Algae
Volume 18(1): 65-69, 2003

A Simple Method for DNA Extraction from Red Algae

Yoo Kyung Lee* and Hong Kum Lee

National Research Lab for Marine Microbial Diversity, Korea Ocean Research and Development Institute, Ansan P.O. Box 29, Seoul 425-600, Korea

A simple method is described for extracting DNA from marine red algae from which it has been difficult to isolate DNA because of their polysaccharides and phenolic compounds. In this DNA extraction method, the red algae were lysed mechanically by freezing and grinding in liquid nitrogen, and chemically by SDS. Then, the DNA was purified in phenol/chloroform, and precipitated in isopropanol. This method was applied to *Antithamnion sparsum, Campylaephora crassa, Gelidium amansii, Griffithsia japonica, Polysiphonia morrowii, Porphyra yezoensis,* and *Symphyocladia latiuscula.* The DNA extracted by this method is high yield and high quality for molecular analyses, such as PCR, sequencing, restriction enzyme digestion, and genomic DNA library construction. This method is reproducible, simple, and fast for routine DNA extraction from red algae.

Key Words: *Antithamnion, Campylaephora,* DNA extraction, *Gelidium, Griffithsia, Polysiphonia, Porphyra, Symphyocladia*

INTRODUCTION

Isolation of good quality DNA is required for molecular taxonomy and gene cloning of red algae. Even though DNA extraction from red algae started more than 30 years ago (Nasatir and Brooks 1966), it has been difficult because of their polysaccharides and phenolic compounds. Polysaccharides precipitate with DNA resulting in hindering isolation of high-quality nucleic acids, in poor yields, and in interference with restriction enzyme digestion (La Claire and Herrin 1997; Nair *et al.* 1999). To overcome this problem, Xing and Gibor (1988) precipitated polysaccharides of brown algae under selected conditions. Addition of CTAB (cetyltrimethylammonium bromide) to the extraction buffer (Liaud *et al.* 1994; Coyer *et al.* 1995), and CsCl (cesium chloride) density gradient ultracentrifugation (MacKay and Gallant 1991; Roell and Morse 1991) were also used to minimize polysaccharides contamination. But these methods need expensive chemicals such as CTAB and CsCl, and ultracentrigation method requires the use of sophisticated equipment and takes more than one day. The extracted DNA was also purified with CTAB treatment (Kitade *et al.* 1996), or QIAGEN Genomic-tip 100/G (Nakajima *et al.* 2000) to remove polysaccharides. However, these methods result in loss of considerable amounts of DNA.

In this report, we describe an effective method for DNA extraction from red algae. This method is reproducible, simple, and fast for routine DNA extraction from red algae.

MATERIALS AND METHODS

Red algae, *Campylaephora crassa, Gelidium amansii, Polysiphonia morrowii, Porphyra yezoensis,* and *Symphyocladia latiuscola* were collected from Yungheong Island and Jeju Island in Korea. The algae alive were transported to the laboratory and washed with fresh seawater to remove epiphytes. *Antithamnion sparsum* and *Griffithsia japonica* were culture samples as described in the previous papers (Lee *et al.* 1995; Lee *et al.* 2001). The samples were blotted with paper towel to remove extra moisture, but *Porphyra yezoensis* was air-dried. They were weighed, quickly frozen in liquid nitrogen, and stored at –70°C until they were used.

The DNA extraction method presented in this paper is a modification of that used for higher plant DNA extraction (Junghans and Metzlaff 1990). To extract DNA, 0.2-2.0 g of algal samples were ground in a mortar and pestle with liquid nitrogen and the powders were transferred to 40-m*l* tubes; 30 m*l* of lysis solution [50 mM

*Corresponding author (leeyk@kordi.re.kr)

