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A simple and efficient protocol for isolation of high molecular weight DNA from the cyanobacterium *Spirulina platensis*

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An efficient protocol which involved mechanical destruction of cell walls by freeze thawing and removal of polysaccharide by cetyltrimethylammonium bromide precipitation is described for the rapid isolation of high molecular weight (≥ 50 kb) DNA from the *Spirulina platensis*. The DNA yield ranged 1.0—1.54 μg/mg dry wt with the purity measured at $A_{260/280}$ of 1.77–1.82. The DNA preparation was suited as substrate for restriction and PCR.

Introduction

Although methods for isolating DNA from cyanobacteria have been published (Smoker and Barnum, 1988 and references therein; Kallas, 1988; Tiboni *et al.*, 1984), most of them were slight modifications of the procedures designed to be used with *E. coli*. Most common problems encountered when making preparation of DNA from *Spirulina platensis* with these methods are the extraction of insufficient amounts of DNA and high production of polysaccharide contaminants in DNA preparations. The low yields may be attributed to incomplete lysis of the *S. platensis* cells. The spiral-shaped chains of cells (trichomes) of *S. platensis* are enclosed in a thin gelatinous sheath (Van Eykelenburg, 1979; Ciferri and Tiboni, 1985) which can seriously obstruct the action of lysozyme (Peng *et al.*, 1996). In our hands, neither 2 mg lysozyme/ml at 37°C for 30 min (adapted from Tiboni *et al.*, 1984) nor 15 mg lysozyme/ml at 37°C for 1 h (adapted from Smoker and Barnum, 1988) could destroy the cell walls completely (determined by microscopic examination, data not shown). In this study, we broke down the cell walls of *S. platensis* efficiently by combining mechanical destruction by freeze thawing with enzymolysis by lysozyme. The polysaccharides were removed from the DNA preparation with cetyltrimethylammonium bromide (CTAB) (Rogers and Bendich, 1988).

Materials and methods

Organism and growth

An axenic and short filament isolate of strain SP51 of *S. platensis* was grown in flasks containing XS liquid medium (Xu *et al.*, 1997) at 30°C in an orbital shaker under fluorescent lights.

DNA isolation

Steps in the procedure

1. Cultures were grown until $OD_{560} = 0.5 - 1.0$ (ca. 0.4–0.8 mg dry wt/ml) and centrifuged for 10 min at 8,000 *g*. Pellets were washed two times with TES (50 mM Tris, 10 mM EDTA, 50 mM NaCl, pH 8.3) and resuspended in TES.
2. Lysozyme was added to give 2 mg/ml. The mixture was frozen in liquid N_2 for 10 min and incubated for 30 min at 37°C after thawing at 65°C.
3. After the addition of 1% of SDS and of 50 μg proteinase K/ml (both are final concentration), the suspension was again frozen in liquid N_2 for 10 min and incubated for 30 min at 65°C.
4. The lysates were extracted with an equal volume of chloroform/isoamyl alcohol (24:1, v/v).
5. One volume of 2 X CTAB(cetyltrimethylammonium bromide) solution [2% CTAB (w/v), 100 mM Tris (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, 1% PVP (polyvinylpyrrolidone, Mr 40,000), 1% 2-mercaptoethanol] was added to the aqueous phase. The mixture was incubated for 15 min at 65°C after gentle mixing.
6. The chloroform/isoamyl alcohol treatment was repeated once.
7. DNA was precipitated from the aqueous phase by the addition of one volume of CTAB precipitation mix [1% CTAB, 50 mM Tris, 10 mM EDTA, pH 8.0]. The pellets recovered by centrifugation

(10,000 $g \times 10$ min) at room temperature was dissolved in high-salt TE (10 mM Tris, 1 mM EDTA, 1 M NaCl, pH 8.0).

