



DNA extraction protocol from Brown Algae

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Abstract

There are various methods published about DNA extraction from marine algae. These methods are the modifications of several DNA extraction methods from other organisms. Extraction of DNA from seaweeds are difficult processes because of the polysaccharide and polyphenole compounds of their thallus. In this study, DNA is extracted from a brown alga (*Scytosiphon lomentaria* and *Cystoseira sp.*, *Ectocarpus sp.*) collected from the Bay of Izmir by using modified CTAB (cetyltrimethylammonium bromide) protocol and used in PCR analysis. This modified method was also found efficient and applicable for other molecular purposes.

Key words: DNA extraction, CTAB, Brown Algae

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Özet

Denizel alglerden DNA izolasyonu için çeşitli yöntemler bulunmaktadır. Ancak bu yöntemler diğer canlılar için kullanılan DNA izolasyon yöntemlerinin modifikasyonlarıdır. Alglerden DNA izolasyonu talluslarında bulunan polisakkaritler ve fenolik bileşiklerden dolayı zor bir işlemdir. Bu çalışmada, İzmir Körfezi'nde toplanan kahverengi alglerden (*Scytosiphon lomentaria* ve *Cystoseira sp.*, *Ectocarpus sp.*) CTAB (cetyltrimethylammonium bromide) yöntemiyle DNA izolasyonu gerçekleştirilmiş ve elde edilen DNA PCR yöntemi ile çoğaltılmıştır. Bu modifiye yöntem ile moleküler amaçlı çalışmalar için uygun DNA eldesi sağlanmıştır.

Anahtar Kelimeler: DNA izolasyonu, CTAB, kahverengi algler

1. Introduction

The application of molecular tools in studies of marine algae has often been hindered by difficulties in acquiring suitable DNA from them. In particular, polysaccharides and secondary metabolites represent an obstacle to DNA isolation, since DNA often copurifies with them, thus inhibiting downstream enzymatic reactions such as PCR (Shioda et al., 1987; Vidal et. Al., 2002).

Phaeophyceae is an important algae class which contains economically important organisms. Also they have antimicrobial activity, usage in cosmetics, medicine, textile industry and paper production. Generally the identification methods based on observing morphological characteristics. But this method has problems because of the morphological changes according to habitats, climate and growth level in the same species. Also some different species may share same morphological characteristics. Because of these reasons, certain and sensitive methods were needed for identification of the algae species. In recent years molecular techniques used in so many studies such as identification of the species, phylogenetics, understanding relationships within the species, monitoring costal waters, genotoxicity etc. The first step for molecular analysis is extraction of the DNA of the organism. The quality and quantity of extracted

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DNA effects the the result of the molecular analysis. All organisms even species have different kinds of DNA extraction methods. Monitoring costal waters for searching harmful algae involves microscobic examination of the plankton. But this method is time consuming and requires taxonomic experience because identification based on morphological characters (Godhe et al., 2001). Methods on Polymerase Chain Reaction (PCR) are popular for detection toxic dinoflagellates. Detection of *Gymnodinium mikimotoi* Miyake & Komimami ex Oda and *Alexandrium minutum* Halim by PCR based methods in field samples were described (Godhe et al., 2001).

The main problems in DNA extraction from macroalgae are the large amount of polysaccharides (Sosa and Oliveira, 1992) such as sulfated polysaccharides and carboxylic polysaccharides (Bold & Wynne, 1978) in their thallus and high nuclease activity (Wee et al., 1992). DNA extraction from brown algae has problems because of their large amount of phenolic components and polysaccharides. Also the procedure requires large amount of material.

The multinucleate cells of green algae in the orders Caulerpales, Charales, Dasycladales and Siphonocladales are valuable experimental organisms for investigating fundamental cellular and developmental phenomena (Coleman et al., 1989). The giant cells possessed by these algae have several advantages for cellular, molecular and biochemical work. Their coenocytic nature permits extraction of thousands of nuclei after disturbing a single cell (Staves & LaClaire, 1985). Also they typically lack significant quantities of the polyphenolic compounds that interfere with nucleic acid purification from Brown algae and many higher plants (John, 1992).

