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PROTOCOLS AND METHODS

A rapid and efficient DNA extraction method suitable for marine macroalgae

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Abstract Macroalgae are a diverse group of organisms. Marine macroalgae, in particular, have numerous medicinal and industrial applications. Molecular studies of macroalgae require suitable concentrations of DNA free of contaminants. At present, numerous protocols exist for DNA extraction from macroalgae. However, they are either time consuming, expensive or work only with few species. The method described in this study is rapid and efficient and applicable to different types of marine macroalgae. This method yields an average of 3.85 µg of DNA per 50 mg of algal tissue, with an average purity of 1.88. The isolated DNA was suitable for PCR amplification of universal plastid region of macroalgae.

Keywords Chlorophyta · DNA extraction · Genomic DNA · Macroalgae · Phaeophyceae · Rhodophyta

Introduction

Macroalgae are a vital part of the marine ecosystem. They contribute to the food web and provide a habitat for various marine organisms (Klinger 2015). Macroalgae or seaweed is also important from an industrial and medical perspective. They have been used for biorefinery and as feedstock for biofuel production (Bruhn et al. 2011; Baghel et al. 2014). Marine macroalgae contain sulphated polysaccharides such as fucans, carrageenans and ulvans, which have been reported to have antioxidant, antitumour, immunostimulatory, anticoagulant, and antimicrobial properties (Patel 2012). Studies have shown that macroalgal species like *Laminaria japonica* contain essential oils, which have antioxidant and antibacterial properties (Patra et al. 2015). Hydromethanolic extracts of the brown seaweed *Padina tetrastromatica* have been proven to have antihyperglycemic and antihyperlipidemic effects on rat models on a high-calorie diet (Mohan et al. 2014).

Various molecular studies have been reported for macroalgae including DNA barcoding (Kazi et al. 2013; Zhao et al. 2013), microsatellite library construction (Varela-Álvarez et al. 2006), phytoplankton community composition (Wallace and Gobler 2015) and whole genome sequencing (Cock et al. 2010). Good quality DNA is a prerequisite for most molecular studies involving macroalgae. The extraction of genomic DNA from marine algae is a challenging task owing to the various contaminants that are co-extracted with the DNA such as polysaccharides and polyphenols (Hoarau et al. 2007). These contaminants can inhibit the action of enzymes such as Taq polymerase, rendering the DNA useless for downstream applications (Jin et al. 1997). Macroalgae have a complicated cellular structure composed of various polysaccharides such as cellulose, sulphated fucans, laminarins and alginates (Mabeau et al. 1990; Michel et al. 2010), which hinder the DNA extraction process. Their morphology is also an important factor to consider during DNA extraction because homogenization of algal tissues is a tough task. Hence, an efficient homogenization method applicable for morphologically different algae is essential for efficient DNA extraction. A number of homogenization methods have been reported to disrupt the complicated algal cell wall structure including treatment with an enzyme cocktail consisting of β-glucuronidase,

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β-glucanase, xylanase, cellulase and agarase (Joubert and Fleurence 2005), grinding with liquid nitrogen (Shivji et al. 1992), and bead beating (Greco et al. 2014). However, a few of these methods require a large amount of biomass, and some methods are time-consuming and expensive, and most of the methods have been standardized for a single organism or only for a few species.

In our recent study on metagenomic DNA extraction, cell lysis was performed using sterile glass powder (Devi et al. 2015). The method was found to be rapid and efficient. In the current study, we have used glass powder for the homogenization of representative species of Rhodophyta, Phaeophyceae and Chlorophyta along with other components for DNA extraction. This is the first report on using glass powder as a homogenization agent for marine macroalgae, and the developed method was found to be efficient, rapid and suitable for other downstream applications.

Materials and methods

Sample collection and morphological identification

Fresh samples of representative species of Rhodophyta, Chlorophyta and Phaeophyceae were collected from Nochiurani (N09°16′16.0″; E79°01′02.0″) and Rameswaram (N09°09′09.6″; E78°39′39.5″) along the Gulf of Mannar region of Tamil Nadu, India. The collected samples were morphologically identified at Central Salt and Marine Research Institute (CSMCRI), Mandapam, Tamil Nadu, India (Fig. 1). The samples were stored at − 80 °C for further studies.

