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METHODOLOGY

A SIMPLIFIED METHOD FOR THE ISOLATION OF HIGH MOLECULAR WEIGHT DNA FROM *Aspergillus nidulans*

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ABSTRACT

We have developed a simple method for obtaining DNA from mycelium of the fungus *Aspergillus nidulans*. A single isopropanol preparation yields good quality high molecular weight DNA preparations that are not contaminated with proteins or salts, and that are easy to solubilize and to digest with restriction enzymes. High yields (approximately 1.6 mg DNA per gram of wet mycelium) are obtained. Contamination with RNA is minimal and there is no need to use RNase. It has been successfully used in our laboratory for many molecular biology experiments.

INTRODUCTION

Many strategies have been used to purify high-molecular weight genomic DNA from filamentous fungi (Ballance and Turner, 1986; Cenis, 1992; Möller *et al.*, 1992; Zhu *et al.*, 1993; Graham *et al.*, 1994; Lecellier and Silar, 1994). However, these techniques are usually time consuming, require the use of expensive chemicals and apparatii, and yield DNA that is randomly nicked. Furthermore the DNA preparations are contaminated with salts, and/or residual organic solvents or detergents that interfere with the activity of many of the restriction enzymes. Also the DNA obtained from these preparations is sometimes contaminated with RNA.

METHODOLOGY

Conidia obtained from strains of the mould *Aspergillus nidulans* were cultured in appropriate culture medium conditions. After growth for a convenient period of time, the harvested mycelium was washed with distilled water, blotted to remove excess liquid and one gram of wet mycelium was freezed in liquid nitrogen and then ground in a mortar with pestle. DNA extraction was made in a 250 ml erlenmayer flask with 25 ml of TSE buffer (150 mM NaCl, 100 mM EDTA, 50 mM Tris-HCl) containing 2% SDS and 0.2 volumes of toluene. After incubation for 72 hours at room temperature with shaking (rotatory shaker at approximatelly 1 cycle per sec) the samples were centrifuged at 2000 x g for 15 minutes and the supernatant collected. DNA was then purified with a classical phenol-chloroform extraction involving 1 volume of phenol, followed by 1 volume phenol-1 volume chloroform and a final 1 volume of chroloform. When 0.6 volumes of isopropanol is added to the supernatant, DNA forms a clot which is removed with a bent Pasteur pipette, rinsed gently with 70% ethanol, dried in a desiccator and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

DISCUSSION

The method yielded good quality high molecular weight DNA preparations that are not contaminated with protein (A_{260}/A_{280} ratio between 1.85 and 1.90) or salts, that are easy to solubilize and to digest with restriction enzymes (Figure 1) and has been successfully used in our

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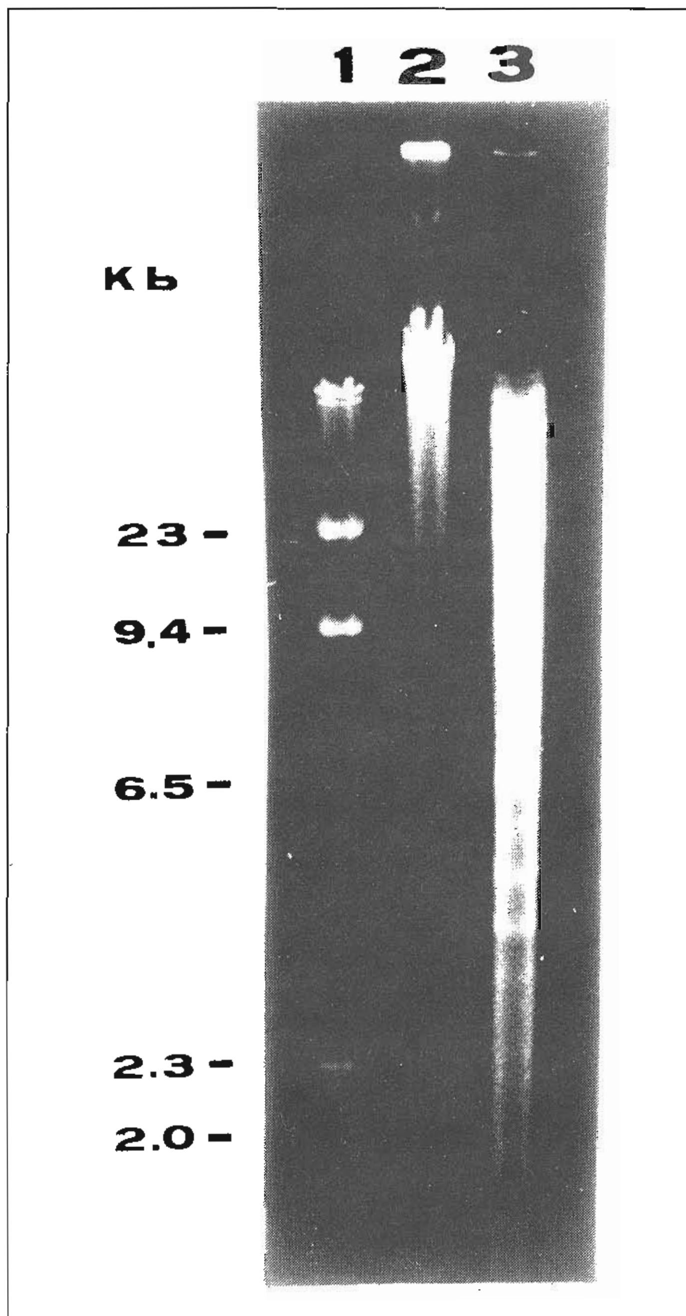

Figure 1 - Agarose gel electrophoresis of DNA isolated from the *paba*A1 strain of *Aspergillus nidulans*. Lane 1. Molecular weight markers (λ DNA digested with restriction enzyme Hind III); Lane 2. Total DNA isolated by the method described in this work; Lane 3. Same sample as in lane 2 but digested with restriction enzyme EcoRI.

laboratory for many molecular biology experiments. Furthermore, contamination with RNA is minimal and there is no need to use RNase.

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RESUMO

Nós desenvolvemos um método simples para preparar DNA micelial do fungo *Aspergillus nidulans* envolvendo uma única precipitação com isopropanol. Este método permitiu a preparação de DNA de alto peso molecular não contaminado com proteínas ou sais. O DNA obtido é facilmente solubilizado e digerido por enzimas de restrição. Além disto, contaminação com RNA é mínima, não havendo necessidade de utilizar RNase. DNA preparado segundo o procedimento proposto tem sido utilizado com sucesso em nossos laboratórios para vários propósitos em experimentos de biologia molecular.

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