There are so many DNA prufication methods to enhance the DNA quality. These are CsCl ultracentrifuge method (Sosa and Oliveira, 1992), agarose gel-electrophoresis purification (Saunders, 1993) and hydroxyapatite column purification (Dutcher et al., 1930). Although these methods improved the DNA quality, they are complex, time consuming and expensive methods. None of them are compatible with large number of sample associated with population based studies (Wattier et al., 2000).

In this study, we use four different methods to extract DNA from one of the brown algae, *Scytosiphon lomentaria* (Lyngbye) Link, *Cystoseira sp.* and *Ectocarpus sp.*

2. Materials and Methods

Plant Material:

S. lomentaria *Cystoseira sp.* and *Ectocarpus sp.* samples collected from Bay of Izmir. The samples identified based on their morphological characteristics. Then they rinsed with distle water. The epifites cleaned under stereoscope and air-dried on filter paper.

Chemicals:

CTAB buffer: 1 M Tris, pH 8, 100 ml
 5 M NaCl, 280 ml
 0.5 M EDTA, 40 ml
 CTAB, 20 g
 dH₂O, 680 ml
 TE buffer: 10 mM Tris, pH 8
 1 mM EDTA

CTAB buffer and β -ME incubated in 65°C water bath. The dried material ground in liquid nitrogen into 1.5 ml eppendorf tube containing 600 μ l CTAB buffer and β -ME. The samples were mixed and incubated for 45 minutes at 65°C. This step lyses the cells. After incubation 500 μ l chloroform: isoamylalchol (C:IA) was added. The samples mixed for 10 minutes and centrifuged for 5 minutes for precipitate the proteins. The supernatant was transferred to a new tube and the CIA extraction was repeated. The supernatant transferred to a new tube again and approximately 125 μ l ice-cold isopropanol used to precipitate the DNA. After this step the supernatant was discard and 300 μ l. TE buffer was added and the tube incubated at 37°C for 1 hour. DNA was washed with 20 μ l 3 M NaAc and ethanol, centrifuged for 10 minutes at 1.844 x g. and the supernatant was discard again. The pellet was washed with ethanol again and centrifuged for 2 minutes at 1.844 x g. Supernatant was discard after centrifufetion. After that the pellet dried on the air and solved in 100 μ l TE buffer. DNA could kept for 18 months at +4°C (Steen, 1999).

In the other DNA extraction method used for *S. Lomentaria*, *Cystoseira sp.* and *Ectocarpus sp.*, PVP was used addition to these chemicals. According to this protocol 100 mg. dried thallus ground in CTAB buffer with 4 g PVP without pre-heating. Then the tubes left incubation overnight. After incubation C:IA added and centrifuged for 8 minutes at 14462 x g. The first layer of the sample pipetted in to new eppendorf tube. Then 8 μ l ice-cold 7.5 M ammonium acetate and 80 μ l ice-cold isopropanol added into the tube. After shaking by hand the tubes were left overnight incubation at - 20°C. After incubation the samples centrifuged at max. speed for 3 minutes. The supernatant

discard and ice-cold absolute ethanol was added into the tubes. The pellet dried after centrifuge for 1 minute. Finally, the pellet dissolved in 100 μ l TE (Steen, 1999).

In the third protocol that we experimented for *S. Lomentaria*, *Cystoseira sp.* and *Ectocarpus sp.*, the CTAB buffer pre-heated before starting to extraction. 2 μ l β -ME used per 1 ml CTAB buffer. Following extraction steps applied as in the previous methods. The samples centrifuged at low speed in centrifuge steps (approximately 1.844 x g. for each steps) (Colosi & Schall 1993).

In the fourth method liquid nitrogen was not used before CTAB treatment. This is the only different point among others. Fresh plant tissue directly extracted in CTAB buffer with β -ME (1 ml CTAB/2 μ l β -ME). The other steps including CIA, isopropanol and ethanol treatment applied as usual. Finally the pellet resuspended in 20-30 μ l TE.

