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# Optimization of RNA Purification Method from *Ecklonia cava* Kjellman (Laminariales, Phaeophyceae)

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A more rapid and efficient method to extract RNA from *Ecklonia cava* Kjellman (Laminariales, Phaeophyceae) was introduced in this study. Each step of the procedure was evaluated and the optimal concentration of each chemical in the lysis solution was determined. Tissue pulverization with PVPP and β-mercaptoethanol in the lysis solution were not essential for RNA extraction of this species. The highest yield and purity of *E. cava* RNA were obtained by the lysis solution containing 1% CTAB, 1 M NaCl, 0.7% PVP, 10 mM EDTA and 100 mM Tris-Cl (pH 9.0). Approximately 8 μg of RNA was obtained from 200 mg of ground tissue. The ratios of the absorbance at 260 nm and 280 nm were from 1.6 to 1.8 and those of at 230 nm and 260 nm were from 1.8 to 2.0. The extracted RNAs obtained in this study turned out to have a sufficient quality for cDNA synthesis.

**Key Words:** CTAB, *Ecklonia cava*, EDTA, NaCl, PVP, RNA purification

## INTRODUCTION

The nucleic acid extraction from macroalgal tissue has been intricate technique because of the existence of sulfated or carboxylic polysaccharides and polyphenols in the tissues (Su and Gibor 1988). Although various extraction buffers and techniques have been suggested to solve the problems (Mayes *et al.* 1992; Apt *et al.* 1995; Hong *et al.* 1995a; Hong *et al.* 1995b; Kitade *et al.* 1996; Lee and Lee 2003), it still remained to be further improved because of its time consuming procedure, high cost and lack of applicability to various seaweed species. For instances, the RNA isolation method developed by Su and Gibor (1988) consisted three main stages takes three days. The LiCl-guanidium method (Hong *et al.* 1995b) for RNA extraction from a red alga *Porphyra perforata* includes uneconomical DNA digestion step by adding RNase-free DNase I and ribonuclease inhibitor.

The sulfated polysaccharides of brown algae have been known to have various physiological activities. Recently inhibitory effects on HIV-1 reverse transcriptase and HIV-1 integrase were found from *Ecklonia cava* during the screening of antiviral effect from various algal species (Ahn *et al.* 2002, 2004). Here we report an efficient method of RNA purification for this species using appropriate combination of lithium chloride, cetyltrimethylammonium bromide (CTAB), ethylene diamine tetraacetate (EDTA) and polyvinylpyrrolidone (PVP).

## MATERIALS AND METHODS

### Plants

Sporophytes of *Ecklonia cava* were collected from 10 m in depth at Seogwipo, Jeju, Korea, and kept in seawater and transported to the laboratory. Thalli of the plant were cut into pieces about 3 cm x 5 cm and quickly frozen in liquid nitrogen and stored at -80°C.

### Total RNA isolation

Algal tissues were mortar-pulverized in liquid nitrogen. The PVPP was added to the algal powder in the final stage of grinding. Approximately 200 mg of algal powder were homogenized in 750 μl of lysis solution [2% CTAB, 20 mM EDTA, 1.4 M NaCl, 1% PVP, 100 mM Tris-Cl (pH 9.0), 0.2% β-mercaptoethanol, as a

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**Table 1.** Ratios of absorbance indices and RNA concentrations purified in four independent conditions

| | $A_{260}/A_{230}$ | $A_{260}/A_{280}$ | RNA Conc. (ng/$\mu l$) |
|---|---|---|---|
| Control | 1.07 ± 0.097 | 1.93 ± 0.098 | 74.7 ± 27.74 |
| w/o PVPP | 1.36 ± 0.371 | 1.91 ± 0.024 | 150.7 ± 19.01 |
| w/o $\beta$-mercaptoethanol | 1.33 ± 0.031 | 1.86 ± 0.166 | 94.7 ± 5.03 |
| w/o Incubation at 65°C | 0.86 ± 0.016 | 1.55 ± 0.106 | 85.3 ± 28.45 |

