Vol. 84: 197-203, 1992 | MARINE ECOLOGY PROGRESS SERIES Mar. Ecol. Prog. Ser. | Published July 30

# Rapid isolation of high molecular weight DNA from marine macroalgae

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ABSTRACT: Application of molecular techniques to study marine macroalgae is in its infancy, and is likely to be facilitated by the ability to routinely isolate high quality DNA from these plants. The generally high polysaccharide and polyphenol content in macroalgae, however, often interferes with the isolation and subsequent enzymatic manipulation of their nucleic acids. We describe the use of a CTAB method for the isolation of high molecular weight DNA from marine macroalgae. The method is rapid, simple, inexpensive, does not require density gradient ultracentrifugation, and has general applicability to red, brown and green seaweeds. The isolated DNA appears sufficiently pure for application of most commonly used molecular techniques such as restriction endonuclease digestion, Southern blot hybridization, cloning, and amplification using the polymerase chain reaction. The method was also tested on the marine angiosperm *Zostera marina* (eelgrass).

## INTRODUCTION

Although the application of recombinant DNA technology to study macroalgae is in its infancy, the use of these techniques promises to yield biologically interesting, and possibly commercially useful discoveries. 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 & Gibor 1988, Parsons et al. 1990, Roell & Morse 1991). Although DNA that is sufficiently pure for enzymatic manipulation has been isolated from some seaweeds, the methods employed involve ultracentrifugation and are time-consuming, labor-intensive and expensive (Fain et al. 1988, Goff & Coleman 1988, Parsons et al. 1990, Shivji 1991). Research in systematics and population biology of seaweeds often requires analysis of large sample sizes, and would benefit from inexpensive and more rapid methods of DNA isolation.

We have earlier reported on a CTAB (hexadecyltrimethylammonium bromide) method to isolate DNA from very small amounts of higher plant tissue (Rogers & Bendich 1985). We now describe a modified version of this method to extract high molecular weight DNA from marine macroalgae. The procedure is rapid, economical, does not require cesium chloride ultracentrifugation, and yields DNA of sufficient purity for use in restriction enzyme analysis, Southern blot hybridization, cloning, and the polymerase chain reaction (PCR).

## MATERIALS AND METHODS

*Cladophoropsis membranaceae* (UWCC 190), *Caulerpa vanbosseae* (UWCC 179), *Acetabularia crenulata* (UWCC 672), *Derbesia* sp. (UWCC 274), *Sphacelaria*

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0171-8630/92/0084/0197/$ 03.00

sp. (UWCC 666) and *Griffithsia pacifica* (UWCC 238) were obtained from the University of Washington, Seattle, Washington (USA) culture collection. All other algae (Table 1) and the eelgrass *Zostera marina* were collected from intertidal or subtidal areas in either Puget Sound, Washington, the outer coast of Washington, or areas in southern British Columbia, Canada.

**DNA isolation methods.** Plants collected from nature were wrapped in paper towels moistened with seawater and transported to the laboratory on ice. An effort was made to collect healthy, young plants that were free of epiphytes. In the laboratory, plants were rinsed briefly in running tap water and gently scrubbed with paper towels to remove most of the surface microbial and epiphytic organisms. Excess moisture was removed by blotting between paper towels. The plants were then wrapped in aluminum foil and frozen at −70 °C until further use.

For DNA extractions, pieces of algal tissue were frozen in liquid nitrogen, mixed with a small amount of dry ice, and ground to a fine powder using a mortar and pestle. The ground tissue dry ice mixture was quickly transferred to sterilized 1.5 ml microcentrifuge tubes, which were then placed at −70 °C with the tops open. Tubes were capped after sublimination of the dry ice, and stored at −70 °C until needed for DNA extraction, at which time an approximately equal volume of 2X CTAB isolation buffer [2 % w/v CTAB (Sigma), 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1 % (w/v) polyvinylpyrrollidone (MW 40 000), 1.4M NaCl], pre-heated to 65 °C in a water bath was added to the ground algal sample. The tube contents were mixed thoroughly to ensure the algal tissue was completely hydrated, and placed at 65 °C for 5 to 15 min. The

