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ISOLATION OF DNA FROM ASPERGILLUS NIDULANS*

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A procedure for isolation of DNA from Aspergillus nidulans on a preparative scale is described. Mechanical disruption of lyophilized material in high-salt medium and treatment with proteinase K, followed by sedimentation of the lysate into saturated CsCl solution yielded pure, highly polymerized DNA.

The main problems in isolation of highly purified DNA in large quantities from filamentous fungi are: breakage of the cell wall and removal of proteins and RNA without degradation of DNA. Enzymatic digestion of the cell wall has been used to form protoplasts of Aspergillus nidulans (Peterdy & Gibson, 1971). However, Gealt et al. (1976) have been unable to process a sufficient amount of mycelia for biochemical analysis. Mechanical disruption of lyophilized material at a low temperature proved to be satisfactory to break A. nidulans cells in our experiments. Most of the conventional techniques for isolation of purified DNA require enzymatic treatment combined with repeated deproteinization, precipitation and redissolving. However, this leads to degradation and considerable loss of DNA.

This work presents a simple and efficient way for isolation of DNA from A. nidulans avoiding the use of phenol, RNase treatment and ethanol precipitation. For comparison purposes, DNA was isolated by the classical method of Marmur (1963) and by a modified procedure of Britten et al. (1969).

MATERIALS AND METHODS

Organism and cultures. The auxotrophic strain of Aspergillus nidulans designated paba A2 bi A1 was used throughout. The mycelial cultures were initiated from heavy conidial suspension in flasks with 600 ml of minimal medium (Cove, 1966)

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containing (per litre) 5 mg of Tween 80, 0.2 ml of silicone antifoam emulsion, and supplemented with 35 μg of biotin and 50 μg of p-aminobenzoic acid. The cultures were grown for 20 - 22 h in a rotary incubator (120 rev/min) at 37°C. Mycelial filaments were collected through several layers of cheese cloth, washed with cold water, pressed free of excess liquid, frozen and lyophilized. About 10 - 15 g wet weight of mycelia were obtained per flask.

3H-labelled DNA. Cells of an adenine requiring mutant of A. nidulans were grown in minimal medium supplemented with 100 μg/ml adenine + 1 μCi/ml [3H]adenine (Amersham, spec. act. 21 Ci/m mole) and 3H-labelled DNA was isolated. The specific activity of the preparation was 650 c.p.m./μg of DNA.

Reagents. All reagents were of analytical grade. Enzymes were purchased from Merck. Ribonucleases were heated for 10 min at 85°C, and pronase was incubated for 1 h at 37°C before use (Marmur, 1963).

Assays. Protein determinations were carried out according to Lowry (1957), RNA was estimated by the orcinol method (Schneider, 1957) and DNA was determined by the modified diphenylamine test (Giles & Myers, 1965).

DNA extraction. In the first procedure according to Marmur (1963) the lyophilized cells were added quickly to 4 vol. of SSC containing 0.5% SDS. Deproteinization was repeated twice with an equal volume of freshly distilled phenol saturated with SSC, nucleic acids were precipitated with 2 vol. of cold 96% ethanol and redissolved in SSC. Pancreatic RNase and RNase T1 were added to a final concentration of 100 μg/ml and 2000 U/ml, respectively, and the digestion was carried out for 30 min at 37°C. After RNase treatment, pronase E was added to a final concentration of 150 μg/ml and the digestion was allowed to proceed for 1 h at 37°C. Pronase was removed by one extraction with phenol and DNA was precipitated with 1.5 vol. of isopropanol.

In the second method, lysis was performed as described by Britten et al. (1969). The homogenate was centrifuged for 10 min at 2500 g and the supernatant was deproteinized with a mixture of phenol/chloroform/isoamyl alcohol (25:24:1). Then solid NaClO4 was added to a final concentration of 1 M and the remaining proteins were pelleted by centrifugation at 10 000 g for 15 min. Chromatography of DNA on hydroxyapatite column was performed according to Markov & Ivanov (1974); finally, the DNA solution was centrifuged in Beckman model L2 65B ultracentrifuge at 100 000 g for 20 h and the DNA pellet was dissolved in 10 mm-phosphate buffer, pH 7.

In the third procedure, 10 - 15 g of lyophilized mycelium was ground in a cold mortar with acid-washed powdered glass. About 150 ml of extraction buffer (10 mM-urea, 1% SDS, 0.1% Triton X-100, 5 mM-EDTA, 10 mM-Tris, pH 7.5) was added to the homogenate. After gentle stirring, the mixture was warmed rapidly to 55°C and incubated at this temperature for 30 min. Proteinase K (200 μg/ml) was added and the extract was transferred to a dialysis bag, previously boiled in 5% Na2CO3.

1 Abbreviations used: SDS, sodium dodecyl sulphate; EDTA, ethylenediamine tetraacetic acid; SSC, 0.15 M-NaCl, 0.015 M-sodium citrate.
for 15 min and in 10 mM-EDTA for 15 min. The digestion was carried out in the bag at 37°C against 4 M-urea, 10 mM-Tris, pH 7.5, for 4 - 5 h. The dialysed extract was carefully pipetted to 60-ml polystyrene tubes containing 3 ml of saturated CsCl, centrifuged in SW 25.2 rotor for 22 - 24 h at 23 500 rev/min (25°C) and samples of 8 - 12 ml were collected from the bottom of the tubes. Solid CsCl and ethidium bromide were added to a final concentration of 1.56 g/ml and 200 μg/ml, respectively, and the mixture was recentrifuged for 35 - 40 h at 45 000 rev/min in 75 Ti rotor. DNA was collected with a pasteur pipette under u.v. lamp. Ethidium bromide was removed by extraction with isopropanol and DNA was dialysed overnight against 10 times diluted SSC.

