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A Simple Procedure for DNA Isolation Using Small Quantities of Lichen Thallus

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A simple protocol for DNA isolation from lichen thalli using small quantities (2 × 2 mm^2) of samples is presented. The abundant polysaccharides and secondary metabolites present in a lichen thallus interfere with not only DNA isolation but also PCR amplification. The isolation procedure from the lichen thallus consists of the following short steps: (1) a fine-grind in a microcentrifuge tube; (2) extraction of ground tissue with the simple extraction buffer; (3) chloroform or CIA (chloroform and isoamylalcohol) extraction to remove impurities; and (4) adsorption of DNA with glass wool. DNA was successfully isolated from species of *Sticta*, *Lobaria*, *Nephroma*, and *Pseudocyphellaria*. DNA samples prepared by the present method were consistently amplifiable in LA-PCR as well as standard PCR reactions. This protocol also can be applicable for DNA isolation from other plant groups such as fungi, filamentous green algae and bryophytes.

Key words: lichens, fungi, green algae, bryophytes, DNA isolation, protocol, small quantities

Introduction

In many previous studies, DNA has been isolated from lichen thalli using the CTAB method (Murray & Thompson 1980) or commercially available extraction kits modified for lichens. The abundant polysaccharides and secondary metabolites present in lichen thalli interfere with not only DNA isolation but also PCR amplification. DNA extracts from lichens, using the CTAB method, are rich in polysaccharides, and yield poor templates for amplification using the polymerase chain reaction (PCR). Although manufactured extraction kits are convenient, their cost becomes prohibitive when examining multiple or large samples. Alternative methods (e.g., Tel-Zur *et al.* 1999) are not always suitable for lichens when limited to small amounts of sample. A protocol for DNA isolation using small quantities of material from very small fragments of bryophytes has already been designed by Tsubota *et al.* (2002). We have made some modifications for lichen material, and present here a simple protocol for DNA isolation with low levels of impurities using small quantities of lichen thallus and a DNA-glass binding interaction.

Materials and Methods

Reagents and solutions

- Extraction buffer: TE buffer (10 mM Tris pH 8.0, 1 mM EDTA pH 8.0) or TE20 buffer (50 mM Tris pH 8.0, 20 mM EDTA pH 8.0) [Note 1].
- Chloroform (100%) or chloroform : isoamyl alcohol (CIA; 24 : 1 v/v) [Note 2].
- Sodium acetate solution (3 M adjusted to pH 5.2).
- Ethanol (100%) or isopropanol (100%).
- Ethanol (70 75%).
- Glass wool.

Protocol

This protocol is for DNA isolation using small quantities of materials, and a modification of the phenol-chloroform method devised by Tsubota *et al.* (1999, 2002).

The method consists of the following steps:

1. Pick up a small amount of thallus (ca 2 × 2 mm^2) under a dissecting microscope [Note 3].
2. Grind the thallus with 30 µl extraction buffer (TE or TE20 buffer; see recipe, above) using a polypropylene pestle (Bel-Art Products, Pequannock) in a 1.5 ml microcentrifuge tube.
3. Add 170 µl of extraction buffer and mix gently [Note 4, 5].
4. Add a half volume of CIA (chloroform : isoamylalcohol = 24 : 1) and mix gently by brief shaking for 2 5 min.
5. Centrifuge the tubes at 20,000 × g for 5 min at 4°C.
6. Transfer the aqueous upper phase to a new 1.5 ml microcentrifuge tube with some glass wool (10 30 mg) [Note 6].
7. Extract with 2.5-volume of cold absolute ethanol and 20 µl of 3M sodium acetate solution and mix gently.
8. Place on ice for 5 min and decant the supernatant.
9. Rinse the glass wool with cold 70% ethanol [Note 7].
10. Decant the ethanol and dry the glass wool under incubation in a heat block at 65°C for ca 10 minutes.
11. Resuspend the DNA into 1/2 TE buffer and incubate at 65°C for 5 minutes.
12. Transfer the DNA suspension to a new 1.5 ml microcentrifuge tube and cool down quickly at frozen [Note 8, 9].

Notes:

1. TE20 buffer has some advantages in buffering capacity.
2. No preference.
3. Samples larger than 2 × 2 mm^2 are not suitable for this procedure.
4. A total amount of the solution should be added up to ca 200 µl.
5. At this stage the procedure may be stopped and the solution may be stored frozen for extended periods.
6. Keep the glass wool at the bottom of the microcentrifuge tube to avoid losing the DNA adsorbed on the wool.
7. The DNA is adsorbed on the glass wool.
8. At this stage, keep the suspension at near 65°C so as not to re-adsorb the DNA with glass wool.
9. Quicker cooling down is better to minimise the activity of DNA polymerase.