trial version] and incubated at 65°C for 5 min. The supernatant was retained after centrifugation at 14,000 rpm for 10 min and added the same volume of water-saturated phenol and 1/3 volume of chloroform and centrifuged at 14,000 rpm for 10 min. The aqueous phase was retained and mixed with 1/3 volume of 8 M LiCl and kept at 4°C for 2 h. RNA was precipitated after centrifugation at 14,000 rpm for 30 min. The precipitate was resuspended in 300 $\mu l$ of DEPC-treated water. RNA was precipitated with 1/10 volume of 3 M sodium acetate (pH 5.2) and the same volume of isopropanol. The precipitated RNA was rinsed with 70% ethanol (diluted in DEPC-treated water) and dissolved in an appropriate volume of DEPC-treated water and kept at -20°C.

**Gel electrophoresis and RNA visualization**

Total RNA was separated and resolved through 1.0% formaldehyde-denatured agarose gel (Sambrook and Russell 2001). After electrophoresis, the gel was stained in methylene blue (0.04% in 0.5 M sodium acetate, pH 5.2) or ethidium bromide (0.5 $\mu$g/ml) and destained to detect 28S and 18S RNA. An alternative way to visualize two major ribosomal RNAs could be done by adding 1 $\mu l$ of ethidium bromide to the RNA sample prior to loading on the agarose gel.

**Optimization of RNA extraction condition**

The composition and concentration (CTAB, EDTA, NaCl and PVP) of lysis solution were evaluated and optimized. Each step mentioned in 'Total RNA isolation' was tested independently single chemical at a time to criticize that was critical for the RNA purification.

**RNA quantification and qualification**

An ultraviolet spectrophotometer (GeneQuant pro, Pharmacia) was used for quantifying and qualifying the isolated RNA. RNA concentration was estimated by measuring the absorbance at 260 nm. Protein or polyphenolic contamination during RNA preparation was presumed by the ratio of the absorbance at 260 nm and 280 nm ($A_{260}/A_{280}$, Sambrook and Russell 2001), and 260 nm and 230 nm ($A_{260}/A_{230}$, Su and Gibor 1988), respectively.

**Fig. 1.** Total RNA extracted from *Ecklonia cava* on 1% formaldehyde-denatured agarose gel under three conditions. C, control; 1, algal tissue was pulverized without PVPP; 2, $\beta$-mercaptoethanol was not added to lysis solution; 3, incubation at 65°C was omitted.

## RESULTS

The necessity of PVPP in tissue pulverization, $\beta$-mercaptoethanol in lysis solution and the homogenate incubation at 65°C was evaluated independently (Fig. 1). The purity and the concentration of each RNA extract were calculated and compared in Table 1. RNA concentration at the final stage was relatively higher when PVPP was not added to the algal powder. It was assumed that the algal powder without PVPP would contain more amount of true algal tissue per unit weight of the raw material than the algal powder with PVPP. Thus, the polyphenol content based on the ratio of the absorbance at 260 nm and 230 nm ($A_{260}/A_{230}$) was slightly higher in the PVPP added sample. With these two results, PVPP addition in the pulverization of algal tissue seemed unnecessary to enhance the RNA purity. The addition of $\beta$-mercaptoethanol made no difference in *E. cava* RNA extraction when we compared the optical densities of extracted RNA. Therefore in the subsequent experiments, PVPP addition was omitted from tissue pulverization and $\beta$-mercaptoethanol excluded from the buffer composition. On the other hand, the incubation at 65°C appeared to be effective to decrease protein and polyphenol contamination. Various concentrations of each component (i.e. CTAB, EDTA, NaCl and PVP) of the buffer were also examined. CTAB was tested at various concentration levels from 0.5 to 5.0% (Fig. 2). The

