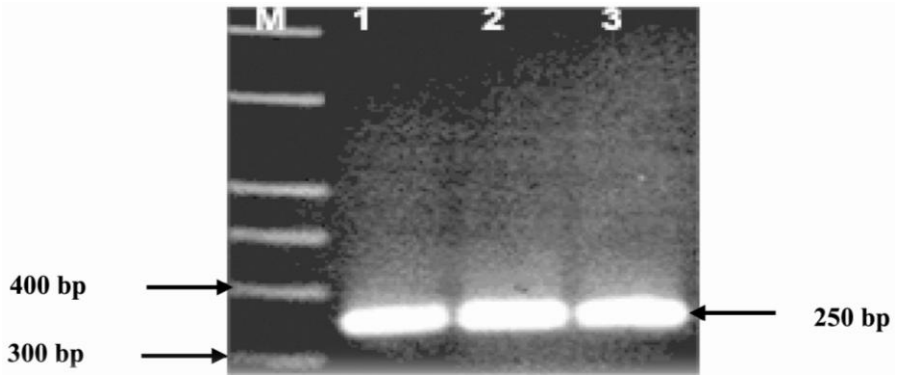

Figure 2. Detection of microcystin producing *Microcystis* spp. by amplification of a region of *mcyE* gene of the microcystin synthetase gene cluster. Lane M: DNA marker; Lanes 1 and 2: PCR amplified gene fragments from water samples; Lane 3: (+) DNA control of *Microcystis aeruginosa* (PCC 7941).

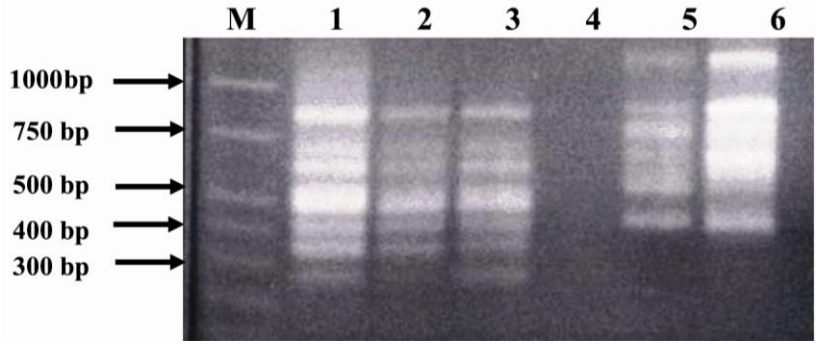

Figure 3. Amplification patterns obtained with the two RAPD primers OPI 20 and OPF 9 for *D. giganteus*. Lane M: DNA marker; Lanes 1, 2 and 3: PCR amplified gene fragments for the RAPD OPI 20 primer; Lanes 5 and 6: Amplification patterns obtained with OPF 9 primer.

DISCUSSION

Separation of pure intact DNA in sufficient quantities from cyanobacteria, other micro algae and plants is considerably difficult, due in part to the rigid cell wall and high carbohydrate content in tissues. The mechanical forces required to break cell walls may also shear DNA while co-isolation of viscous polysaccharides, polyphenols, tannins and other contaminants that may cause damage to DNA or lead to poor PCR amplification and will also inhibit restriction enzymes and DNA polymerases. There are numerous DNA isolation methods, such as Murray and Thompson's (1980) CTAB method and mini prep (Khan *et al*., 2004) protocols. Yet none of which is optimally suited for different plant species. There are many modifications of the Murray and Thompson's CTAB method for isolation of plant and fungal DNA (Borges *et al*., 2009; Tel-Zur *et al*., 1999; Brandfass and Karlovsky, 2008).

In this study we have described a protocol for purifying DNA that makes it possible to remove of contaminants and DNA elution in one step from organisms belonging to different taxonomic groups. In this method cyanobacteria were lysed by three sequential freezing (-5 ^{0}C) and heating (95 ^{0}C) followed by the enzymatic treatments of lysozyme and proteinase K. The extraction and purification of DNA was achieved with guanidinium isothiocyanate and silica. The cells / tissue material is lysed in the presence of high concentrations of the chaotropic agent guanidinium isothiocyanate and DNA binds to silica particles. Then the silica with adsorbed DNA is washed, to remove salt and impurities from the original sample and finally purified DNA is eluted in Tris EDTA buffer.

This method has several advantages over the other DNA extraction and purification methods; (a) The entire procedure can be completed in 2 h; (b) traditional method of sample grinding and cell disruption in liquid nitrogen is not required; (c) The pellet does not have to be further purified by phenol / chloroform extraction and or by commercial kits; (d) RNase treatment is not necessary to obtain DNA free of RNA (e) This method of isolation of DNA is faster and easier

to perform than the other organic extraction methods.

The technique used here to extract and purify genomic DNA is the Boom's method or guanidinium isothiocyanate /silica DNA extraction method which has a wide application in clinical microbiology (Boom *et al*., 1990; Magana-Arachchi *et al*., 2008). In this study we have shown that it is also an efficient and rapid method for isolating high quality DNA from a variety of bacterial and bamboo species.

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