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DNA isolation protocol for different types of seaweeds from seashore areas of Rameshwaram

Babita Kumari
Department of Biosciences and Biotechnology, Banasthali University, Rajasthan
*To whom for corresponding author address: bbtmshr@yahoo.co.in

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Abstract

In this paper, DNA isolation protocol for development of seaweeds collected from seashore areas of Rameshwaram. The protocol was applied to 10 seaweed species. The protocol yields about 2.5µg of high molecular weight DNA collected from 5mg of dried material of different type of seaweeds, with no RNA. No sign of degradation was observed after agarose gel electrophoresis for both freshly extracted DNA and DNA stored for 18 months at 4°C. The development of DNA isolation protocol was suitable for genomic library construction (tested for one species), endonuclease restriction, and PCR amplification for all species. Quantification of DNA concentration was performed using UV spectrophotometric measurement of the A_{260}/A_{280} ratio.

Keywords: CTAB; DNA extraction; nuclei isolation; Seaweed

Introduction

Isolation of DNA from seaweeds has proven quite difficult (Sosa and Olieveira, 1992, Chesnick and Cattolico, 1993), mostly due to co-isolation of hydrocolloids (agars and carrageenans). A requirement for the application of such techniques to study macroalgae is the ability to isolate high molecular weight nucleic acids of sufficient purity for enzymatic manipulations. Isolation of high quality nucleic acids from seaweeds is, however, hampered by the fact that these plants have cell walls, and often possess copious amounts of mucilaginous polysaccharides, polyphenolic compounds, diverse pigments and other secondary metabolites (McCandless, 1981; Ragan, 1981). Many of these compounds Co-purify with the nucleic acids during extraction procedures, and often interfere with subsequent enzymatic processing of the nucleic acids for molecular biological studies (Su and Gibor, 1988; Parsons *et al*., 1990, Roell and Morse,1991). Although DNA that is sufficiently pure for enzymatic manipulation has been isolated from some seaweed, the method involves time consuming.

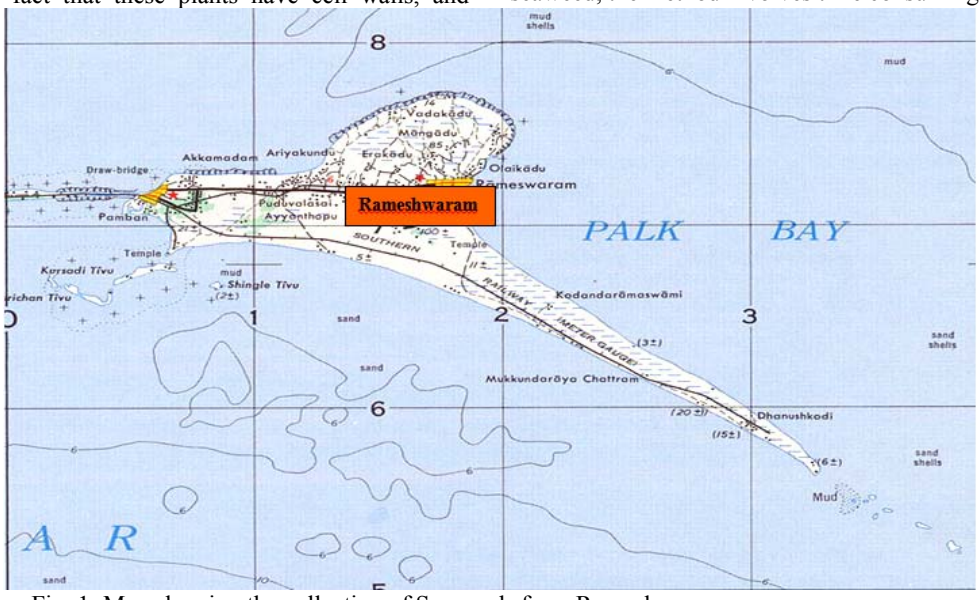


Fig. 1: Map showing the collection of Seaweeds from Rameshwaram

Materials and Methods

Collection of Samples

The tide of Rameshwaram coast (9º14`N, 79º14`E) was low, having rocky seashore areas (Fig.1) enriched with luxuriant growth of *Sargassum jhonstonii, S. vulgare, S.wightii, S. illcifolium, S. tenerrimum, Caulerapa racemosa, Enteromorpha intestinalis, Palmaria palmate, Graetloupia filicina, Ulva rigida, U. fasciata, Codium fragile, Padina racemosa, Kappaphycus alverzii, Chondria crispus* etc. The thallus of each species attached strongly on rocks and coral stones having minute initiation of young plants.