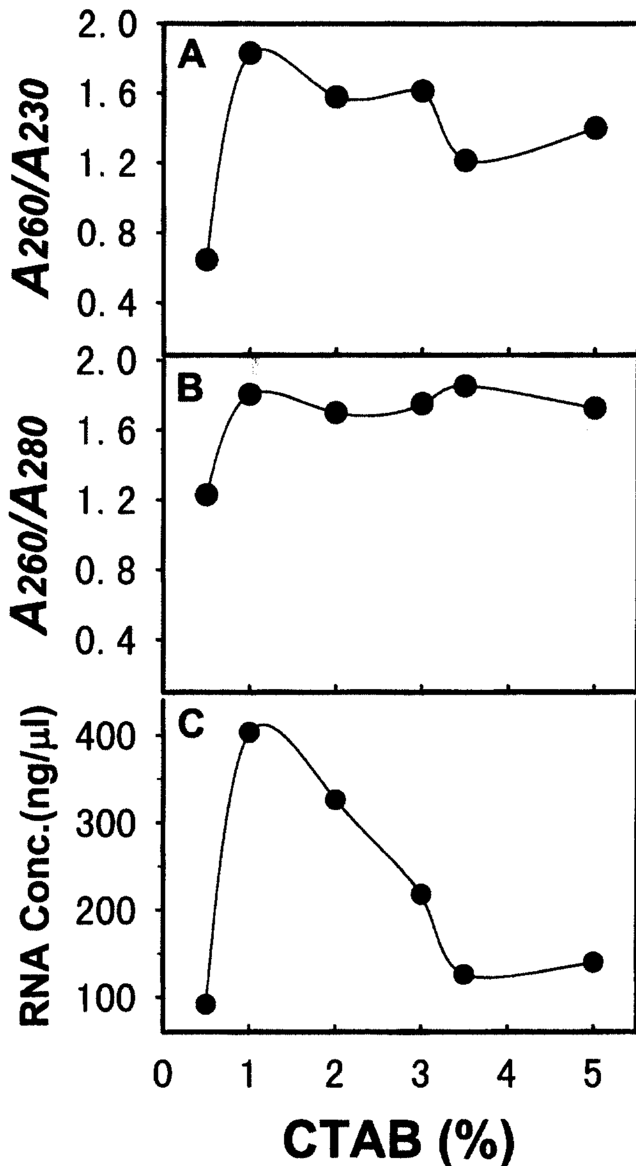


**Fig. 2.** Effect of CTAB concentration of lysis solution on RNA purification. A, ratio of absorbance at 230 nm and 260 nm; B, ratio of 260 nm and 280 nm; C, concentration of extracted RNA.