Table 1. Susceptibility of algal DNAs to restriction endonuclease digestion. DNAs were digested overnight at 37 °C with 30 units of each enzyme. +: complete digestion; +/−: variable results (i.e. partial or complete digestion depending on DNA preparation). nt: enzyme not tested

| Species | Restriction endonuclease | | | |
|---|---|---|---|---|
| | *Eco*RI | *Pst*I | *Hind*III | *Bam*HI |
| **Red algae** | | | | |
| *Iridaea cordata* (Turner) Bory | + | + | + | nt |
| *Gracilaria* sp. Greville | + | + | + | + |
| *Branchioglossum* sp. Kylin | + | + | nt | + |
| *Bossiella* sp. Silva | + | + | +/− | nt |
| *Gastroclonium coulteri* (Harvey) Kylin | + | + | + | nt |
| *Smithora naiadum* (Anderson) Hollenberg | + | + | + | + |
| *Porphyra thuretii* Dawson | + | + | nt | + |
| *Porphyra torta* Krishnamurthy | + | + | + | + |
| *Porphyra miniata* C. Agardh | + | + | nt | + |
| *Porphyra nereocystis* Anderson | + | + | + | + |
| *Gigartina exasperata* Harvey & Bailey | + | + | + | nt |
| *Griffithsia pacifica* Kylin | + | + | + | nt |
| *Rhodymenia* sp. Greville | + | + | + | nt |
| *Neoagardhiella baileyi* Wynne & Taylor | + | + | + | nt |
| **Brown algae** | | | | |
| *Nereocystis luetkeana* Postels & Ruprecht | + | + | + | + |
| *Macrocystis integrifolia* Bory | + | + | +/− | + |
| *Costaria costata* (C. Agardh) Saunders | + | + | + | + |
| *Laminaria saccharina* (L.) Lamouroux | +/− | + | + | nt |
| *Alaria marginata* Postels & Ruprecht | + | + | + | nt |
| *Hedophyllum sessile* (C. Agardh) Setchell | + | +/− | + | nt |
| *Agarum fimbriatum* Harvey | + | + | + | nt |
| *Sargassum muticum* (Yendo) Fensholt | +/− | +/− | +/− | nt |
| *Sphacelaria* sp. Lyngbye | + | + | + | nt |
| *Petalonia debilis* (C. Agardh) Derbes & Solier | + | + | + | nt |
| *Scytosiphon lomentaria* (Lyngbye) J. Agardh | + | + | + | nt |
| *Fucus* sp. (L.) | +/− | + | nt | nt |
| **Green algae** | | | | |
| *Acetabularia crenulata* Lamouroux | + | + | + | nt |
| *Caulerpa vanbosseae* Lamouroux | + | + | + | nt |
| *Cladophoropsis membranaceae* Borgesen | + | + | + | nt |
| *Derbesia* sp. Solier | + | + | + | nt |

sample was then extracted with an equal volume of chloroform-isoamyl alcohol (24:1, v:v) by mixing thoroughly enough to form a complete emulsion. The mixture was centrifuged at 11 000 × *g* in a microfuge for 30 to 60 s to separate the 2 phases. The upper phase (containing the DNA), was carefully transferred to a new 1.5 ml sterilized microfuge tube. One-fifth volume of a 5 % CTAB solution (5 % CTAB, w/v, 0.7M NaCl), pre-heated to 65 °C, was added and the sample mixed thoroughly. The sample was then re-extracted with an equal volume of chloroform: isoamyl alcohol, centrifuged at 11 000 × *g* for 30 s, and the upper phase transferred to a new 1.5 ml sterilized microfuge tube. Between 25 and 50 µg of yeast tRNA were added to the sample as a carrier to aid in precipitation of the nucleic acids. Between 1 and 1.5 volumes of CTAB precipitation buffer [1 % CTAB, w/v, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA] was added very slowly (drop by drop) and the tube contents mixed very gently by swirling. The tube was placed on dry ice for 5 to 10 min until the sample became viscous or frozen, and then centrifuged (11 000 × *g*) for 3 to 5 min. The supernatant was removed and the pellet resuspended in 50 to 100 µl of warm (65 °C), high-salt TE buffer (10 mM Tris-HCl, 1 mM EDTA, 1 M NaCl, pH 8.0). Incubating the sample at 65 °C for 2 to 10 min sometimes facilitated dissolving the pellet. After the pellet was completely dissolved, 2 volumes of cold 95 % ethanol were added and the sample placed in dry ice for 10 to 15 min or at −20 °C overnight. The sample was then centrifuged (11 000 × *g*) for 10 min, the pellet washed in 70 % ethanol, re-centrifuged for 1 min, and dried under a vacuum for 20 to 30 min. The dried pellet was re-suspended in 300 µl TE (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and precipitated for a second time by the addition of half volume 7.5 M ammonium acetate and 2 volumes cold 95 % ethanol. The sample was centrifuged (11 000 × *g*) for 30 min, washed in cold 70 % ethanol, and dried under vacuum. The final, dry pellet was resuspended in 20 to 200 µl of TE buffer, depending on its size.