8. The high molecular weight RNA was removed by centrifugation at 10,000 g for 10 min at 4°C after the addition of an equal volume of 5 M LiCl.
9. The supernatant was incubated at 37°C for 20 min and at 65°C for 15 min with 50 μg RNase A/ml (Sigma).
10. The solution was deproteinized with the same volume of phenol/chloroform/isoamyl alcohol (25:24:1, by vol.), and the remaining phenol was removed by extraction with chloroform/isoamyl alcohol (24:1, v/v).
11. DNA was dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) after precipitation with 2/3 volume of isopropanol and being washed with 80% ethanol at room temperature.

Results and discussion

The washing of cells (step 1) is necessary because of the high pH (ca. 9.2–9.4) and salts in the growth medium which may interfere with subsequent reactions. Quick thawing of the frozen at 65°C (both of step 2 and 3) can improve efficiency of mechanical destruction of cells. The contaminants of RNA in DNA preparation can be completely eliminated by using of LiCl together with RNase A (step 8 and 9). The isopropanol precipitation at room temperature instead of ethanol precipitation at low temperature at the last step (step 11) was employed to minimize the co-precipitation of residual trace polysaccharides with DNA. Typically the DNA yield was 1.0–1.54 μg/mg dry wt depending on the isolates. Absorbance ratios of DNA preparations at $A_{260/280}$ were in range of 1.77–1.82. The length of genomic DNA of *S. platensis* isolated with the above protocol was on the order of 50 kb or better (Fig. 1, lane 3). The DNA was free of contaminants that interfere with digestion of restriction endonuclease (Fig. 1, lane 5) and suited as substrate for restriction (Fig. 1, lane 4) and PCR (data not shown).

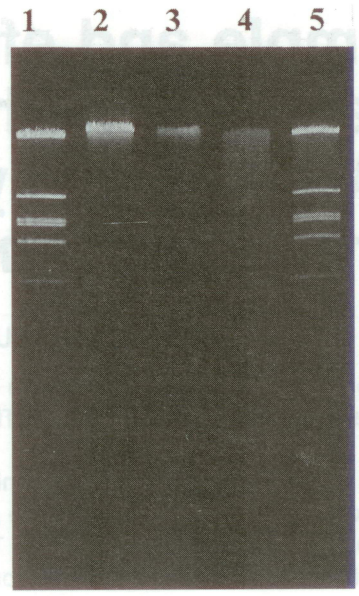

Fig. 1 Electrophoretic analysis (0.7% agarose gel) of DNA isolated from *S. platensis*. About 300 ng λDNA and 200 ng *S. platensis* DNA was loaded respectively. Lane 1, 2: λDNA; lane 3, 4: *S. platensis* DNA; lane 5: λDNA and *S. platensis* DNA mixture; lane 1, 4, 5: cut with *Eco*RI; lane 2, 3: uncut.

References

Ciferri, O. and Tiboni, O. (1985). *Ann. Rev. Microbiol.* 39, 503–526.

Kallas, T. (1988). *Methods Enzymol.* 167, 779–794.

Peng, G., Shi D., Fei, X. and Zeng, C. (1996). *Acta Botanica Sinica* 38, 861–866.

Rogers, S. O. and Bendich, A. J. (1988). Extraction of DNA from plant tissues. In: *Plant Molecular Manual*. S. B. Gelvin, R. A. Schilperoort and D. P. S. Verma, eds. pp. A6: 1–10, Dordrecht: Kluwer Academic Publishers.

Smoker, J. A. and Barnum, S. R. (1988). *FEMS Microbiol. Lett.* 56,119–122.

Tiboni, O., Di Pasquale, G. and Ciferri, O. (1984). *J. Bacteriol.* 159, 407–409.

Van Eykelenburg, C. (1979). *Ant. Leeuwenhoek* 45, 369–390.

Xu, Z., Liu, X., Cao, J., Qiu, G. and Li, B. (1997). *Acta Scientiarum Naturalium Universitatis Sunyatseni* 36 (in press).

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