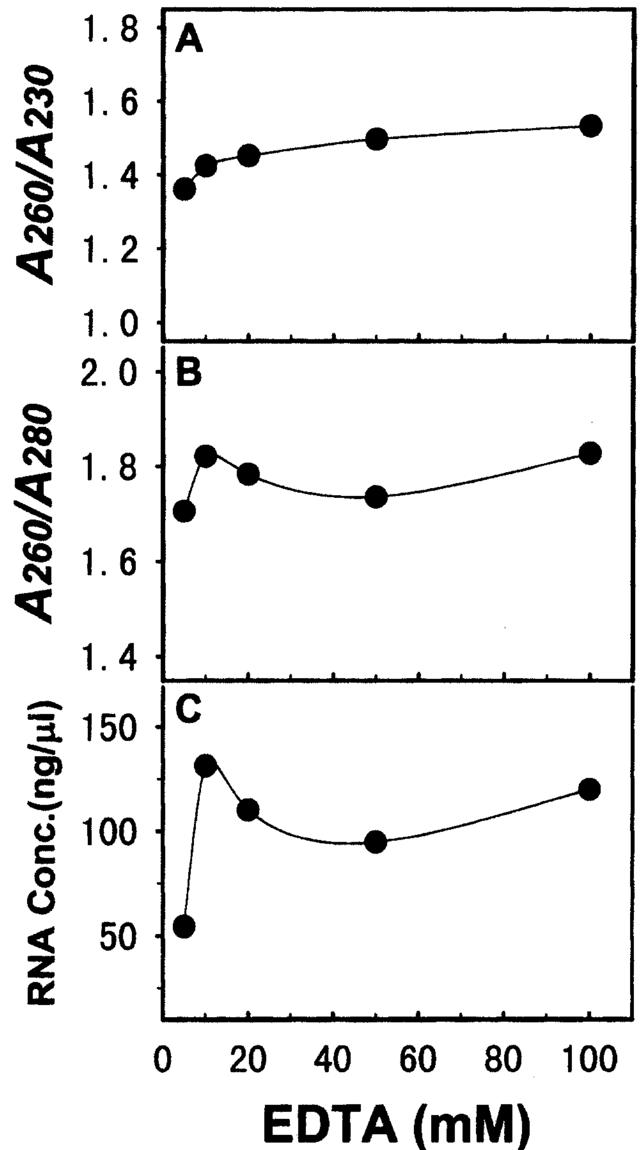


**Fig. 3.** Effect of EDTA concentration of lysis solution on RNA purification. A, ratio of absorbance at 230 nm and 260 nm; B, ratio of 260 nm and 280 nm; C, concentration of extracted RNA.

highest yield of RNA extraction was achieved at 1.0% of CTAB concentration (Fig. 2C). Approximately 400 ng/$\mu l$ of RNA was purified when the extract dissolved in 20 $\mu l$ of DEPC-treated water. In total, 8 $\mu g$ of RNA was obtained from 200 mg of ground tissue. Therefore, RNA yield of current purification method was 40 ng/mg. Polyphenol and protein contents of the extracts were considerably low at 1.0% of CTAB (Fig. 2A and 2B). The effect of EDTA concentrations (5~100 mM) on the purity and concentration of RNA was tested, and the optimum level was found at the concentration of 10 mM (Fig. 3). Regarding to NaCl levels, the largest amount of RNA with high purity was obtained at 1.0 M NaCl (Fig. 4). Finally, on PVP concentrations in the lysis solution, the highest purity and concentration of RNA extracts were obtained at 0.7% PVP (Fig. 5).

## DISCUSSION

We introduce an efficient method for algal RNA purification using thalli of *Ecklonia cava* in this study. The procedural tips described here were also the products of our extensive preliminary experiments, including various kinds of extraction buffers and methods, which