The average size and concentration of DNA extracted from the various algal species was estimated by comparing the migration and fluorescence intensity of undigested algal DNA with standardized amounts of undigested bacteriophage lambda DNA on agarose gels (Maniatis et al. 1982).

**Molecular methods.** Restriction endonucleases and T4-Ligase (Bethesda Research Laboratories, Gaithersburg, MD, USA) were used according to the supplier's specifications. RNase A and RNase T1 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The probe used for Southern blot hybridizations was the plasmid pBD4, which contains the yeast *Saccharomyces cerevisiae* 5S, 18S, 5.8S and 25S ribosomal RNA genes (Bell et al. 1977). The probe was labelled with $^{32}$P dCTP (New England Nuclear, Boston, MA, USA) using the random primer method of Feinberg & Vogelstein (1983). Gels were blotted onto Nytran membranes (Schleicher and Schuell, Keene, NH, USA), according to the manufacturer's instructions. DNA blots were hybridized with the probe at 55 °C in 2X SSC (0.3M sodium chloride/0.03M sodium citrate), 1 % SDS (sodium dodecyl sulfate), 1M sodium chloride, for 16 to 24 h. After hybridization, the blots were washed twice in 2X SSC at room temperature, followed by two 30 min washes in 2X SSC, 1 % SDS at 55 °C, and two 30 min washes in 0.1X SSC at room temperature. Autoradiography using intensifying screens (DuPont Company, Boston, MA) was carried out at −70 °C for 1 to 5 d.

To determine if the extracted DNA was of sufficient purity for cloning, DNA from the kelp *Alaria marginata* was digested with *Eco*RI and ligated into the plasmid vector pIC-7 (Marsh et al. 1984) using the shotgun method outlined by Maniatis et al. (1982). Twenty-six white, recombinant *Escherichia coli* colonies were randomly selected from LB-ampicillin-Xgal plates and screened for cloned algal DNA inserts. The *E. coli* plasmids were isolated using the boiling lysis method of Maniatis et al. (1982), digested with *Eco*RI to liberate the cloned *A. marginata* DNA fragments, and subjected to electrophoresis on a 0.8 % agarose gel.

The primer designed for PCR amplification consisted of the randomly chosen sequence GCATCACTGG. Amplifications were performed in 50 µl reactions with 1 ng of template DNA, 1 pM primer, 1.25 units of DNA polymerase (Taq polymerase, Perkin-Elmer/Cetus), and 0.2 mM of each dNTP in reaction buffer [50 mM KCl, 10 mM Tris (pH = 8.3), 1.5 mM $MgCl_2$, 0.01 % BSA]. The reaction mix was overlaid with mineral oil, denatured for 3 min at 93 °C, and amplified through 25 cycles in a Biocycles (Bios Corporation) thermal cycler using the following temperature profile: 25 s at 93 °C, 30 s primer annealing at 40 °C, and 1 min extension at 72 °C. A final extension for 2 min at 72 °C was performed after completion of the 25 cycles.

## RESULTS

### DNA isolation and yields

Using the method described, DNA was obtained from all the species examined. DNA yields were variable, ranging from approximately 10 to 70 ng per mg of frozen algal tissue, and depended on the species as well as the age and overall condition of the tissue. Older and thicker tissues generally gave lower yields than younger tissues, although this relationship seemed to be reversed in the case of the kelp *Nereocystis luetkeana*.

The DNAs obtained from most of the algae were substantially of high molecular weight, migrating in approximately the same position as undigested lambda DNA [48 kb (kilobases)] in agarose gels (Fig. 1). The only exceptions were the green alga *Ulva* sp., and the red articulated coralline alga *Bossiella* sp., which consistently yielded degraded DNA. Lyophilization of the algal tissue before DNA extraction also seemed to increase DNA degradation, at least in the few species tested (Fig. 1). This observation is consistent with our findings using higher plants and fungi (data not shown).