Tris-HCl (pH 7.6), 100 mM NaCl, 50 mM EDTA, 2% SDS, 0.2% PVP, 0.1% β-mercaptoethanol] was added to each sample. The homogenates were incubated at room temperature for 15-30 min, and the cellular debris were removed by centrifugation at 4,000 rpm for 5 min. Each supernatant was split in half to two 40-m*l* tubes, and 7.5 m*l* of phenol was added. The phases were mixed gently for 5 min at room temperature and 7.5 m*l* of chloroform was added. The phases were mixed gently and then separated by centrifugation at 4,000 rpm for 10 min. The aqueous phases were transferred with the cut-off 5 m*l* tips into new 40-m*l* tubes. The phenol/chloroform extraction step was repeated. The nucleic acids in the aqueous phases were precipitated by adding 0.6 volumes of isopropanol, and collected by centrifugation at 8,000 rpm, 4°C for 10 min. The pellets were washed with 70% ethanol and dried. The dry pellets were dissolved in 100 μ*l* to 1 m*l* of TE [10 mM Tris (pH 8.0), 1 mM EDTA] solution and stored at 4°C for further use. The purity of the DNA was assessed spectrophotometrically by calculating the A_{260} / A_{280} ratio to determine protein impurities. The DNA yield was calculated from the A_{260} for DNA samples showing between 1.8-2.0 of A_{260} / A_{280}. The DNA was loaded on a 1.0% agarose gel to determine size and concentration.

The primers selected to amplify the SSU rDNA gene fragment were: G01, 5'-CACCTGGTTGATCCTGCCAG-3' and G10, 5'-CCGCGGCAGCTGGCACCAGAC-3' (Choi *et al.* 2001). The PCR mixture consisted of 5 μ*l* of 10 ×-PCR mix [final concentrations: 50 mM KCl, 0.01% gelatin, 10 mM Tris-HCl (pH 9.0)], 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 1 μ*l* each of the two primers, 1 μ*l* of *Griffithsia japonica* DNA sample, and 2.5 units of *Taq* polymerase (TaKaRa, Japan) in a final volume of 50 μ*l*. The PCR was performed in a thermal cycler (Biometra, Germany) using cycling conditions that consisted of an initial denaturation at 95°C for 5 min and then 30 cycles with denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 min. A final extension was performed at 72°C for 5 min. The amplified SSU rDNA of *G. japonica* was analyzed by agarose gel electrophoresis and sequenced using ABI PRIZM 3100 Genetic Analyzer (Applied Biosystems, USA). The SSU rDNA sequences of Ceramiacean species were obtained from GenBank data release and aligned with the default settings of CLUSTAL W (Thompson *et al.* 1994).

The *G. japonica* DNA was digested with *Sal*I, *Hin*dIII, *Pst*I, and *Eco*RI restriction enzymes at 37°C for 2 h. The

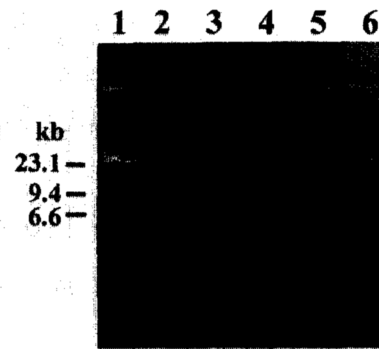

Fig. 1. Total DNA extracted from red algae on a 0.7% agarose gel. 1. *Porphyra yezoensis*; 2. *Gelidium amansii*; 3. *Antithamnion sparsum*; 4. *Campylaephora crassa*; 5. *Polysiphonia morrowii*; 6. *Symphycocladia latiuscola*.

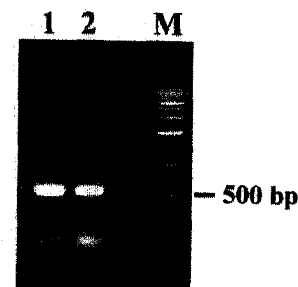

Fig. 2. Partial SSU rDNA fragments of *Griffithsia japonica* amplified from the extracted DNA as templates. Ethidium bromide stained DNA bands resulting from PCR amplification of target DNA were readily detected following electrophoresis of agarose gels. 1. *G. japonica* male gametophytes DNA; 2. *G. japonica* female gametophytes DNA used as templates; M = 500 bp size marker.

digested DNA was analyzed by agarose gel electrophoresis and photographed by Polaroid camera with Polaroid 667 Black and White film.