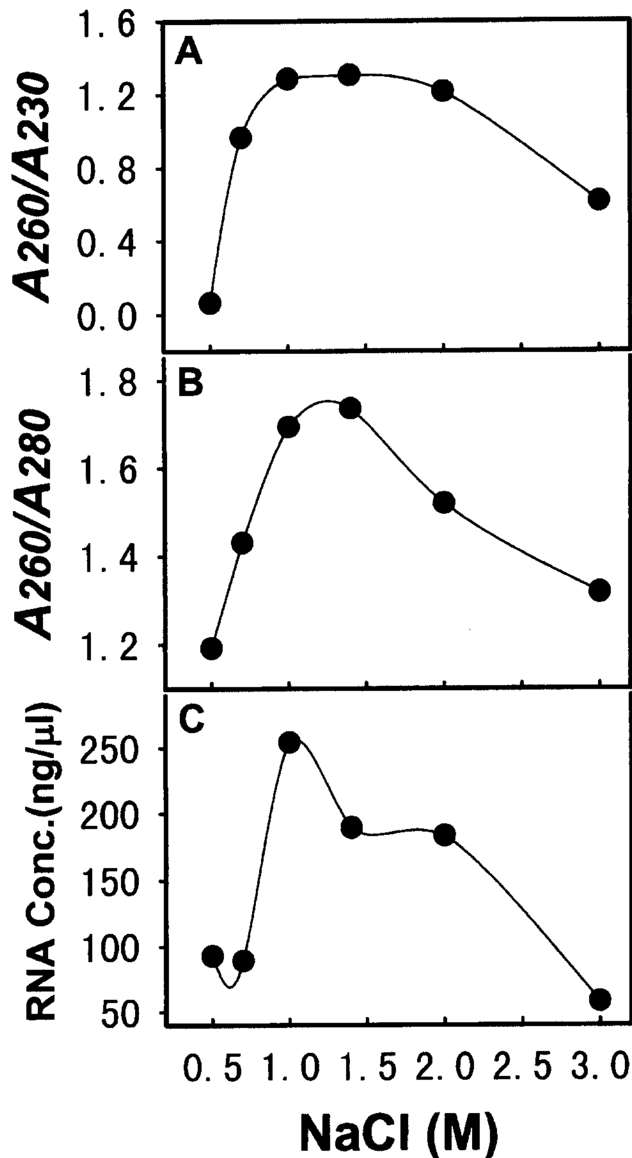


**Fig. 4.** Effect of NaCl concentration of lysis solution on RNA purification. A, ratio of absorbance at 230 nm and 260 nm; B, ratio of 260 nm and 280 nm; C, concentration of extracted RNA.

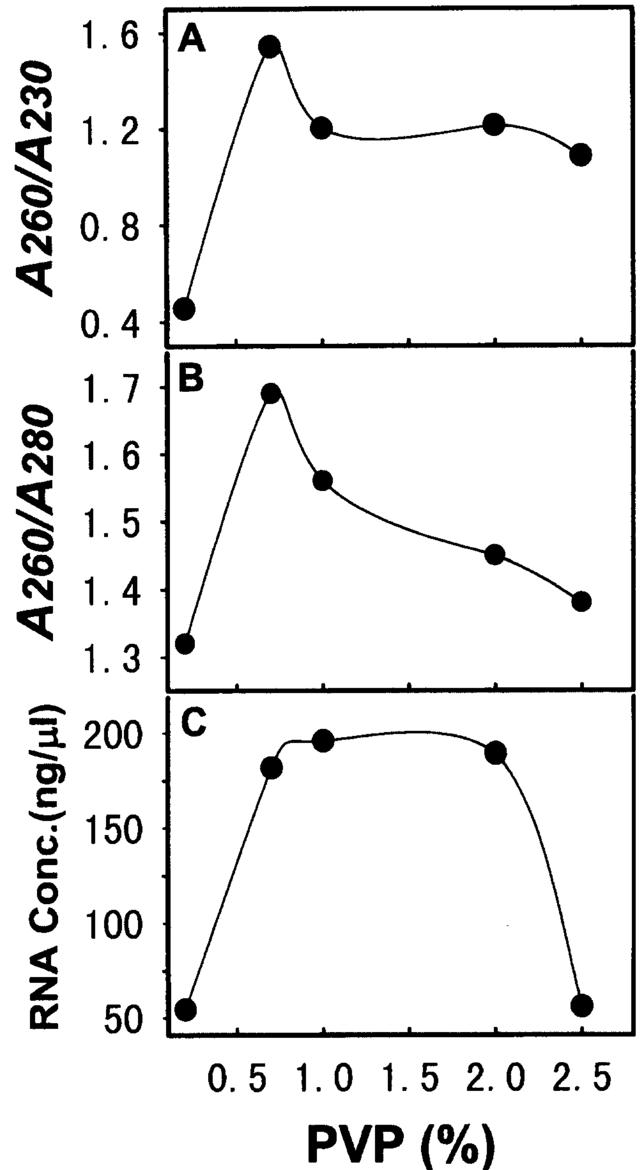


**Fig. 5.** Effect of PVP concentration of lysis solution on RNA purification. A, ratio of absorbance at 230 nm and 260 nm; B, ratio of 260 nm and 280 nm; C, concentration of extracted RNA.