In the fifth method we tried three different modifications of the protocol that Coyer et al. published in 1995. In our first experiment we extracted the cleaned samples with extraction buffer as described as the second group of second experiment in Coyer et al., 1995. This extraction buffer includes %2 CTAB, %0.1 PVP, 100mM Tris-HCl, 20mM EDTA, %0.1 SDS. In our second experiment we add NaCl to the extraction buffer. In the last experiment we used another extraction buffer that consist of 20 g CTAB, NaCl (5 M), Tris-HCl (1 M), EDTA (0.5 M) and β -ME. All the tubes incubated at room temperature for a week. After incubation the protocol continued by addition of 500 μ l C:IA. Samples mixed vigorously and centrifuged for 10 minutes at 14462 x g. Three layers are formed after centrifugation. DNA is excised in the upper layer. The upper layer transferred to a new eppendorf. 24 μ l ice-cold 7.5 M ammonium acetate and 175 μ l 2-propanol added to the tubes then incubated at -20°C overnight. The tubes centrifuged for 3 minutes after incubation. The aquaous part discarded and the pellet was washed 2 times with absolute ethanol and centrifuged for 2 minutes. The supernatant discarded and the pellet left for an hour for air drying. The pellet resuspended in 100 μ l TE buffer. DNA can stored at -20°C for years or 18 months at +4°C.

3. Results

After extraction protocols the DNA samples run in %0.8 agarose gel at 80V for two hours (Figure 1). Also we examined the DNA samples in spectrofotometer at 260 and 280 nm. The DNA samples used in PCR with RAPD (Random Amplified Polymorphic DNA) primers for checking the quality of the DNAs' for molecular analysis (Figure 2).

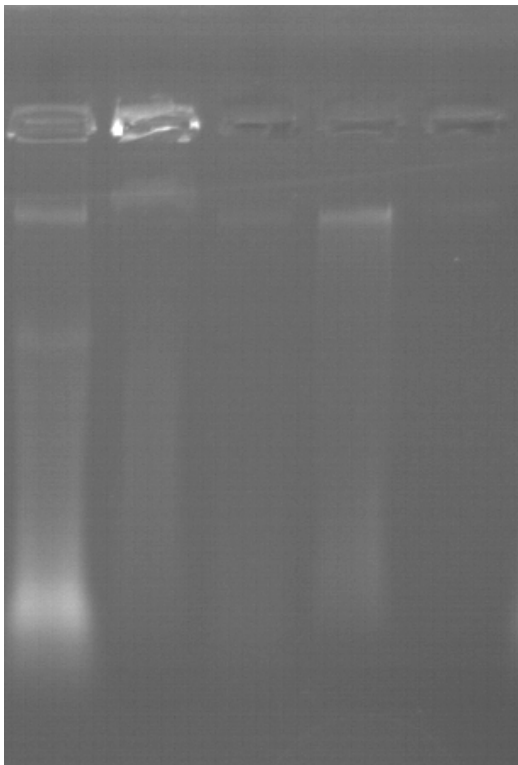


Figure 1. Agarose gel electrophoresis results of the *Cystoseira sp.* DNAs by different extraction methods.