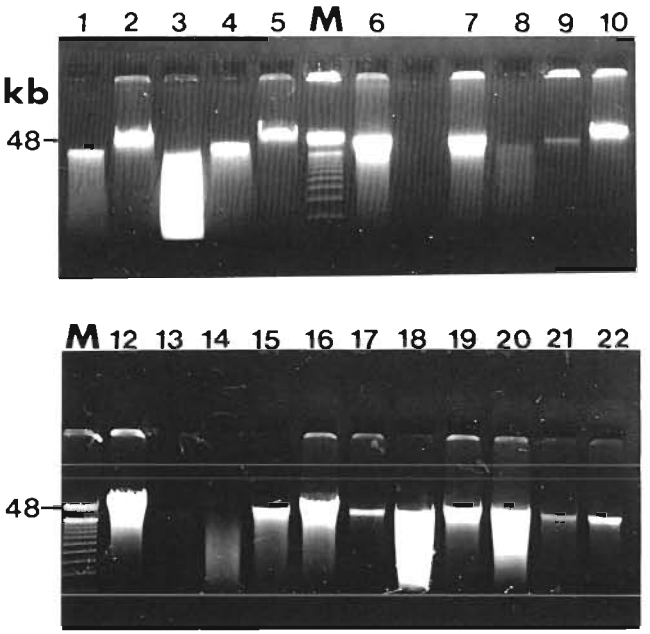


Fig. 1 Agarose gel of undigested total DNA isolated from various marine macroalgae and the eelgrass *Zostera marina*. M: molecular size standards [undigested bacteriophage lambda DNA and 1 kb ladder marker (BRL)]; lt: lyophilized tissue; asterisk: repeated attempts to isolate DNA from these algae. Lanes: 1, *Iridaea cordata* (lt); 2, *Gracilaria* sp. (lt); 3, *Gastroclonium coulteri* (lt); 4, *Sargassum muticum* (lt); 5, *Nereocystis luetkeana* (lt); 6, *Iridaea cordata*; 7, *Gracilaria* sp.; *8, *Bossiella* sp.; 9, *Gastroclonium coulteri*; 10, *Nereocystis luetkeana*; 12, *Petalonia debilis*; *13, *Ulva* sp.; *14, *Ulva* sp.; 15, *Cladophoropsis membranaceae*; 16, *Caulerpa vanbosseae*; 17, *Acetabularia crenulata*; *18, *Bossiella* sp.; 19, *Derbesia* sp.; 20, *Sphacelaria* sp.; 21, *Smithora naiadum*; 22, *Zostera marina* (eelgrass)

### Utility of DNA for molecular biological studies

Susceptibility of the various algal DNA samples to digestion by 4 commonly used restriction endonucleases are shown in Figs. 2 & 3 and Table 1. With few exceptions (indicated in Table 1), the DNAs are sufficiently pure for restriction endonuclease digestion and Southern blot hybridizations. The DNA isolated from the eelgrass *Zostera marina* had a dark brown pigmentation that did not seem to interfere with digestion by the endonuclease *Bam*HI. No other endonuclease enzymes were tested on this species however.

The yeast ribosomal DNA (rDNA) probe detected homologous DNA sequences in all the plants tested, except the green alga *Acetabularia crenulata* (Figs. 4 & 5). Ribosomal DNA restriction fragment length polymorphisms (RFLPs) were readily detected among species of the red algal genus *Porphyra* (Fig. 4). Use of the rDNA probe also revealed RFLPs among individual plants obtained from different *Nereocystis luetkeana* populations separated by short geographic distances. The north Seattle population differs in its hybridization patterns from the more southern Vashon Island and Tacoma Narrows populations, when using DNAs

