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Rapid small-scale DNA isolation from filamentous cyanobacteria

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1. SUMMARY

A rapid small-scale DNA isolation procedure is described for the filamentous cyanobacteria, which yields enough chromosomal and plasmid DNA for restriction endonuclease digestions, Southern hybridizations, and electroelution from gels for further manipulation. DNA from seven strains of cyanobacteria were isolated and analyzed on agarose gels.

2. INTRODUCTION

Cyanobacteria are Gram-negative, photosynthetic prokaryotes, with some species capable of fixing atmospheric dinitrogen. There has been increasing interest in the regulation of these and other processes in cyanobacteria at the molecular level. Methods are being developed to transfer cloned DNA to cells [1–7] so that regulation can be studied using molecular genetics.

Although methods for isolating DNA from cyanobacteria have been published [8–11], many are time-consuming and require large amounts of material. Separate isolations for both plasmid and chromosomal DNA are used, as well as different procedures depending on the plasmid size. There are many instances where it is necessary to rapidly obtain DNA for various screenings or other manipulations. In this study, we have isolated both plasmid and chromosomal DNA from a number of filamentous cyanobacteria using a simple lysozyme method which yields chromosomal DNA and plasmids rapidly in one preparation. This method is useful for examining plasmid profiles, for Southern blot hybridizations, and for use in cloning of both plasmids and chromosomal DNA.

3. MATERIALS AND METHODS

3.1. Cyanobacteria strains and growth conditions

Plectonema strains, *P. boryanum* 594, 581, 1566, and *P.* sp. 1541 were purchased from the University of Texas Culture Collection (UTEX). *Oscillatoria tenuis* 1566 and *Phormidium autumunale* 1580 also were obtained from UTEX. Two cultures were obtained from the American Type Culture Collection, the LPP strains 27913 and 29117. Cells were grown in Erlenmeyer flasks containing 50 ml BG-11 liquid medium [12] at 30 °C in an orbital shaker-incubator under fluorescent lights.

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3.2. DNA isolation

Cultures were grown until mid to late log phase and centrifuged for 10 min at 10000 rpm. Pellets were washed in 50 mM Tris-HCl (pH 8.0)–5 mM EDTA–50 mM NaCl and resuspended in 2 ml 50 mM Tris-HCl–50 mM EDTA, pH 8.0. Lysozyme (45 mg/ml stock resuspended in ^2H_2O) was added (1 ml) to give a final concentration of 15 mg/ml. Cells were incubated at 37°C for 1 hour. An equal volume of 30 mM Tris–10 mM EDTA–2% sarkosyl then was added and the tubes were mixed gently. Pretreated Ribonuclease A was immediately added at a final concentration of 10 μg/ml and the lysates were incubated at 37°C for 45 minutes. This was followed by two phenol, two chloroform, and two ether extractions. DNA was precipitated in 2.5 volumes of ethanol in a dry-ice ethanol bath for 15 minutes. Precipitation can be done overnight at −20°C. DNA then was pelleted at 12000 rpm for 15 min in a microfuge. The pellets were washed in 70% ethanol, centrifuged again, and resuspended in 10 mM Tris-HCl (pH 8.0)–1 mM EDTA. Absorbance ratios at $A_{260/280}$ were in range of 1.7–1.9. Up to 100 μg of DNA (or more) were obtained from each isolation.