Washing and Cleaning of Thallus

The thallus contaminated with sand particles and epiphytes were removed by successive washings with seawater and distilled water. The thallus were then frozen and kept at -20°C.

The following method was employed for DNA Estimation (Fleurence *et al*.,2005):

EQUIPMENTS

- Water bath with shaking
- 15-mL tubes, 20-mL Nalgene tubes, 2- and 1.5-mL Eppendorf tubes
- High-speed refrigerated centrifuge
- Spectrophotometer

Chemicals, Solutions and Buffers

- Enzymatic digestion buffer: 50 mM sodium acetate (pH 5) stocked at 4°C.
- Enzymes from Novozymes: Ultraflow L (β-glucuronidase), Finizym 250L (β-glucanase), Shearzyme 500L (xylanase), Celluclast 1.5L (cellulase); from Sigma:Agarase from Pseudomonas Atlantica.
- Stock DNA extraction buffer (pH 8): 100 mM Tris HCl, 100 mM NaCl, 10 mM EDTA; autoclave and store at 4°C
- SDS solution, 20% w/v in water; sore at room temperature
- Sarcosyl, 10% w/v in water; store at room temperature
- Proteinase K, 20 mg/mL of deionized water; store at –20°C
- Phenol-chloroform-isoamylalcohol (pH 7.7)
- Isopropanol
- 70% ethanol
- H_2O, molecular grade
- Agarose
- Tris-acetate-EDTA 0.5× buffer
- Ethidium bromide
- Long-wave UV light.

Protocol

- Cut 1 g of seaweed plant material.
- Add 12.5 mL of enzymatic digestion buffer.
- Add enzymes at various concentrations.
- Incubate for 6 h in a water bath at 40°C with shaking.
- Centrifuge at 28,600gfor 30 min at 4°C.
- Transfer supernatant to a new tube.
- Frost the pellet at –20°C.
- Transfer 50 mg of pellet in a 2-mL Eppendorf tube.
- Add 2 mL of stock DNA extraction buffer.
- Incubate for 2 h in a water bath with shaking.
- Separate into 2-mL Eppendorf tube.
- Add 1 mL of phenol-chloroform-isoamylalcohol.
- Mix by inversion to form an emulsion.
- Centrifuge at 20,800gfor 15 min.
- Carefully pipet the upper aqueous phase (70 µL) to a new Eppendorf tube.
- Add 700µL of isopropanol at room temperature and mix by inversion for3 min.
- Spin at 20,800gfor 15 min.
- Discard isopropanol.
- Wash pellet twice with cold 70% ethanol.
- Spin at 13,000 rpm at 4°C.
- Dry pellet at 37°C in a thermobloc for 10 min.
- Redissolve the DNA in distilled water molecular grade.
- Allow enough time for complete resuspension.
- Calculate the DNA concentration by measuring absorbance at 260 nm and purity by measuring absorbance at 280 nm.

Removal of RNA contamination

The RNA contamination was removed by treating the DNA sample with RNase A (10 mg/ml) for 20 min at 37^0C and DNA was retrieved by following steps 7 and 9 of the above protocol.

Agarose gel electrophoresis

Following the isolation of DNA samples, the electrophoresis was done according to the following procedure (Sambrook *et al*., 1989)

a. Weigh 250mg of agarose and dissolve it in 25 ml of TAE buffer (4.84g Tris base, pH 8.0, 0.5 M EDTA/1 ltr) by boiling.