RESULTS

In this DNA extraction method, the red algae were lysed mechanically by freezing and grinding in liquid nitrogen, and chemically by SDS. Then, the DNA was purified in phenol/chloroform, and precipitated in isopropanol. This method gave good reproducible yields of high-quality DNA from both dried and fresh samples (Fig. 1). The average DNA yield was 14.9 mg/g (dry) from *Porphyra yezoensis*, 163 μg/g (wt) from *Gelidium amansii*, 312 μg/g (wt) from *Antithamnion sparsum*, 302 μg/g (wt) from *Campylaephora crassa*, 427 μg/g (wt) from *Polysiphonia morrowii*, 480 μg/g (wt) from *Symphycocladia latiuscola*, and 142 μg/g (wt) from *G. japonica*.

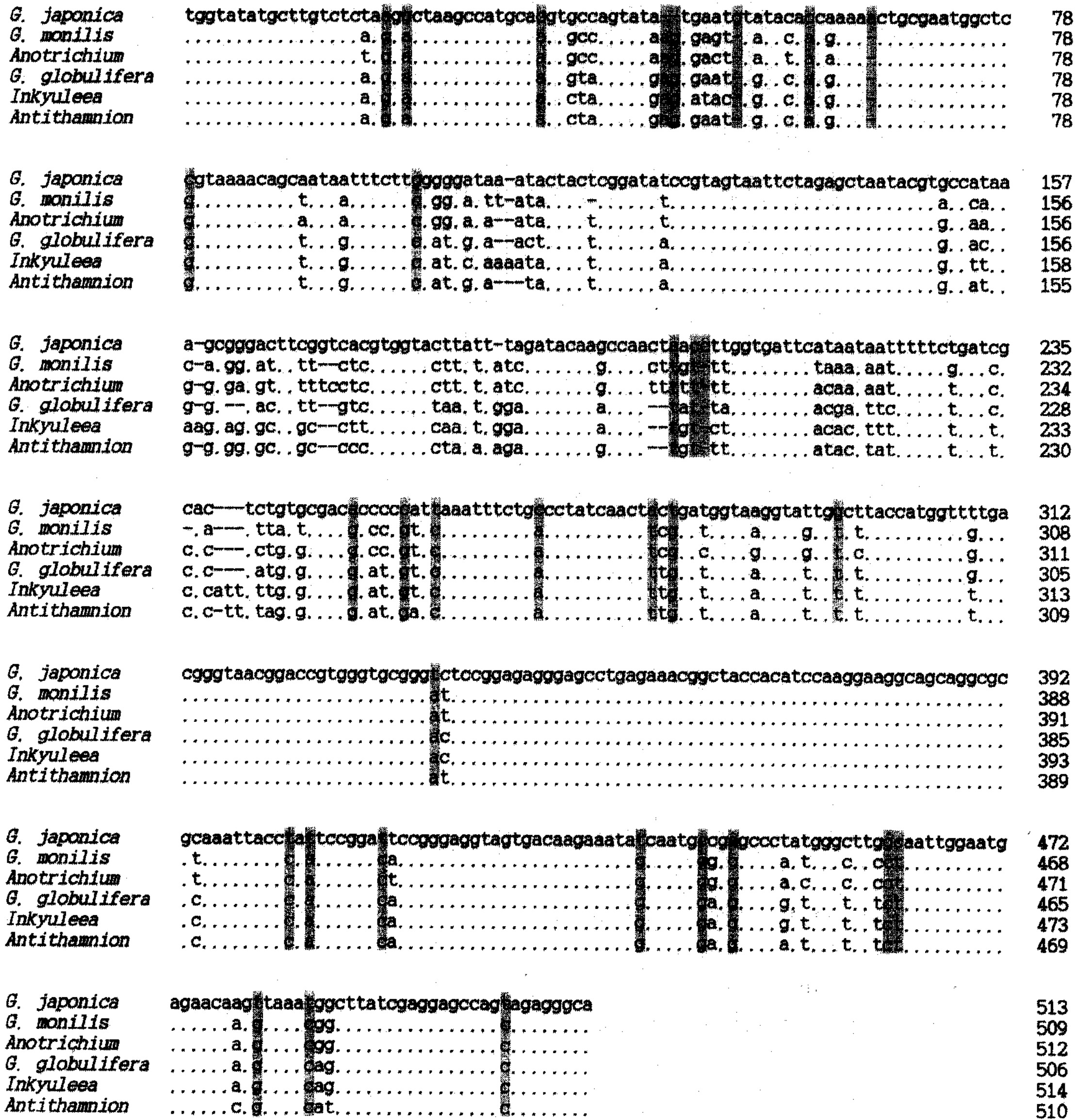

Fig. 3. DNA alignment of the partial SSU rDNA sequences of *Griffithsia japonica* (AY161284), *G. monilis* (U32565), *Anotrichium furcellatum* (U32561), *G. globulifera* (L26192), *Inkyuleea mariana* (AF236792), and *Antithamniom sparsum* (AF236787). Gaps indicated by hyphen (-) were introduced to improve alignment, and identical nucleotides are marked by a dot (·). Shadows mark positions that contain unique nucleotides in *G. japonica*.

The DNA extracted by this method is of good quality for molecular analyses, such as PCR, sequencing, restriction enzyme digestion, and genomic DNA library construction. A partial SSU rDNA of *G. japonica* was amplified by PCR using the extracted DNA as templates (Fig. 2). The SSU rDNA of *G. japonica* was sequenced and compared with those available for Ceramiacean species in the GenBank (Fig. 3). Sequences were different each other in 135 positions; out of them, 32 positions (23.7%) showed unique nucleotides in *G. japonica*, 12 positions (8.9%) in *G. monilis*, 