Journal of Biological Research **3**: 109 – 111, 2005
J. Biol. Res. is available online at http://www.jbr.gr

First protocol for DNA isolation in Indian charophyta

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Received: 3 November 2004 Accepted after revision: 10 March 2005

A protocol for the isolation of genomic DNA from two Indian charophyta (genera *Chara* and *Nitella*) has been developed for the first time, in order to clarify the profuse biodiversity and aid the molecular characterization of the studied genera.

Key words: Charophyta, DNA, protocol, *Chara, Nitella*, molecular characterization.

INTRODUCTION

Charophyta is a highly diversified assemblage of six macrophytic algal genera, namely *Chara, Nitella, Tolypella, Lychnothamnus, Lamprothamnium* and *Nitellopsis*. Several attempts have been made to resolve the biodiversity of this group on the basis of morphology, sexual dimorphism, cytotaxonomy, palaeobiogeography and fossil history (Sundaralingam, 2002). Bhatnagar *et al*. (1996) tried to resolve charophycean biodiversity and trace the phylogeny of this group on the basis of the Giemsa C-banding technique. In view of the revision of Characeae by Wood & Imahori (1965), in which the nomenclature of many taxa was altered without adequate experimental verification, molecular characterization of charophyta taxa became inevitable.

Several researchers have isolated DNA from various algal species (Bancroft & Smith, 1988; Dutcher & Kapraun, 1994; Alberto *et al*., 1997; Satoh *et al*., 1997; Winkler *et al*., 2002). Among charophyta, although the isolation of cDNA from *Chara corallina* (Nakanishi *et al*., 1999) and mitochondrial DNA from *Chara vulgaris* (Turmel *et al*., 2003) are on record, protocols for genomic DNA isolation from Indian Charophyta are not yet available. Hence, this study is aimed at developing an efficient protocol for DNA isolation from Indian charophyta.

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MATERIALS AND METHODS

The CTAB (cetyltrimethylammonium bromide) method has been proposed by Rogers & Bendich (1988) for the extraction of genomic DNA in plants. However, in Indian charophyta, intact genomic DNA could not be isolated by this method. This and other protocols provided by Sambrook *et al*. (1989) were taken into consideration in order to optimize an extraction protocol for charophyta. Seven species of charophyta, 4 from the genus *Chara* and 3 from the genus *Nitella*, were used in this study.

Preparation of samples for DNA isolation

The epiphyte-free charophyta taxa were initially bleached for over seven days and soaked on tissue paper for 15 min. An amount of 0.5 g of dried plant material was subsequently grinded with a pestle in a pre-cooled mortar containing 5 ml of liquid nitrogen until a fine powder of plant material was obtained.

The crushed plant material was then transferred into a microfuge tube and 1 ml of extraction buffer (100 mM Tris, 100 mM EDTA, 250 mM NaCl) was added. This was kept at room temperature for 30 min and then 50 μl of 10% sodium dodecyl sulphate (SDS), an anionic detergent, were added to remove the lipid molecules and disrupt the cell membranes. After 10 min, 4 μl of proteinase K (25 mg ml^{-1}) were added to degrade the DNA-associated proteins. This mixture was thoroughly stirred for 2 min and incubated for 16 h at 37°C in a water bath. DNA was isolated using the optimized phenol extraction method, as described by Sambrook *et al*. (1989).

Protocol for DNA isolation and purification

The incubated samples were mixed thoroughly with an equal volume of Tris-saturated phenol, which separates denatured proteins leaving the nucleic acid in an aqueous phase. The samples were centrifuged at 5000 rpm for 5 min at 4°C. The aqueous phase was collected in a fresh microfuge tube and this step was repeated twice. It was then extracted with a solution of phenol: chloroform: isoamyl alcohol (25:24:1) by thorough mixing and centrifuging at 5000 rpm for 5 min at 4°C. The aqueous phase was collected again in a fresh tube. The latter step was repeated twice.

A solution of chloroform: isoamyl alcohol (24:1) was added in the aqueous phase and mixed well. It was then centrifuged at 5000 rpm for 5 min at 4°C and the upper aqueous phase was collected. This step was also repeated twice. The chloroform-isoamyl alcohol solution was used in order to remove impurities and traces of phenol.

Water saturated ether was added to the aqueous phase in order to remove traces of chloroform and isoamyl alcohol. It was mixed thoroughly and centrifuged at 5000 rpm for 5 min at 4°C. The upper layer of ether was removed and traces of it were allowed to evaporate by keeping samples at room temperature for 1-2 h. The lower layer, which contained DNA in an aqueous form, was precipitated by adding 2-2.5 volumes of ice-cold absolute ethanol. It was kept overnight at -20°C for complete precipitation. DNA can be stored for many months in alcohol at -20°C.