have already been developed for nucleic acids extraction, mostly for macroalgal DNA. However, most previous methods were not worked properly for RNA purification of this species. For examples, SDS or guanidium-HCl containing (Su and Gibor 1988) and guanidium thiocyanate and PVPP containing extraction buffer (Hong *et al.* 1995b) were not effective for RNA purification of this species. Moreover, even a method for nucleic acids isolation from a brown alga *Macrocystis pyrifera* (Apt *et al.* 1995) was not applicable for *E. cava* RNA. RNA with a considerable purity and concentration is successfully extracted by the lysis solution including CTAB, EDTA, NaCl and PVP specifically. After deciding effective contents of the RNA extraction buffer, we have tried to optimize concentration of each component, subsequently. RNA extraction performed with various concentrations of each chemical (CTAB: 0.5, 1, 2, 3, 3.5 and 4%; EDTA: 5, 10, 20, 50 and 100 mM; NaCl: 0.5, 0.7, 1.0, 1.4, 2.0 and 3.0 M; PVP: 0.2, 0.7, 1.0, 2.0 and 2.5%). Based on the values of optical densities, purity and concentration of extracted RNAs of each condition were compared. In conclusion, optimal concentration of each component for *E. cava* RNA extraction buffer was 1% CTAB, 1 M NaCl, 0.7% PVP, 10 mM EDTA, 100 mM Tris-

Cl (pH 9.0). With this buffer, a value of $A_{260}/A_{230}$ indicating degree of polyphenol contamination could be achieved 1.8~2.0 and a value of $A_{260}/A_{280}$ indicating degree of protein contamination could be achieved 1.6~1.8. It is believed that the extracted RNA with these values reaches at a sufficient purity level to proceed on further experiments, such as mRNA isolation and/or first strand cDNA synthesis. We have been constructing a considerable titer of cDNA libraries using the RNA extracted by this current method (data not shown).

Study with RNA opens special interests in expressed gene analyses. The establishment of good purity and enough amount of RNA can make many of such works possible; analyses of tissue specific genes, life stage specific genes, and non-biological and biological stress responsive genes. Recently, extensive expressed gene profiling approaches, namely EST (expressed sequence tag) projects, have been initiated in various model systems, such as crops and domestic animals, to understand the organisms at the molecular level and to secure useful genes of special interests in applied biology. However such studies on macroalgae are far left behind or under the progress in very limited ranges, because RNA purification are extremely difficult due to the existence of polysaccharides and polyphenols, even though they possess supreme importance as primary producers in marine ecosystem. In this study, we established the RNA purification condition from *E. cava*. The method introduced for RNA purification is relatively easy to manipulate and can be applicable to other algal species that have been difficult in RNA purification with previous methods.

## REFERENCES


Ahn M.-J., Yoon K.-D., Kim C.Y., Min S.-Y., Kim Y.-U., Kim H.J., Kim J.H., Shin C.-G., Lee C.-K., Kim T.G., Kim S.H., Huh H. and Kim J. 2002. Inhibition of HIV-1 reverse transcriptase and HIV-1 invertase and antiviral activity of Korean seaweed extracts. *J. Appl. Phycol.* **14:** 325-329.

Ahn M.-J., Yoon K.-D., Min S.-Y., Lee J.S., Kim J.H., Kim T.G., Kim S.H., Kim, N.-G. and Kim, J. 2004. Inhibition of HIV-1 reverse transcriptase and protease by phlorotannins from a brown alga, *Ecklonia cava*. *Biol. Pharm. Bull.* **27:** 544-547.

Apt K.E., Clendennen S.K., Powers D.A. and Grossman A.R. 1995. The gene family encoding the fucoxanthin chlorophyll proteins from the brown alga *Macrocystis pyrifera*. *Mol. Gen. Genet.* **246:** 455-464.

Hong Y.-K., Kim S.-D., Polne-Fuller M. and Gibor A. 1995a. DNA extraction conditions from *Porphyra perforata* using LiCl. *J. Appl. Phycol.* **7:** 101-107.

Hong Y.-K., Sohn C.H. Polne-Fuller M. and Gibor A. 1995b. Differential display of tissue-specific messenger RNAs in *Porphyra perforata* (Rhodophyta) thallus. *J. Phycol.* **31:** 640-643.

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.

Lee Y.K. and Lee H.K. 2003. A simple method for DNA extraction from red algae. *Algae* **18:** 65-69.

Mayes C., Saunders G.W., Tan I.H. and Druehl L.D. 1992. DNA extraction methods for kelp (Laminariales) tissue. *J. Phycol.* **28:** 712-716.

Sambrook J. and Russell D.W. 2001. Moclecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor Laboratory Press, New York.

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


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