6 positions (4.4%) in *Anotrichium furcellatum*, 5 positions (3.7%) in *G. globulifera*, 5 positions (3.7%) in *Inkyuleea mariana*, and 4 positions (3.0%) in *Antithamniom sparsum*, respectively. The DNA extracted in this study was cut with the restriction enzymes *Sal*I, *Hind*III, *Pst*I, and *Eco*RI very well (Fig. 4). A genomic DNA library was constructed using the DNA extracted in this study from *G. japonica*.

We applied this DNA extraction method to prepare genomic DNA from several microalgae including *Oscilatoria* sp., *Nostoc* sp., *Chroococcus* sp., *Cochlodinium polykrikoides*, and obtained good results (data not shown).

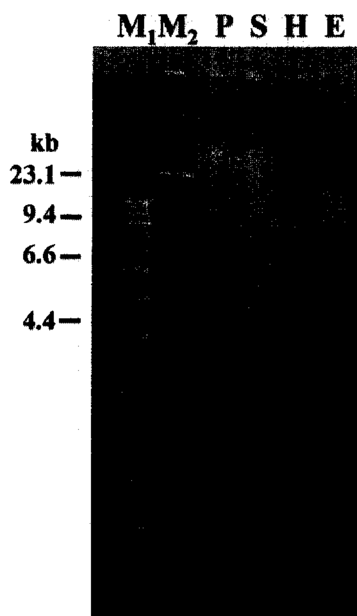

Fig. 4. Restriction enzyme digestion of *Griffithsia japonica* DNA by *Pst*I (P), *Sal*I (S), *Hind*III (H), and *Eco*RI (E). M_1 = 1 kb size marker; M_2 = *Hind*III digested lamda DNA size marker.

DISCUSSION

This simple DNA extraction method has several advantages: (1) the extraction steps are minimized, and DNA extraction is completed in three hours; (2) the cells are disrupted at the temperature of liquid nitrogen, minimizing nucleic acid degradation (Volossiouk *et al.* 1995); (3) this method does not use harsher chemical, mechanical, and enzymatic treatments, such as guanidine isothiocyanate, sonication, bead-beating, or lysozyme, which all increase DNA fragmentation (Krsek and Wellington 1999; Niemi *et al.* 2001); and (4) this method is less costly, since CTAB, guanidine isothiocyanate, and proteinase K are not used. Treating cells with lytic enzymes (proteinase K) can result in a high yield of DNA, but may inhibit the PCR if the denatured enzymes are not removed subsequently (Abolmaaty *et al.* 1998).