Genomic DNA extraction

Sterile glass powder was prepared by crushing broken borosilicate glass to a fine powder using a mortar and pestle. Extraction buffer was prepared having the following composition 100 mM Tris–HCl, 20 mM EDTA, 1.5 M NaCl, 1% sarkosyl, 2% PVP and 0.2% β mercaptoethanol (added fresh just before extraction). Algal tissue (50 mg) was homogenized in a mortar and pestle with extraction buffer and sterile glass powder (50 mg). The ground mixture was centrifuged to remove cell debris at 14,200×*g*, 4 °C, for 10 min. Absolute ethanol (1/9 volume) and 3 M potassium acetate (pH 4.8) (1/4 volume) were added to the supernatant to remove polysaccharide contamination. One volume of chloroform: isoamyl alcohol (24:1) was added to the solution. The tube was vortexed vigorously for a few seconds and incubated at − 20 °C for 20 min with constant mixing. The tube was then centrifuged at 14,200×*g*, 4 °C for 20 min. RNase A (50 µg) was added to the aqueous phase, and the tube was gently mixed by inversion. The tube was incubated at 37 °C for 30 min. An equal volume of chloroform: isoamyl alcohol was added; the tube was vortexed vigorously for few seconds and incubated at − 20 °C for 20 min with constant mixing. The tube was centrifuged at 14,200×*g*, 4 °C for 20 min. Isopropanol (0.8 volume), 3 M sodium acetate (pH 5.2) (0.1 volume), and β mercaptoethanol (0.2%) were added to the aqueous phase. The tube was kept at − 80 °C for 1 h and centrifuged at 14,200×*g*, 4 °C for 20 min. The pellet was washed with 1 ml of 70% ethanol and centrifuged at 14,200×*g*, 4 °C for 10 min. The pellet was air-dried and dissolved in 50 µl of sterile distilled water.

Agarose gel electrophoresis of DNA

The extracted DNA was loaded on an agarose gel (1% w/v), stained with ethidium bromide (10 µg/mL) and subjected to electrophoresis at 100 V. After electrophoresis, the gel was visualized using gel documentation system (Gelstan 1012, Mediccare, India) to check the quality and integrity of the DNA.

Assessment of yield and purity of the extracted DNA

Concentration of the extracted DNA was determined using Biophotometer (Eppendorf, NY). The total yield of DNA was calculated using the following equation:

$$\text{Yield of DNA } (\mu g/50 \text{ mg of sample}) = [\text{Concentration of DNA}(ng/\mu L) \times \text{Volume used to suspend DNA}(\mu L)].$$

The purity of DNA was determined by measuring the absorbance ratio at A_{260}/A_{280}.

PCR amplification of universal plastid amplicon region

The extracted DNA was used as template for PCR amplification to assess its suitability for downstream applications. Universal Plastid Amplicon [UPA] (400 bp) region was selected as a reference gene for PCR amplification. To amplify the UPA sequences, the primers p23S1 (forward, 5′-GGACAGAAAGACCCTATGAA-3′) and p23S2 (reverse, 5′-TCAGCCTGTTATCCCTAGAG-3′) were used (Zhao et al. 2013). The PCR reaction was carried out using the following reaction conditions: initial denaturation of 94 °C for 2 min, followed by 35 cycles of 94 °C for 20 s, 55 °C for 30 s, and 72 °C for 30 s, with a final extension of 72 °C for 10 min. PCR products were run on an agarose gel (1% w/v) and visualized using gel documentation system (Gelstan 1012, Mediccare, India) after staining with

Fig. 1 Representative species of macroalgae used in this study. **a** *Gracilaria corticata*, **b** *Acanthophora spicifera*, **c** *Champia parvula*, **d** *Sargassum tenerrimum*, **e** *Padina tetrastromatica*, **f** *Sargassum wightii*, **g** *Sargassum polycystum*, **h** *Caulerpa scalpelliformis*, **i** *Caulerpa racemosa*, **j** *Chaetomorpha aerea*

ethidium bromide (10 μg/mL). The reaction without the template served as a non-template control (NTC).

Results

Genomic DNA extraction

Genomic DNA was extracted using the developed method from representative samples of red, brown and green macroalgae. A distinct, intense band was seen on the agarose gel for all the species tested in this study (Fig. 2).