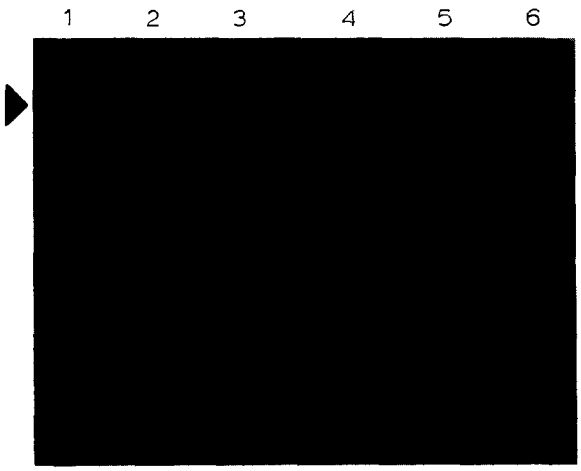

Fig. 1. DNA (1/200 of total sample) from six isolations were run on a 0.8% mini gel. Lane 1, PB581; Lane 2 PB594; Lane 3, OT1566; Lane 4, P1541; Lane 5, 29117; Lane 6, 27913. Both 27913 and 29117 do not contain plasmids. *Plectonema* 581 and 594 contain a 12.9 kb and a 1.6 kb plasmid, although only the small plasmid is seen since the large plasmid is in very low copy number. OT1566 contains two plasmids and P1541 contains at least three plasmids.

Fig. 2. Agarose gel (0.8%) of total *Phormidium autumunale* 1580 DNA. Intact chromosomal DNA and two plasmids (arrows), 16.7 kb and 2.7 kb, are clearly visible.

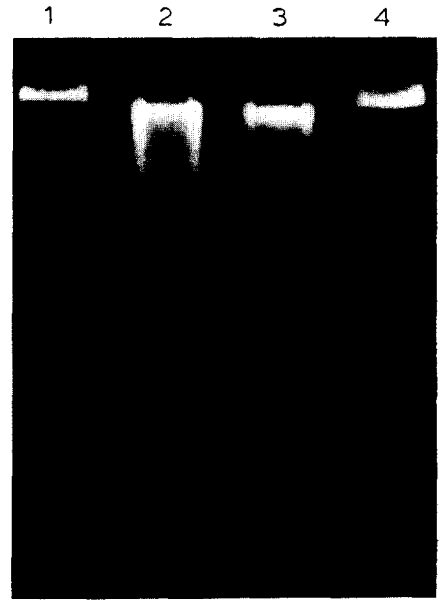

Fig. 3. Mini gel of total DNA from PB581 (Lane 1, uncut; Lane 2, *Cla*I digested), and PB594 (Lane 3, *Cla*I digested; Lane 4, uncut). Plasmid DNA was not visible due to the small amount of DNA loaded onto the gel. The gel has been overstained.

3.3. Restriction endonuclease digestions

Restriction endonucleases were purchased from Promega Biotec and New England Biolabs, Inc. and were used according to manufacturers' specifications.

4. RESULTS AND DISCUSSION

We have obtained high-molecular weight chromosomal DNA from cells that can be lysed with lysozyme using a small-scale, rapid isolation procedure (Fig. 1, Lanes 1–6). Although plasmids are present in very low copy number, they were visualized on gels (Fig. 1, Lanes 1–4; Fig. 2). These profiles have been confirmed using a small-scale plasmid purification procedure [13]. Chromosomal DNA was observed as a discrete, high-molecular weight band; however, upon digestion with a variety of restriction enzymes, the chromosomal DNA band was readily digested into smaller fragments as demonstrated by smearing (Fig. 3, Lanes 2–3; Fig. 4, Lanes 2–4). Although cyanobacterial plasmids are resistant to cleavage by many restriction enzymes, digested plasmid DNAs were visualized as discrete bands (Fig. 4).

Fig. 4. Agarose gel (0.8%) of DNA isolated from *Plectonema* 1541. Lane 1, lambda DNA cut with *Hin*dIII; Lane 2, uncut total DNA; Lane 3, *Cla*I; Lane 4, *Hpa*I; Lane 5, *Fok*I. A small plasmid is visualized in Lanes 2 and 5 (arrow). Larger plasmids are barely detectable in the gel (Lane 2). Digested plasmids are seen in Lanes 3–5 as discrete bands.

This method has the advantage of being rapid and yielding enough DNA (both plasmid and chromosomal) for manipulations such as Southern blot hybridizations, electroelution, cloning, and determination of plasmid profiles, etc. Chromosomal DNA may mask plasmid profiles due to shearing, especially if plasmids are low in copy number, or there is broadening of the DNA band in the gel [10,14,15]. Other methods such as spooling and ultracentrifugation also produce some breakage (unpublished observations). In the method described here, plasmids at least 17 kb in size, are not obscured since the procedure is gentle enough so that chromosomal and plasmid DNAs are not sheared.

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