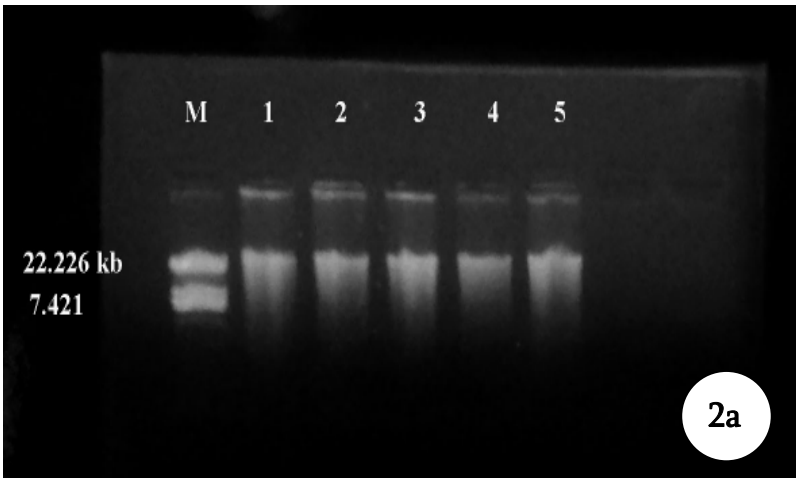
b. When the gel attains ~550C, pour it into the gel cassette fitted with comb to solidify Gel.
c. Carefully remove the comb, place the gel in the electrophoresis chamber flooded with TAE buffer.
d. Load 20 µl of DNA sample (mixed with bromophenol blue dye @ 1:1 ratio), carefully into the wells, along with standard DNA marker and pass the constant 50 V of electricity for around 30 min.
e. Remove the gel and carefully stain with Ethedium Bromide solution (10 µg/ml) for 10-15 min and observe the bands under UV transilluminator (UVP, Germany).

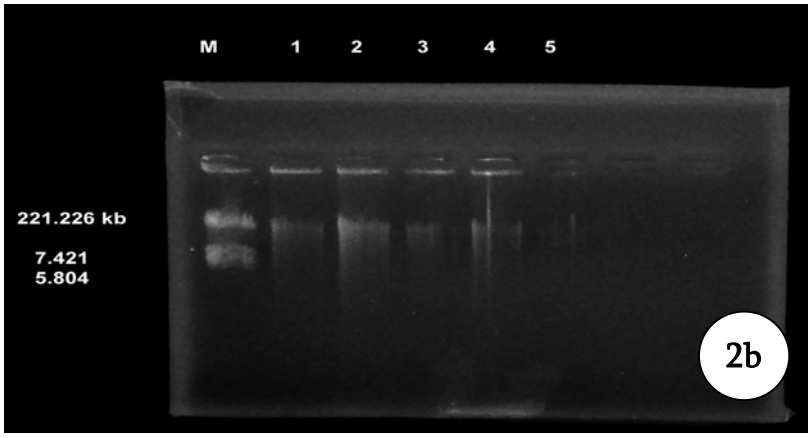
Results and Discussion

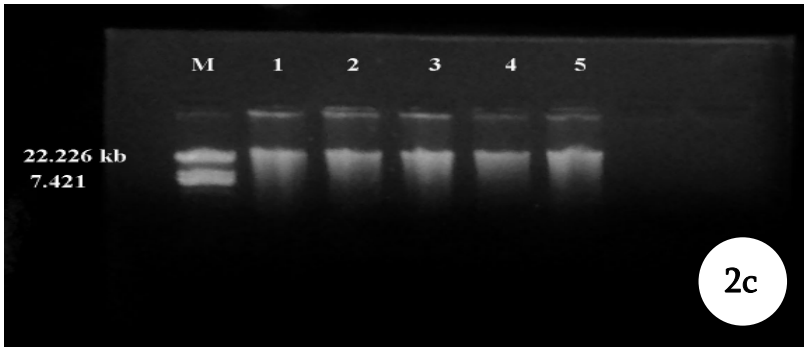
The different types of seaweeds were collected from Mandapam coastal area of south India for DNA isolation. The process was simplified in such a way that it could be used to process large sample numbers and to minimize polysaccharide co-isolation. In general, there are several valuable methods in the literature for seaweed DNA extractions, but for some algal taxa these methods yield DNA that is not useful for PCR amplification or restriction enzyme digestion. Hong *et al*., (1992) developed a simple method using LiCl for the rapid extraction of seaweed nucleic acids suitable for PCR analysis. However, the LiCl protocol did not work in the same way in all the tested species. Hong et al., (1997) found that DNA extracted from most seaweed species by the LiCl method were of sufficient quality to be used as a template for PCR amplification, with the exception of DNAs from a few species, which yielded large quantities of DNA, but did not show any PCR product, probably due to the presence of inhibitors of the DNA polymerase. Jin *et al*., (1997) tested 70 species of brown, red, and green algae for PCR inhibitors. Species such as *Colpomenia bullosa* (Saunders) Yamada, *Sargassum thunbergii* (Mertens ex. Roth) Kuntze, *Symphyocladia latiuscula* (Harvey) Yamada, and *Ulva sp*. showed very high inhibitory activity in PCR reactions. This inhibitory activity by cytosolic inhibitors in PCR reactions in DNA extracts of seaweeds has been associated with antiviral and antitumor effects (Kim *et al*.,1997; Cann *et al*.,2000; Eitsuka and Nakagawa, 2004). The protocol was applied to green, red and brown alga. The protocol yields about 5µg of high molecular weight DNA from 1gm of dried material, with no RNA. No sign of degradation was observed after 0.8 % agarose gel electrophoresis for both freshly extracted DNA (Fig. 2a, b, c & d). After genomic DNA was isolated from fresh material, the quality was checked by agarose gel. Quantification of DNA concentration was performed using UV spectrophotometric measurement of the A_{260}/A_{280} ratio.The detailed was summarized in Table -3. In conclusion, the protocol presented is highly recommended for seaweed DNA extractions. The procedure is a combination of two modified protocols developed previously (Triboush *et al*., 1998) for nuclei isolation, and Doyle and Doyle (1990) for DNA isolation in land plants. The procedure is rapid, requires few solutions, and is effective in isolating genomic DNA from *Caulerpa* species and *S. muticum*, where other methods failed. As the main advantage, this procedure provides genomic DNA of high quality with no degradation and with high yields from a small amount of material. The DNA isolated by this method has been successfully used in PCR, cloning, hybridization, and in other techniques used in the construction of genomic libraries.