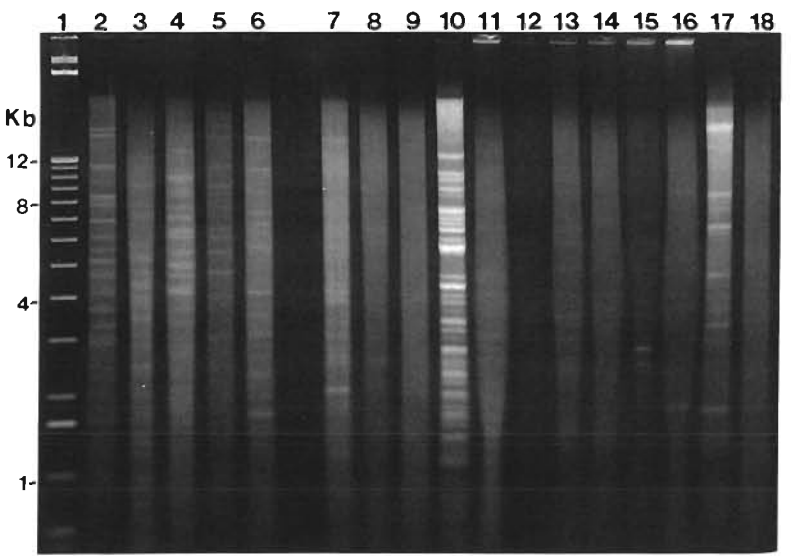


Fig. 2. Agarose gel of restriction endonuclease digested red algal DNAs. DNAs in Lanes 2 to 6 and 9 to 13 were digested with *Eco*RI, and in Lanes 7, 8, and 14 to 18 with *Bam*HI. Lanes 10 and 17 contain non-stoichiometric amounts of cesium chloride gradient purified nuclear, chloroplast and mitochondrial DNAs from *Porphyra yezoensis* (see Shivji 1991 for methods). Lanes: 1, molecular weight markers; 2, *Griffithsia pacifica*; 3 and 8, *Smithora naiadum*; 4, *Rhodymenia* sp.; 5, *Branchioglossum* sp.; 6, *Iridaea cordata*; 7, *Gracilaria* sp.; 9 and 18, *Porphyra torta* conchocelis; 10 and 17, *Porphyra yezoensis* conchocelis; 11 and 16, *Porphyra thuretii*; 12 and 15, *Porphyra nereocystis*; 13 and 14, *Porphyra miniata*

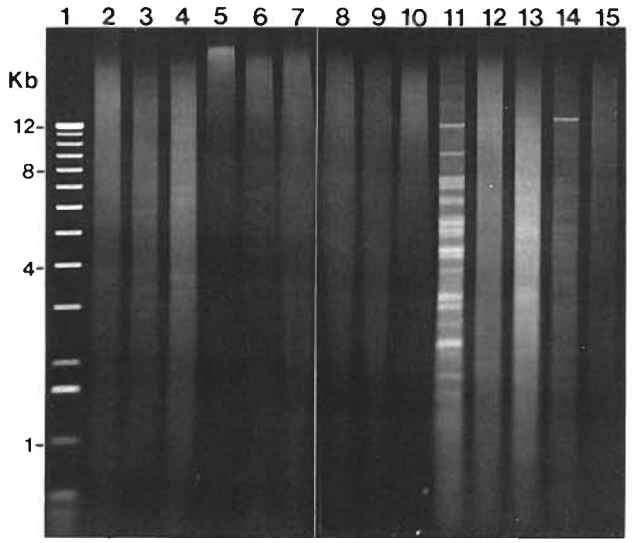


Fig. 3. Agarose gel of restriction endonuclease digested DNAs from brown and green algae and the eelgrass *Zostera marina*. All DNAs digested with *Eco*RI, except for *Z. marina* (*Bam*HI). Lanes: 1, molecular weight markers; 2, *Alaria marginata*; 3, *Petalonia debilis*; 4, *Sphacelaria* sp.; 5, *Laminaria saccharina*; 6, *Macrocystis integrifolia*; 7, *Costaria costata*; 8, *Nereocystis luetkeana* blade; 9, *Nereocystis luetkeana* stipe (lyophilized); 10, *Fucus* sp.; 11, *Caulerpa vanbosseae*; 12, *Cladophoropsis membranaceae*; 13, *Derbesia* sp.; 14, *Acetabularia crenulata*; 15, *Zostera marina*