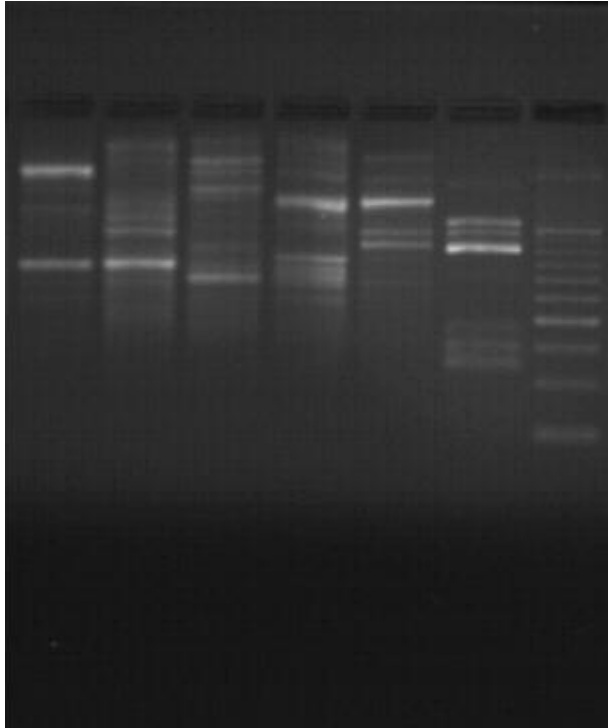


Figure 2. Agarose gel electrophoresis results after RAPD-PCR analysis. Line 1-6: *Cystoseira barbata*, *C. compressa*, *C. mediterranea*, *C. crinita*, *Ectocarpus sp.* and *Scytosiphon lomentaria* respectively, Line 7: DNA ladder 100 bp.

4. Conclusions

The efficient DNA extraction protocol from brown algae for molecular analysis described in this study. The main differences of the DNA extraction protocols from algae and other organisms is the chemicals that used for elimination of the polysaccharides. These chemicals are β -ME, PVP (Polivinilpirrolidon) and SDS (Sodiumdodecil sulfate). Increased consantrations of this chemicals used for brown algae because of the high contents of polysaccharides and phenolic compounds of the thallus. We used only β -ME and PVP among these chemicals. According to the results the efficient DNA extraction methods are first and the second protocols. The only method that has PVP step is the second one. One of the reason that second protocol yielded efficient DNA for the PCR analysis could be this feature. If we compare the third method with the first one which include same chemicals we could see the concantration differences about β -ME between them. The first method has brighter bands than third one in the agarose gel electrophoresis. This could be the consequence of the high concantration of β -ME that first protocol has. The fourth method which does not include liquid nitrogen grinding step gave the lowest DNA yield.

After the spectrophotometry and agarose gel electrophoresis analysis we could say that the most efficient methods are the first and second protocols.

These two DNA isolation protocols tested again by changing their incubation extentions in different steps. The target was to find the efficient incubation time with low contamination and high DNA quantity. Incubation with CTAB buffer for a short period and isopropanol, for a long period gave good DNA yield for molecular analysis. Coyer et al. (1995) searched the efficiency of grinding with liquid nitrogen step in DNA extraction protocols. In their study 12 algae samples tested in two different experiment. In the first experiment the first group of samples incubated in extraction buffer with SDS for 45 minutes after grinding in liquid nitrogen. The second group incubated by same way without liquid nitrogen step. In first group of second experiment the samples incubated in extraction buffer for a week after grinding in liquid nitrogen. In the second group this process done without liquid nitrogen step. In the first experiment they found differences between the two groups. The first group yielded 64% much DNA than second group. In the second experiment they couldn't find noticeable differences between the two groups. According to that study, the time and labour consuming liquid nitrogen step of DNA extraction protocol gave the same result with 1 week incubation step. By that study a field-competible DNA extraction method was constituted.

In our study the extraction method with liquid nitrogen step was preferred instead of one week incubation period. Although one week incubation seems long period for incubation, it is competible for laboratory away field studies. Because of the chemicals and the equipments are eassly available in all laboratory, makes this extraction method

suitable for the molecular studies with marine algae. Also this protocol requires less amount of algae sample for DNA extraction.

PCR analysis with RAPD primers has shown that the first DNA extraction method is appropriate for molecular analysis. In this RAPD-PCR analysis four *Cystoseira* species, *Ectocarpus sp.* and *Scytosiphon lomentaria* used by four RAPD primers.

The aim of this study is to describe the efficient DNA extraction protocol from macro marine algae for the purpose of molecular analysis. Different DNA extraction protocols have been tested by modifying them. After getting results the best protocols have been tested again by changing their incubation times. After all these analysis, the liquid nitrogen application found necessary for DNA isolation. The most effective protocol tested in this study is the first one which includes liquid nitrogen grinding step. The effectiveness of this protocol probably depends on the β -ME content of the extraction buffer. This methodology should prove to be applicable to an even wider variety of algae especially the sample material is limited.

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