We modified the original method: (1) we used the method in large scale with 15 m*l* of lysis solution for 1g of fresh samples and 0.1 g of dry samples to reduce polysaccharides concentration in homogenate, because dilution of homogenate with enough lysis solution prevents polysaccharides contamination (Nakajima *et al.* 2000); (2) we increased the SDS percentage from 0.5% (in original method) to 2.0% to penetrate extracellular materials; (3) we added PVP to prevent hindrance of phenolic compounds, because PVP or PVPP complex with phenolic compounds and effectively remove them from the homogenate (John 1992, Krsek and Wellington 1999); (4) we eliminate the resuspension in TNE buffer and re-precipitation by ethanol. Because the ethanol precipitation and drying steps may actually enhance the association of polysaccharides to the DNA (Wu *et al.* 2000), and these steps are not effective to reduce polysaccharides contamination in our experiences (data not shown).

This method removes effectively polysaccharides from red algae. When we applied an ultracentrifugation method to extract DNA from *G. japonica*, a gelatinous pellet of polysaccharides was co-precipitated with nucleic acids. But there were no gelatinous materials in the DNA extracted in this study. PCR and sequencing reaction also showed clear results.

This method provides high yield of DNA. For example, the average DNA yields of *Porphyra yezoensis* in the previous reports were 11.8 μg/g (wt) (17.7 μg DNA from 1.5 g fresh algae; Kitade *et al.* 1996) and 15 μg/g (wt) (Nakajima *et al.* 2000), but 14.9 mg/g (dry) in this study. Traditional methods for DNA extraction from red algae require large amounts of tissue due to low yields (Coleman and Goff 1991; Roell and Morse 1991), but this method needs only 1-2 g of fresh materials because average DNA yield is several hundreds μg/g (wt).

The DNA extracted in this study was well digested by restriction enzyme (Fig. 4). Because polysaccharides inhibit enzyme digestion (Demeke and Adams 1992), *Porphyra* DNA extracted by LiCl method was not digested by some restriction enzymes (Hong *et al.* 1992). When we applied CTAB or ultracentrifugation methods to extract DNA from *G. japonica*, we couldn't also digest the DNA using some restriction enzymes.

The SSU rDNA sequences of *G. japonica* showed much more unique nucleotides than other species. Further phylogenic analysis on SSU full sequences of more species is needed to make clear the status of *G. japonica* in Ceramiacean algae.

In conclusion, this study has established a simple and cost-effective method for extracting high-quality DNA from red algae that is suitable for further molecular analyses.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Basic Research Program of the Korea Science and Engineering Foundation (KOSEF). We thank Dr. Han-Gu Choi for

providing primers G01 and G10.

REFERENCES

Abolmaaty A., El-Shemy M.G., Khallaf M.F. and Levin R.E. 1998. Effect of lysing methods and their variables on the yield of *Escherichia coli* O157:H7 DNA and its amplification. *J. Microbial. Meth.* **34:** 133-141.

Choi H.-G., Kim M.-S., Guiry M.D. and Saunders G.W. 2001. Phylogenetic relationships of *Polysiphonia* (Rhodomelaceae, Rhodophyta) and its relatives based on anatomical and nuclear small-subunit rDNA sequence data. *Can. J. Bot.* **39:** 1465-1476.

Coleman A.W. and Goff L.J. 1991. DNA analysis of eukaryotic algal species. *J. Phycol.* **27:** 463-473.

Coyer J.A., Steller D.L. and Alberte R.S. 1995. A field-compatible method for extraction of fingerprint-quality DNA from *Macrocystis pyrifera* (Phaeophyceae). *J. Phycol.* **31:** 177-180.

Demeke T. and Adams R.P. 1992. The effects of plant polysaccharides and buffer additives on PCR. *Biotechniques* **12:** 332-334.