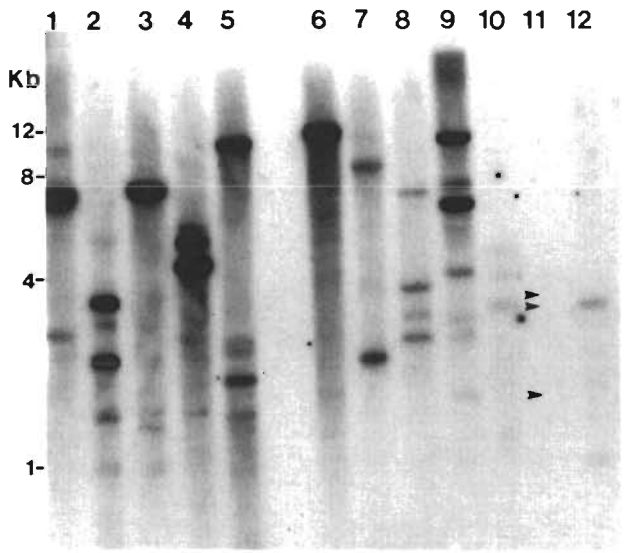


Fig. 4. Autoradiograph showing hybridization of *Saccharomyces cerevisiae* ribosomal DNA gene probe to red algal DNAs. All DNAs digested with *Eco*RI, except for Lanes 6 and 7 (*Bam*HI). Lanes: 1, *Griffithsia pacifica*; 2, *Smithora naiadum*; 3, *Rhodymenia* sp.; 4, *Branchioglossum* sp.; 5, *Iridaea cordata*; 6, *Gracilaria* sp.; 7, *S. naiadum*; 8, *Porphyra torta*; 9, *Porphyra yezoensis*; 10, *Porphyra thuretii*; 11, *Porphyra nereocystis*; 12, *Porphyra miniata*. Arrowheads in Lane 11 indicate positions of hybridizing bands evident upon longer exposures

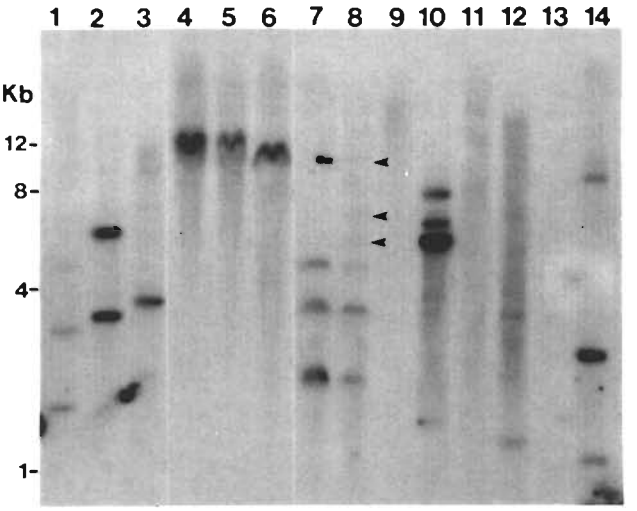


Fig. 5. Autoradiograph showing hybridization of *Saccharomyces cerevisiae* ribosomal DNA gene probe to DNAs from brown and green algae and the eelgrass *Zostera marina*. Arrowheads indicate the 3 hybridization bands evident with *Nereocystis luetkeana* stipe tissue, but absent with blade tissue. DNA in Lanes 1 to 3 and 7 to 13 digested with *Eco*RI. DNA in Lanes 4 to 6 and 14 digested with *Bam*HI. Lanes: 1, *Alaria marginata*; 2, *Petalonia debilis*; 3, *Sphacelaria* sp.; 4, *N. luetkeana* (Vashon Island population); 5, *N. luetkeana* (Tacoma population); 6, *N. luetkeana* (N. Seattle population); 7, *N. luetkeana* (N. Seattle population, blade tissue); 8, *N. luetkeana* (N. Seattle population, stipe tissue); 9, *Fucus* sp.; 10, *Caulerpa vanbosseae*; 11, *Cladophoropsis membranaceae*; 12, *Derbesia sp.*; 13, *Acetabularia crenulata*; 14, *Zostera marina*

digested with the enzymes *Bam*HI (Fig. 5) and *Eco*RI (not shown). Interestingly, rDNA polymorphisms that may be tissue-specific were also detected in blade and stipe tissue from this kelp (Fig. 5).

Shotgun cloning of *Alaria marginata* DNA using the pIC-7 plasmid vector resulted in the successful cloning of numerous *Eco*RI DNA fragments, ranging in size from approximately 1.5 to 6 kb (data not shown), indicating that inhibitors of DNA ligase were not present in the DNA preparation.

Results of PCR amplifications using the arbitrary sequence primer and DNAs from 3 species are shown in Fig. 6. The results indicate no inhibition of the amplification reactions by components of the DNA preparation.