Results and Discussion

Key elements of the new extraction protocol can be summarized as follows:

- DNA isolation from small quantities of lichen thallus is possible utilizing the following short steps: (1) a fine-grind in a microcentrifuge tube; (2) extraction of ground tissue with a simple extraction buffer; (3) chloroform or CIA (chloroform and isoamylalcohol) extraction to remove impurities; and (4) adsorption of DNA with glass wool.
- All the stages can be completed within one hour. It is possible to design efficient experiment plans for DNA isolation utilizing a small centrifuge. This procedure can be successfully carried out even using a hand centrifuge or a small size desktop centrifuge.
- The key steps in this new protocol are (1) the fine-grind of the thallus with extraction buffer; (2) the rapid transfer of the DNA suspension to a new tube keeping it at or near 65°C so as not to re-adsorb the DNA with glass wool; and (3) rapid cooling to minimize the activity of DNA polymerase.
- DNA can be successfully isolated from not only lichen species — we used the genera *Sticta*, *Lobaria*, *Nephroma*, and *Pseudocyphellaria*, — but also from other plant

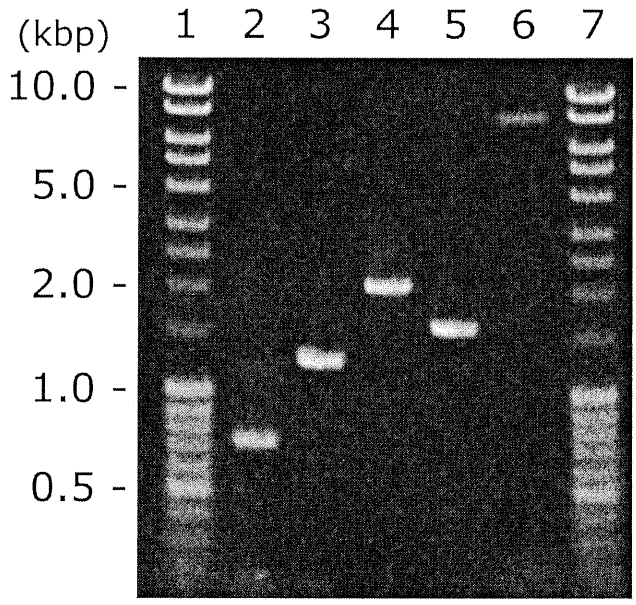

Fig. 1. DNA bands of PCR products from different organisms on a 1.0% agarose gel. Lane 1, 7: Size marker (100 bp DNA Ladder). Lane 2: *Sticta gracilis* (nrITS region). Lane 3: *Lentinula edodes* (nrITS region). Lane 4: *Trentepohlia aurea* (18S rDNA region; nested PCR). Lane 5: *Conocephalum japonicum* (chloroplast *rbcL* region). Lane 6: *Haplomitrium mnioides* (chloroplast *rps4-rbcL* region; ca. 7.5 kbp by LA-PCR).

groups, including macrofungi — such as the basidiomycete Shiitake mushroom, *Lentinula edodes*, filamentous green algae (*Trentepohlia* spp.) and bryophytes (small mosses, and thalloid and leafy liverworts).

- In the case of lichens, the use of small quantities of thallus or reproductive/non-reproductive structures of thallus such as apothecia and cephalodia ensures less contamination with other lichens or lichenicolous fungi as well as minimizing residual matter for PCR amplification.
- DNA samples prepared by the present method were consistently amplifiable in LA-PCR (Long and Accurate PCR) as well as standard PCR reactions (Fig. 1).

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坪田博美 [1)]・高橋奏恵 [1)]・中原美保 [1)]・H. Mohamed [2)]・出口博則 [1)]. 2005. 少量の地衣体からのDNA簡易抽出法. Lichenology 4(1): 25-28.

少量の地衣体からDNAを抽出する方法を考案した．方法の概要は，少量の地衣体をマイクロチューブ内で粉砕後，抽出バッファーに懸濁し，クロロホルムなどで不純物を取り除いた後，ガラスウールにDNAを吸着させて回収するという手間の少ないものである．この方法でDNA抽出を行った場合，地衣体に含まれる夾雑物の影響をほとんど受けることなく，抽出されたDNAを使ってPCR反応を行うことができた．また，この方法を利用して，地衣類だけでなくシイタケのような菌類やスミレモなどの緑藻類，蘚苔類でもDNAの抽出が可能であった．

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