The method for isolating high-quality DNA is presented for the green, red and brown alga in Fig. 2a, b, c & d. Previous methods of extraction, using Cetyl trimethyl ammonium bromide or various commercial kits, were used to isolate genomic DNA but either no DNA or DNA of very low quality was obtained. Elena *et al*., (2006) reported the method for isolating high quality DNA is presented for the green algae *Caulerpa* sp., *C. racemosa*, *C. prolifera*, and *C. taxifolia* and the brown alga *Sargassum muticum*. Previous methods of extraction, using cetyl trimethyl ammonium bromide (CTAB), were used to isolate genomic DNA of very low quality was obtained. The method presented here consists of the rapid isolation of nuclei, followed by DNA extraction. Yields of 6-10 µ*l*g genomic DNA from 1 g fresh blades obtained. After isolation of genomic DNA from fresh material, the quality of DNA was checked by agarose gel. Quantification of DNA concentration was performed using UV spectrophotometric

measurement of the A_{260}/A_{280} ratio. The first one was the recovery of the nuclei pellets, which depends on the speed and length of the centrifugation step, as well as the separation of the nuclei from cell debris and endosymbiotic bacteria associated with these algae. The second critical factor was the use of sodium acetate added to the DNA before precipitation. In this last step, sodium acetate was used instead of NaCl to avoid residual salt problems, which could inhibit DNA ligase.







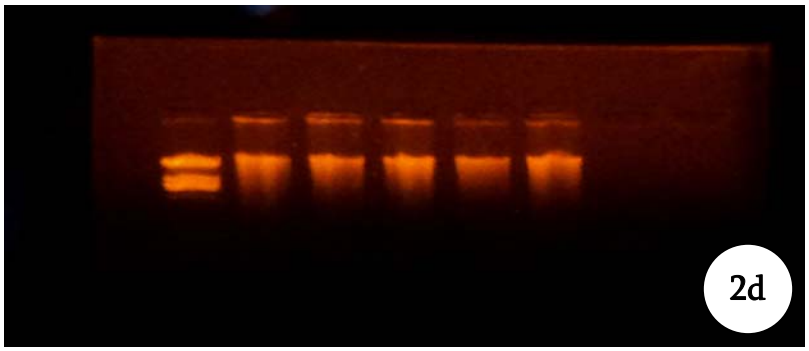


Fig. 2 a, b, c, d : Electrophoresis analysis (0.8% agarose gel in 10Mm TAE buffer, 40mM Tris acetate, 2mM EDTA) of (a) Random samples of *S. wightii, S. jhonstonii, S. muticum, S.tenerimum, S.illcifolium, C. recemosa*, *Enteromorpha sp., Palmaria palmate* etc. genomic DNA extracted with the procedure mentioned here. DNA digested with endonucleases (λ DNA Eco-RI digest, Bangalore Genei, Bengaluru).

In conclusion, the protocol presented is highly recommended for seaweed DNA extractions. The procedure is rapid, requires few solutions, and is effective in isolating genomic DNA from different species of Seaweeds. It provides genomic DNA of high quality with no degradation and with high yields from a small amount of material. The DNA isolated by this method has been successfully used in PCR, cloning, hybridization, and in other techniques used in the construction of genomic libraries.

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