## DISCUSSION

The procedure outlined here allows extraction of high molecular weight DNA from a wide diversity of marine macroalgae. The method is rapid and economical, utilizing only a few microfuge tubes per algal

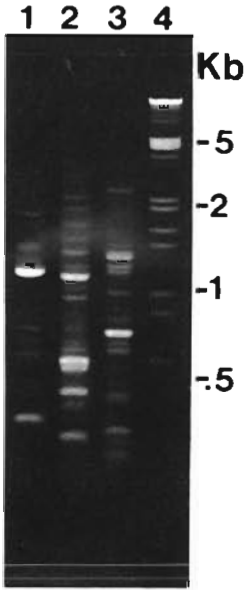


Fig. 6. Fingerprinting algal genomes using PCR and an arbitary sequence primer. Lanes: 1, *Porphyra torta*; 2, *Petalonia debilis*; 3, *Nereocystis luetkeana*; 4, molecular weight markers

sample from beginning to end of the procedure. The DNA yields obtained appear generally higher than those obtained with ultracentrifugation methods (e.g. 1 ng $mg^{-1}$: Fain et al. 1988; 20 ng $mg^{-1}$: Roell & Morse 1991).

Despite the wide diversity of potentially enzyme-inhibiting, secondary compounds found in red, brown and green seaweeds, the method appears to have general applicability, yielding DNA of sufficient purity for enzymatic manipulations used most commonly in molecular biological studies. Our inability to extract undegraded DNA from *Ulva* sp., and the articulated coralline alga *Bossiella* sp., even after repeated attempts with both fresh and frozen tissue, may reflect high nuclease activities in these algae. High levels of nuclease activity have also been found in leaves of wheat and maize (Jones & Boffey 1984). DNA degradation may also have occurred in *Bossiella* sp., due to the extensive grinding required to break open the calcified cells. Alternative methods of tissue grinding, coupled with the addition of higher concentrations of EDTA and/or extra organic-phase extractions, might result in isolation of higher quality DNA from such algae.

The ability to rapidly isolate restrictable and clonable DNA from macroalgae should facilitate studies on the genetics, population biology, systematics and evolution of seaweeds. The utility of the DNAs isolated here for detecting genetic differences among algal populations is illustrated by the discovery of RFLPs among *Nereocystis luetkeana* populations separated by relatively short geographic distances (i.e. the north Seattle population is about 53 and 64 km north of the Vashon Island and Tacoma Narrows populations, respectively).

The difference in rDNA hybridization patterns between blade and stipe tissues of *Nereocystis luetkeana* was unexpected, and warrants some comment. These differences might result from the presence of endophytic algae that occur preferentially on the stipe. Alternatively, we speculate that such differences could also occur as a result of underrepresentation, or loss of some rRNA genes in the blade tissues. Such an occurrence has been described in several higher plants (Grisvard & Tuffet-Anghileri 1980, Cullis 1986, Rogers & Bendich 1987a, b).

Our study also demonstrates the utility of using yeast ribosomal RNA genes as probes for detecting RFLPs in all 3 macroalgal divisions. Plants contain multiple copies of ribosomal RNA genes, usually arranged as tandemly repeated units separated by regions (intergenic spacers) of variable length and DNA sequence (Rogers & Bendich 1987a). The rapid evolution of intergenic spacer regions is indicated by changes in DNA sequence and restriction enzyme recognition sites, thus providing a readily detectable source of genetic variation (polymorphisms) between species, populations, and in some cases individual plants (Appels & Dvorak 1982, Rogers & Bendich 1987a). Because of the highly conserved nature of eukaryotic ribosomal RNA genes, such genes from other organisms can be used as probes to detect RFLPs in the macroalgae. Bhattacharya & Druehl (1989) and Bhattacharya et al. (1990) have demonstrated the utilty of a nematode ribosomal DNA probe to detect genetic differences among populations of the kelp *Costaria costata*. Species differences are readily detectable within the genus *Porphyra* when yeast ribosomal genes are used as the probe (Fig. 4). Such genetic polymorphisms have been found to be useful for resolving taxonomic problems in the phenotypically plastic macroalgae (Goff & Coleman 1988).

The utility of the isolated algal DNAs for use in PCR studies is demonstrated by the successful amplification of DNA segments using a primer of arbitrary sequence. Amplification using short, arbitrary sequence primers has been shown to be useful for detecting genetic variation among higher plant cultivars (Gustavo et al. 1991). This technique may also prove useful for detecting strain and population differences in the macroalgae.