Preparation of DNA solutions

The precipitated DNA was pelleted by centrifuging it at 10000 rpm for 10 min at 4°C, followed by two washes with 1 ml of 80% ethanol. The pellet was detached from the wall by tapping of the tube. It was then centrifuged at 10000 rpm for 10 min. The ethanol was carefully discarded and the DNA was either air-dried or kept in a desiccator for 1-2 h. The pelleted DNA was dissolved in approximately 500 μl of autoclaved double distilled water (ddH_2O) and stored at 4°C. After a period of 3-4 days the DNA was completely dissolved.

RESULTS AND DISCUSSION

This protocol was applied on 7 species, 4 belonging to the genus *Chara* and 3 belonging to the genus *Nitella* (Table 1). The quality of the extracted genomic DNA was tested by agarose gel electrophoresis while its purity and concentration by UV-VIS spectrophotometry.

Quality evaluation of isolated genomic DNA

The isolated DNA was checked for quality by agarose (0.7%) gel electrophoresis. The amount of the DNA per sample loaded in each gel was 12 μl (6 μl genomic DNA, 2 μl bromophenol blue and 4 μl autoclaved ddH_2O). To visualize the DNA, the gels were stained with ethidium bromide and observed under UV light. Strong bands indicated that good quality, unsheared DNA had been extracted (Fig. 1).

Estimation of DNA purity and concentration

Spectrophotometry was used to check the purity and concentration of DNA. The purity of DNA was checked through its absorbance at the wave lengths of 260 and 280 nm (nucleic acids absorb maximum incident radiation at 260 nm while proteins absorb at 280 nm). The OD 260:280 ratio ranged between 1.41 and 2.05 (Table 1) which revealed satisfactory DNA purity for PCR-based applications. In general, an $OD_{260/280}$ ratio of 1.7-1.9 is considered good for Restriction Endonuclease (RE) digestion-based studies. The concentration of DNA was calculated using the following formula:

TABLE 1. Spectrophotometric test of purity and concentration of isolated DNA

Sample	Taxon	OD_{260}	OD_{280}	$OD_{260/280}$	DNA Conc. (μg ml^{-1})
1	*Chara erythrogyna*	0.0167	0.0086	1.94	0.501
2	*Chara socotrensis*	0.0442	0.0263	1.68	1.326
3	*Chara wallichii*	0.0164	0.0094	1.74	0.492
4	*Chara braunii*	0.01	0.0055	1.82	0.3
5	*Nitella furcata*	0.0465	0.0329	1.41	1.395
6	*Nitella microcarpa*	0.014	0.01	1.40	0.42
7	*Nitella roxburghii*	0.0168	0.0082	2.05	0.504

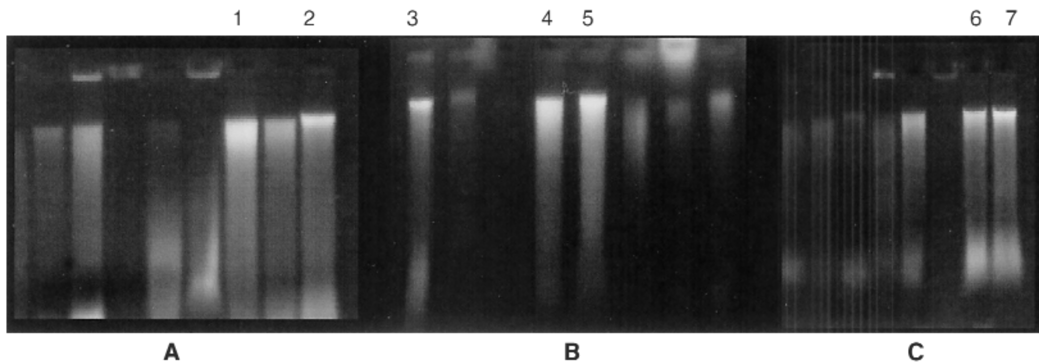

FIG. 1. Agarose gels (A, B, C) showing genomic DNA and its quality in seven samples of charophyta marked as 1, 2, 3, 4, 5, 6 and 7. Other samples showed sheared DNA.

$$\text{Conc. } (\mu g\ ml^{-1}) = OD_{260} \times 50 \times \text{dilution factor}$$

ACKNOWLEDGEMENTS

S.K.B. is grateful to the University Grants Commission, New Delhi for providing an Indo-Greek visiting scientist fellowship.

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