**Sedimentation analysis.** For preparative ultracentrifugation in CsCl density gradient about 350 μg of DNA was mixed with saturated CsCl solution to give a final density of 1.7 g/cm³. The samples were centrifuged to equilibrium at 70 000 g for 65 h at 20°C, then 25-drop fractions were collected by siphoning from the bottom of the tube and analysed for optical density at 260 nm.

Molecular weights of DNA were determined in Beckman model E ultracentrifuge, by band sedimentation analysis according to Studier (1965).

**DNase treatment of DNA.** Solutions of purified [³H]DNA (spec. act. 650 c.p.m./μg) were dialysed overnight against 0.1 M-MgCl₂. The samples were divided into two portions. One (0.5 ml) was incubated at 37°C with 20 μg of DNase. The control was kept without DNase under the same conditions. After 2 h, 0.1 ml samples were withdrawn, mixed with 0.5 mg of cold DNA and precipitated with 4 ml of ice-cold 0.3 M-trichloroacetic acid. The precipitate was filtered through Millipore filter and washed subsequently with 50 ml of 0.3 M-trichloroacetic acid and 25 ml of 95% ethanol. The filters were dried and the radioactivity determined in a Beckman LS-150 scintillation counter after immersion in toluene - permablend III (Packard).

**RESULTS AND DISCUSSION**

The characteristics of the DNA preparations isolated by three different procedures are given in Table 1. As judged from the absorbance ratios (A₂₆₀/A₂₃₀, A₂₆₀/A₂₈₀) and the content of RNA and proteins, the DNA preparation obtained by the method of Marmur showed the lowest purity and yield. The Britten procedure yielded DNA of satisfactory purity but this method presents some technical difficulties. A large amount of lysate strongly decreased the flow rate and led to the clogging of the column. Furthermore, SDS and EDTA in the lysing medium and proteins in the cell lysate decreased the affinity of DNA to hydroxyapatite and caused incomplete retention of DNA (Markov & Ivanov, 1974). Purity of the DNA preparation obtained by the third method was at least equal to that of the preparation isolated by the Britten method. Moreover, the yield was severalfold higher than obtained by the other methods. Sedimentation of the lysate in the presence of proteinase K into saturated CsCl solution offers a simple and mild method for separation of DNA from most of the proteins and low molecular components. Centrifugation in the CsCl-ethidium bromide density gradient results in complete
Table 1

Characteristics of DNA preparations isolated by different procedures

The yield is expressed in µg of DNA obtained from 1 g of freeze-dried mycelial powder. Purity was estimated by the DNase test.

<table>
<thead>
<tr>
<th>Isolation procedure</th>
<th>Yield (µg)</th>
<th>$A_{260/280}$</th>
<th>$A_{230/280}$</th>
<th>RNA Content (%)</th>
<th>Protein Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol extraction*</td>
<td>200-300</td>
<td>1.85</td>
<td>0.55</td>
<td>1.5-2</td>
<td>1-2</td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>400-500</td>
<td>1.9</td>
<td>0.4</td>
<td>1-1.5</td>
<td>2-3</td>
</tr>
<tr>
<td>chromatography**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>95-96</td>
</tr>
<tr>
<td>CsCl centrifugation</td>
<td>800-1000</td>
<td>1.98</td>
<td>0.4</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

** Britten et al. (1969).

separation of DNA from RNA and remaining proteins. The purity of the preparation isolated by the third method is about 99% (as judged from the DNase treatment). The preparations, although heterogeneous, had molecular weight not lower than $8 \times 10^7$ daltons. The content of RNA and proteins was below detection by the colorimetric methods. Thermal denaturation in 10 times diluted SSC showed a hyperchromic effect of about 39%. The optical melting curve (Fig. 1) is typical for fungal DNA containing two components: nuclear and mitochondrial (Dutta & Ojha, 1972). The heterogeneity of the preparations is shown by the buoyant density in CsCl (Fig. 2). The nuclear fraction has a buoyant density of 1.71 g/cm$^3$.

![Fig. 1](image1.png)

Fig. 1. Optical melting curve of DNA from *Aspergillus nidulans*. Absorbance at 260 nm in SSC was recorded by Unicam SP 200 spectrophotometer.

![Fig. 2](image2.png)

Fig. 2. Preparative ultracentrifugation of total DNA from *Aspergillus nidulans* in CsCl density gradient. After centrifugation to equilibrium, fractions of 25 drops were collected and diluted with 0.5 ml of water.
and the mitochondrial fraction 1.69 g/cm³. Similar results were obtained by Pontecorvo (1967). In the DNA preparations isolated by Pontecorvo by the Marmur method, a third component of a density below 1.69 g/cm³ was observed. We found that this fraction (probably a polysaccharide) appeared only in the preparations purified by the classical method.

REFERENCES

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Streszczenie

Opisano metodę preparatyki DNA z Aspergillus nidulans. Homogenizacja liofilizowanej grzybi w obecności wysokiego stężenia soli, trawienie proteinazą K połączone z zagęszczaniem lizatu pozwoliło na otrzymanie wysokocząsteczkowego DNA o bardzo wysokim stopniu czystości.

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