In conclusion, the DNA isolation method described yields DNA of sufficient purity for use in a variety of molecular biological studies, and is of general applicability for isolation of DNA from diverse red, brown, and green macroalgae. The method also has the advantages of being simple, rapid, inexpensive, and only requiring a small amount of algal tissue.

*Acknowledgements.* We thank L. Geselbracht for assistance in field collection of the seaweeds, E. Duffield for the laboratory cultures, and J. Stiller for performing the PCR amplifications. This work was supported in part by the AK Foundation, NSERC (Canada), Tetra Tech., Inc., Washington Sea Grant Program, and the Egtvedt Food Research Fund.

## LITERATURE CITED

Appels, R., Dvorak, J (1982). The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. Theor. appl. Genet. 63: 337–348

Bell, G. I., DeGennaro, L. J., Gelfand, D. H., Bishop, R. J., Valenzuela, P., Rutter, W. J. (1977). Ribosomal RNA genes of *Saccharomyces cerevisiae*. J. biol. Chem. 252(22): 8118–8125

Bhattacharya, D., Baillie, D. L., Druehl, L. D. (1990). Population analysis of the kelp *Costaria costata* (Phaeophyta) using a polymorphic ribosomal DNA probe. Pl. Syst. Evol. 170: 177–191

Bhattacharya, D., Druehl, L. D. (1989). Morphological and DNA sequence variation in the kelp *Costaria costata* (Phaeophyta). Mar. Biol. 102: 15–23

Cullis, C. A. (1986). Plant DNA variation and stress. In: Gustafson, J. P., Stebbins, G. L., Ayala, F. J. (eds.) Genetics, development and evolution. Plenum Publishing, New York, p. 143–155

Fain, S. R., Druehl, L. D., Baillie, D. L. (1988). Repeat and single copy sequences are differentially conserved in the evolution of kelp chloroplast DNA. J. Phycol. 24: 292–302

Feinberg, A. P., Vogelstein, B. (1983). A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Analyt. Biochem. 132: 6–13

Goff, L. J., Coleman, A. W. (1988). The use of plastid DNA restriction endonuclease patterns in delineating red algal species and populations. J. Phycol. 24: 357–368

Grisvard, J., Tuffet-Anghileri, A. (1980). Variations in the satellite DNA content of *Cucumis melo* in relation to dedifferentiation and hormone concentration. Nucleic Acids Res. 8: 2843–2858

Gustavo, C-A., Bassam, B. J., Gresshoff, P. M. (1991). DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 9: 553–557

Jones, M. C., Boffey, S. A. (1984). Deoxyribonuclease activities of wheat seedlings. FEBS Lett. 174: 215–218

Maniatis, T., Fritsch, E. F., Sambrook, J. (1982). Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, New York

Marsh, J. L., Erfle, M., Wykes, E. (1984). The pIC plasmid and phage vectors with versatile cloning sites for recombinant selection by insertional inactivation. Gene 32: 481–485

McCandless, E. L. (1981). Polysaccharides of the seaweeds. In: Lobban, C. S., Wynne, M. J. (eds.) The biology of seaweeds. Blackwell Scientific Publications, Oxford, p. 559–588

Parsons, T J., Maggs, C. A., Douglas, S. E. (1990). Plastid DNA restriction analysis links the heteromorphic phases of an apomictic red algal life history. J. Phycol. 26: 495–500

Ragan, M. A. (1981). Chemical constituents of seaweeds. In: Lobban, C. S., Wynne, M. J. (eds.) The biology of seaweeds. Blackwell Scientific Publications, Oxford, p. 589–626

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

Rogers, S. O., Bendich, A. J. (1985). Extraction of DNA from milligram amounts of fresh, herbarium and mummified plant tissues. Plant Mol. Biol. 5: 69–76

Rogers, S. O., Bendich, A. J. (1987a). Ribosomal DNA in plants: variability in copy number and in the intergenic spacer. Plant Mol. Biol. 9: 509–520

Rogers, S. O., Bendich, A. J. (1987b). Heritability and variability in ribosomal RNA genes of *Vicia faba*. Genetics 117: 285–295

Shivji, M. S. (1991). Organization of the chloroplast genome in the red alga *Porphyra yezoensis*. Curr. Gen. 19: 49–54

Su, X., Gibor, A. (1988). A method for RNA isolation from marine macroalgae. Analyt. Biochem. 174: 650–657

*This article was submitted to the editor*

*Manuscript first received: February 17, 1992*
*Revised version accepted: June 1, 1992*