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Rapid Mini-Preparation of Fungal DNA for PCR

Nucleic acid detection methods such as PCR have become a common tool for microbial identification and diagnosis. Although PCR amplification can be performed directly for various microbial cultures, for filamentous fungi and yeasts, prior isolation of DNA is often preferred. As the DNA extraction process eliminates many unknown interfering substances present in the biological material, it plays an important role in ensuring consistent test results. Toward this end, considerable efforts have been made to enable improved DNA preparation from fungi (1–3). Many of these methods rely on using a grinder (with or without liquid nitrogen) for initial breaking up of the mycelia. This is a significant handicap when dealing with a large number of samples.

Being a clinical diagnostic laboratory, we routinely perform PCR experiments on a variety of fungal pathogens. The availability of a rapid, low-cost, and reliable DNA extraction procedure for fungi not only would reduce the workload considerably but also would decrease the test turnaround time. After experimenting with several DNA purification regimens, we have optimized a rapid, mini-preparation procedure for fungal DNA. This procedure includes the following steps. (i) To a 1.5-ml Eppendorf tube containing 500 μl of lysis buffer (400 mM Tris-HCl [pH 8.0], 60 mM EDTA [pH 8.0], 150 mM NaCl, 1% sodium dodecyl sulfate), a small lump of mycelia is added by using a sterile toothpick, with which the lump of mycelia is disrupted. The tube is then left at room temperature for 10 min. (ii) After adding 150 μl of potassium acetate (pH 4.8; which is made of 60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid, and 28.5 ml of distilled water), the tube is vortexed briefly and spun at $>10{,}000 \times g$ for 1 min. (iii) The supernatant is transferred to another 1.5-ml Eppendorf tube and centrifuged again as described above. After transferring the supernatant to a new 1.5-ml Eppendorf tube, an equal volume of isopropyl alcohol is added. The tube is mixed by inversion briefly. (iv) The tube is spun at $>10{,}000 \times g$ for 2 min, and the supernatant is discarded. The resultant DNA pellet is washed in 300 μl of 70% ethanol. After the pellet is spun at 10,000 rpm for 1 min, the supernatant is discarded. The DNA pellet is air dried and dissolved in 50 μl of 1× Tris-EDTA, and 1 μl of the purified DNA is used in 25 to 50 μl of PCR mixture.

The whole procedure can be completed within an hour and is applicable to various filamentous fungi. Up to now, we have used the technique to isolate DNA from 150 dermatophytes (including 110 *Trichophyton*, 30 *Microsporum*, and 10 *Epidermophyton* isolates) and 10 *Fusarium* isolates. In addition, we also succeeded in generating sufficient DNA from yeasts (10 *Candida* and 10 *Cryptococcus* isolates) as well as bacteria (180 *Staphylococcus* and 10 *Campylobacter* isolates) using this rapid method. The DNA yields from filamentous fungi and bacteria were reasonably high, and a clear DNA band was frequently seen when 10 μl of the 50-μl DNA preparation was run in an agarose gel and stained with ethidium bromide. However, the amount of DNA obtained from *Candida* and *Cryptococcus* spp. was considerably lower, and only a very faint DNA band was observed when the DNA preparations were assessed by agarose gel electrophoresis, particularly those from *Cryptococcus* isolates. Nevertheless, genomic DNA extracted by the procedure, including that from *Candida* and *Cryptococcus* spp., has been readily amplified by PCR. It is likely that this procedure could be applied to the examination of many other fungal cultures and, possibly, clinical specimens. It provides a rapid, reliable, and low-cost alternative to the existing DNA purification protocols used in research and clinical laboratories.

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