Hong Y.-K., Coury D.A., Polne-Fuller M. and Gibor A. 1992. Lithium chloride extraction of DNA from the seaweed *Porphyra perforata* (Rhodophyta). *J. Phycol.* **28:** 717-720.

John M.E. 1992. An efficient method for isolation of RNA and DNA from plants containing polyphenolics. *Nucleic Acids Res.* **20:** 2381.

Junghans H. and Metzlaff M. 1990. A simple and rapid method for the preparation of total plant DNA. *Biotechniques* **8:** 176.

Kitade Y., Yamazaki S. and Saga N. 1996. A method for extraction of high molecular weight DNA from the macroalga *Porphyra yezoensis* (Rhodophyta). *J. Phycol.* **32:** 496-498.

Krsek M. and Wellington E.M.H. 1999. Comparison of different methods for the isolation and purification of total community DNA from soil. *J. Microbiol. Methods* **39:** 1-16.

La Claire J.W., II and Herrin D.L. 1997. Co-isolation of high-quality DNA and RNA from coenocytic green algae. *Plant Mol. Biol. Rep.* **15:** 263-272.

Lee S.-R., Oak J.H., Suh Y. and Lee I.K. 2001. Phylogenetic utility of rbcS sequences: an example from *Antithamnion* and related genera (Ceramiaceae, Rhodophyta). *J. Phycol.* **37:** 1083-1090.

Lee Y.K., Choi H.-G., Hong C.B. and Lee I.K. 1995. Sexual differentiation of *Griffithsia* (Ceramiales, Rhodophyta): nuclear ploidy level of mixed-phase plants in *G. japonica. J. Phycol.* **31:** 668-673.

Liaud M.-F., Valentin C., Martin W., Bouget F.-Y., Kloareg B. and Cerff R. 1994. The evolutionary origin of red algae as deduced from the nuclear genes encoding cytosolic and chloroplast glyceraldehyde-3-phosphate dehydrogenase from Chondrus crispus. *J. Mol. Evol.* **38:** 319-327.

MacKay R.M. and Gallant J.W. 1991. Beta-tublins are encoded by at least four genes in the brown alga *Ectocarpus variabilis. Plant Mol. Biol.* **17:** 487-492.

Nair S., Karim R., Cardosa M.J., Ismail G. and Pang T. 1999. Convenient and versatile DNA extraction using agarose plugs for ribotyping of problematic bacterial species. *J. Microbiol. Methods* **38:** 63-67.

Nakajima M., Kitade Y., Iitsuka O., Fukuda S. and Saga N. 2000. Rapid extraction of high-quality genomic DNA from *Phophyra yezoensis* (Bangiales, Rhodophyta). *Phycol. Res.* **48:** 15-17.

Nasatir M. and Brooks A.E. 1966. DNA of the marine red alga *Griffithsia globilifera. J. Phycol.* **2:** 144-147.

Niemi M.R., Heiskanen I., Wallenius K. and Lindstrom K. 2001. Extraction and purification of DNA in rhizosphere soil samples for PCR-DGGE analysis of bacterial consortia. *J. Microbiol. Methods* **45:** 155-165.

Roell M.K. and Morse D.E. 1991. Fractionation of nuclear, chloroplast, and mitochondrial DNA from *Polysiphonia boldii* (Rhodophyta) using a rapid and simple method for the simultaneous isolation of RNA and DNA. *J. Phycol.* **27:** 299-305.

Thompson J.D., Higgins D.G. and Gibson T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22:** 4673-4680.

Volossiouk T., Robb E.J. and Nazar R.N. 1995. Direct DNA extraction for PCR-mediated assays of soil organisms. *Appl. Environ. Microbiol.* **61:** 3972-3976.

Wu X., Zarka A. and Boussiba S. 2000. A simplified protocol for preparing DNA from filamentous cyanobacteria. *Plant Mol. Biol. Rep.* **18:** 385-392.

Xing S. and Gibor A. 1988. A method for RNA isolation from marine macro-algae. *Anal. Biochem.* **174:** 650-657.

Accepted 28 February 2003