Yield and purity of genomic DNA

The concentration and purity of the extracted DNA was determined by spectrophotometric method. The total yield of DNA was calculated (Table 1). DNA yield varied between different genera of macroalgae. The DNA extracted from the red algae ranged from 1.73 to 6.5 μg/50 mg of biomass. Brown algae yielded DNA ranging from 2.2 to 5.4 μg/50 mg of biomass. DNA extracted from green algae ranged between 2.9 and 6.0 μg/50 mg of biomass. The purity of the extracted DNA ranged from 1.78 to 2.05 for all the samples.

PCR amplification

The extracted DNA was amplified using specific primers designed for the UPA region. The amplified products were separated and visualized on an agarose gel (1% w/v). The amplicons were seen as a distinct band at 400 bp from all the species of macroalgae. The non-template control showed no band (Fig. 3).

Discussion

Methods such as bead beating, detergent-based cell lysis, treatment with enzymes and grinding with liquid nitrogen have been employed for homogenization of macroalgae for DNA extraction and have proven to be effective (Joubert and Fleurence 2005; Wang et al. 2005; Hoarau et al. 2007; Greco et al. 2014). However, most enzymes are expensive and hence cannot be used for a large number of samples. Bead beating requires a thermomixer/bead beater and is a time-consuming process. Glass powder can be made from waste glassware in any laboratory and is economical when compared to the use of enzymes. Additionally, glass grinding is rapid and greatly shortens the time required for

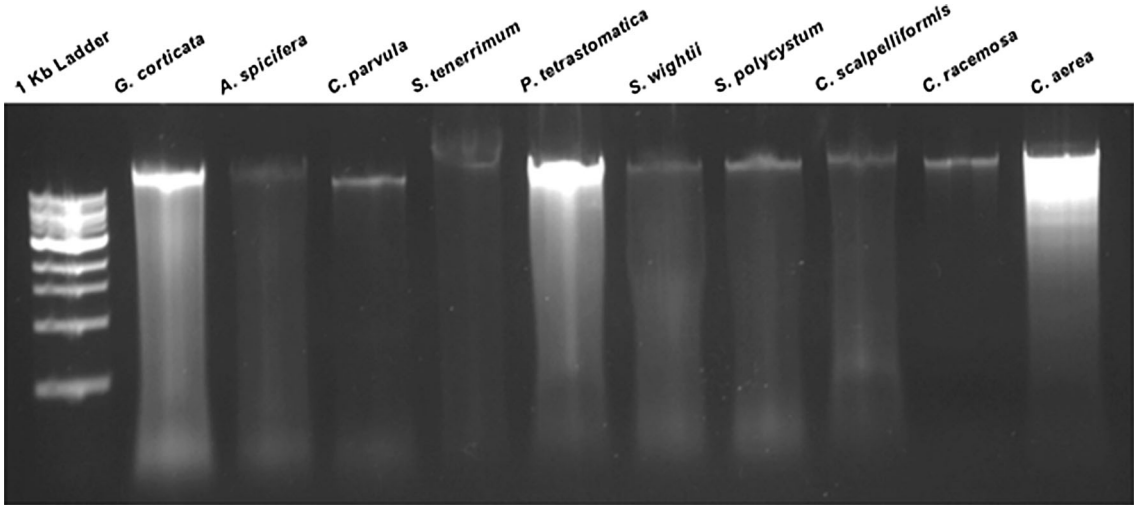

Fig. 2 Genomic DNA isolated using the developed method from various macroalgal samples run on a 1% agarose gel

Table 1 Yield and purity of DNA isolated using the developed method from various macroalgal samples

S. no.	Macroalgae	Classification	Concentration of DNA (ng/μL)	Purity A_{260}/A_{280}	Yield (μg/50 mg of sample)
1	*Gracilaria corticata*	Rhodophyta	130.6 ± 9.29	1.86 ± 0.01	6.5 ± 0.4
2	*Acanthophora spicifera*	Rhodophyta	34.6 ± 4.16	1.81 ± 0.03	1.73 ± 0.2
3	*Champia parvula*	Rhodophyta	37.33 ± 4.5	1.83 ± 0.10	1.86 ± 0.2
4	*Sargassum tenerrimum*	Phaeophyceae	45.33 ± 3.21	1.9 ± 0.05	2.2 ± 0.1
5	*Padina tetrastromatica*	Phaeophyceae	109.6 ± 6.5	1.80 ± 0.06	5.4 ± 0.32
6	*Sargassum wightii*	Phaeophyceae	87.66 ± 3.05	1.78 ± 0.01	4.38 ± 0.15
7	*Sargassum polycystum*	Phaeophyceae	81.6 ± 3.51	1.86 ± 0.09	4.0 ± 0.17
8	*Caulerpa scalpelliformis*	Chlorophyta	72 ± 3.6	1.95 ± 0.02	3.6 ± 0.18
9	*Caulerpa racemosa*	Chlorophyta	59.6 ± 2.88	2.05 ± 0.04	2.9 ± 0.14
10	*Chaetomorpha aerea*	Chlorophyta	120.5 ± 22.5	1.98 ± 0.05	6.0 ± 0.1

