Simultaneous extraction of high quality DNA and RNA from Microsporum canis

The application of molecular techniques to the study of fungal systematics, molecular evolution, analysis of virulence determinants and host parasite interactions has grown over the last few years. A prerequisite for successful studies in this field is the ability to purify high quality nucleic acids which are amenable to a number of downstream applications, e.g. restriction enzyme digestion, ligation, library construction, PCR and reverse transcription.

A number of protocols have been published which result in the isolation of high quality DNA or RNA from fungal material (6). However, to our knowledge no protocol exists for the simultaneous isolation of both types of nucleic acid, and their subsequent separation. We present here a simple protocol, based on the method of Raeder & Broder (5), which was used successfully to obtain excellent quality high molecular mass DNA and undegraded RNA from Microsporum canis NCPF 176.

Molecular studies on M. canis and other dermatophyte fungi are rare (3), while infection with these fungi is extremely common (1). One potential reason for this is the very slow growth rate of these fungi and the resultant difficulty in obtaining sufficient nucleic acids for molecular studies. Simultaneous extraction of RNA and DNA would result in the optimal use of material obtained from these slow-growing pathogens.

M. canis NCPF 176 was cultured on Sabourauds dextrose agar plates at 30 °C for 14 d. Portions of this culture were inoculated into 50 ml 2% (w/v) glucose, 1% (w/v) peptone in a 500 ml Erlenmeyer flask and incubated at 30 °C for 6 d at 100 r.p.m. Mycelia were separated from media by filtration and squeezed dry between sterile sheets of Whatman No.1 paper. The dried mycelial mat was cut roughly into 0.5 cm cubes; these were snap frozen in liquid nitrogen and ground to a fine powder with a mortar and pestle. The ground mycelium was added to 20 ml lysis buffer (200 mM Tris/HCl, pH 8.5, 250 mM NaCl, 25 mM EDTA, 0.5%, w/v, SDS) and vortexed for 30 s. To this 14 ml lysis buffer equilibrated phenol and 6 ml chlorform/isooamyl alcohol (24:1, v/v) were added. This mixture was vortexed for 1 min five times, with 1 min rests on ice between agitations. The aqueous phase was separated by centrifugation at 9500 g for 30 min and nucleic acids precipitated with 0.6 vols isopropanol at −70 °C for 30 min. After thawing, the mixture was centrifuged at 9500 g for 20 min. The pellet was washed in 70% (v/v) ice-cold ethanol, air-dried and re-suspended in 1 ml distilled water prior to separation of DNA and RNA. To precipitate RNA, 60 M LiCl was added to a final concentration of 2.0 M and the mixture was left on ice in a cold room (4 °C) overnight. RNA was pelleted at 9500 g at 4 °C for 30 min. DNA in the supernatant was re-precipitated with 0.6 vols isopropanol at −70 °C for 30 min and pellet by centrifugation at 9500 g for 30 min. Both pellets were washed with 70% (v/v) ice-cold ethanol, air-dried and re-suspended in 0.5 ml distilled water. This LiCl precipitation results in complete separation of RNA (Fig. 1, lane 3) from DNA (Fig. 1, lane 4).

We have enzymically digested DNA prepared by this method and used it as template in a PCR reaction employing primers universally conserved within the fungal kingdom (2) (Fig 1, lane 6). In addition, this DNA is currently being used to prepare a genomic library of M. canis NCPF 176. The purified RNA was not degraded, as shown by the intact rRNA bands (Fig. 1, lane 3), and has been used to synthesize cDNA (data not shown). We are employing this method to extract mRNA from M. canis NCPF 176 and Trichophyton rubrum NCPF 119 grown in the presence or absence of keratin (4) to differentially screen genomic libraries.

Fig. 1. Electrophoretic analysis of DNA and RNA isolated from M. canis NCPF 176 by the described method, and use of the DNA in PCR and restriction digests. Lanes: 1, λ HindIII molecular size markers; 2, total nucleic acids obtained prior to LiCl precipitation; 3, purified, undegraded RNA; 4, purified high molecular mass DNA; 5, 5 μg of Sau3A-digested DNA; 6, PCR product obtained after amplification of 10 ng of purified DNA using the universal primers and conditions described by Fell (2).

GUIDELINES

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Adoption of this simple method should result in the optimization of nucleic acid extraction from dermatophytes, and other slow-growing fungi, and will facilitate molecular studies on these common human pathogens.