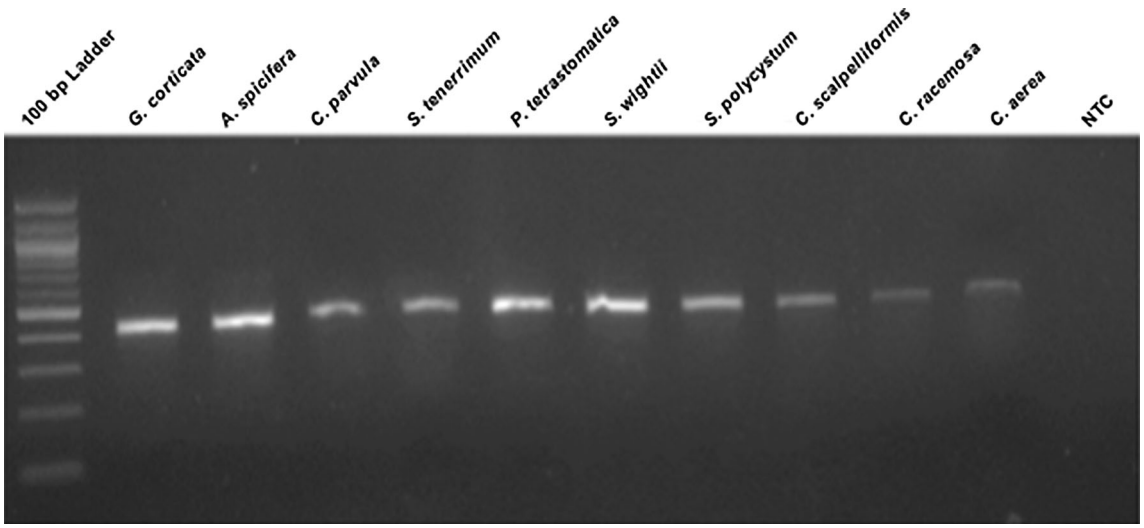

Fig. 3 PCR amplification of UPA region of macroalgae samples run on a 1% agarose gel. NTC—non-template control

homogenization. Glass powder acts as an abrasive agent, resulting in complete maceration of algal tissue. In this study, glass powder together with extraction buffer was used during the homogenization step to enhance cell lysis, leading to the release of DNA into solution.

The method described in this study is rapid and comparable to other fast methods while yielding higher concentrations of DNA (Varela-Álvarez et al. 2006; Hoarau et al. 2007). It also requires just 50 mg of fresh biomass while other protocols require substantial quantities of biomass (Joubert and Fleurence 2005; Varela-Álvarez et al. 2006). Furthermore, our method has been standardized to work for different types of macroalgae, with representatives from Rhodophyta, Chlorophyta and Phaeophyceae.

The DNA extracted using this method yields an average of 3.36 μg of DNA for red algae, 3.9 μg for brown algae and 4.1 μg for green algae per 50 mg of biomass. The yield of DNA obtained was higher compared to several methods proposed previously (Wang et al. 2005; Varela-Álvarez et al. 2006). The variation in the yield of DNA could be attributed to the variation in morphological structure and chemical composition of the algae. The species which possess a soft, fleshy/leafy thallus are easily homogenized and hence released more DNA when ground with glass powder. The average purity of the DNA extracted is 1.88. These values suggest that the DNA is of good quality with low levels of contamination.

Although there was a distinct band of DNA observed on the gel image, there was also some amount of smearing which could be a result of shearing of the DNA. This could be attributed to the harshness of the method. However, the extracted DNA was still applicable for downstream applications. The UPA region of the DNA extracted from all the species was successfully amplified. These results suggest that the isolated DNA is of good quality and can be used for routine molecular biology experiments.

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Author contributions Conceived and designed the experiments: MR. Performed the experiments: GSR and AAF. Analyzed the data: GSR, MR. Wrote the paper: GSR, MR.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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