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NOTES

Simple Chemical Extraction Method for DNA Isolation from *Aspergillus fumigatus* and Other *Aspergillus* Species

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DNA was successfully isolated from numerous *Aspergillus* spp. by use of a commercial kit. DNA that was easily digested and yielded PCR products up to 8.5 kb in size was recovered from broth or agar cultures. The ease and speed of this protocol provide an alternative to physical methods of DNA isolation.

Aspergillus fumigatus is a filamentous acscomycetous fungus that is ubiquitous in nature. In recent years the aspergilli have become an increasingly frequent cause of life-threatening opportunistic infections (7, 8, 11, 12). Consequently, there is a growing interest in the molecular biology of this fungus, which has been accelerated by the genome-sequencing project (6). Although relatively new, a broad range of molecular manipulations of *A. fumigatus* are now possible, including gene disruption, various PCR applications (random amplified polymorphic DNA analysis, microsatellite typing, etc.), and DNA-based epidemiological studies (restriction fragment length polymorphism analysis, fingerprinting, etc.) (1–3, 5, 13–15). Each of these techniques requires the recovery of good-quality genomic DNA.

Most DNA extraction protocols for *Aspergillus* spp. rely on mechanical isolation methods that employ grinding mycelia after freezing them in liquid nitrogen or glass bead disruption, followed by additional purification steps (9). We routinely isolate DNA from many types of fungi by a variety of different techniques. These techniques range from simple boiling, which can yield small amounts of DNA within minutes, to bead bashing, which increases yield but also increases time, to spheroplasting, which can take two or more days but yields large amounts of high-purity high-molecular-weight DNA (4). In an effort to identify a method that was as simple as bead beating, that had as high a yield as spheroplasting, and that had as high a throughput as boiling, we investigated a chemical-based method (MasterPure yeast DNA purification kit [Epicentre, Madison, Wis.]) and found that with modification, this method is convenient and easy to use, does not require physical methods, and works with mycelia or conidia.

Strains and growth conditions. The strains used in this study are listed in Table 1. Stock cultures were grown on potato dextrose agar (PDA) plates (24 g of PD [Difco, Detroit, Mich.]/liter, 20 g of agar/liter) at 30°C. Mycelial cultures were harvested from PD broth grown for 8 to 24 h in 10-ml tubes (3 ml of culture) or 125-ml flasks (40 ml of culture) at 30°C (225 rpm) by filtering them through Whatman paper (Fisher Scientific, Inc., Pittsburgh, Pa.), washed according to the manufacturer's instructions, and then blotted dry. Conidial cultures were prepared from PD agar plates grown for 3 to 11 days at 30°C and harvested by washing with 10 ml of sterile 0.1% Tween 20. The suspension was pipetted into a 15-ml snap cap tube (Fisher) and centrifuged at 4,800 × *g* for 10 min. After the supernatant was discarded, the conidia were transferred to a 1.7-ml microcentrifuge tube and washed once with 500 μl of sterile water and once with 500 μl of 0.1 M $MgCl_2$. Conidial and mycelial preparations were used directly for DNA extraction or kept in a −70°C freezer for later use.

DNA extraction. DNA extraction was performed with an Epicentre kit but with a modification of the manufacturer's protocol. Approximately 200 mg of washed mycelia or one plate of conidia (~10^{10}) was added to a 1.7-ml microcentrifuge tube. The step involving grinding in liquid nitrogen was omitted; instead, 450 μl of yeast cell lysis solution and 1 μl of a 50-μg/ml concentration of proteinase K (where indicated) were added to the tubes. The tubes were vortexed for 10 s, incubated in a 65°C heating block for 1 h, and then chilled on ice for 5 min. Next, 225 μl of protein precipitation reagent was added, and the tubes were vortexed for 5 s. The suspensions were then centrifuged at 20,800 × *g* for 10 min to pellet cellular debris. The supernatant (~500 μl) was transferred to a new tube, spun again to remove any residual cellular material, and then transferred to a new tube. An equal volume of isopropanol was added, and the tubes were gently inverted several times to precipitate the DNA, which was then pelleted by centrifugation at 20,800 × *g* for 10 min. Pellets were washed with 70% ice-cold ethanol, centrifuged, and then vacuum dried. DNA was resuspended in 50 to 100 μl of Tris-EDTA and then treated with 2 μl of a 5-mg/ml concentration of RNase A at 65°C for 1 h. Extraction and reprecipitation after RNase treatment were optional. For *Aspergillus flavus* and *Aspergillus niger*, a phenol-chloroform isoamyl alcohol (PCIA) (Ambion, Austin, Tex.) extraction step was required and was performed directly after the protein precipitation step. Supernatants were

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TABLE 1. *Aspergillus* strains used in this study[a]

Species	Strain designation	Reference
A. fumigatus	WSA-450	Genome sequence strain (293)
A. fumigatus	WSA-270	ATCC 64746
A. fumigatus	WSA-271	ATCC 14110
A. fumigatus	WSA-621	Clinical isolate
A. fumigatus	WSA-622	Environmental isolate
A. fumigatus	WSA-623	Clinical isolate
A. fumigatus	WSA-624	Clinical isolate
A. fumigatus	WSA-625	Clinical isolate
A. fumigatus	WSA-626	Clinical isolate
A. terreus	WSA-174	Clinical isolate
A. versicolor	WSA-175	Clinical isolate
A. nidulans	WSA-176	Clinical isolate
A. flavus	WSA-269	ATCC 26947
A. niger	WSA-272	ATCC 9508

[a] Strains WSA-621, WSA-622, WSA-623, WSA-624, WSA-625, and WSA-626 were received from Mark M. Huycke, Medical Service, Department of Veterans Affairs Medical Center, Oklahoma City, Okla. Strain WSA-450 was received from Rodolfo Aramayo, Department of Biology, College of Science, Texas A&M University, College Station. Strains WSA-174, WSA-175, and WSA-176 were received from Mike Rinaldi, Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio.

added to new tubes and precipitated with 2 volumes of ice-cold ethanol. The DNA was then pelleted, washed in 70% ethanol, and redissolved in 500 μl of Tris-EDTA. The rest of the procedure was the same as that described above, beginning with the RNase treatment. Bead bash DNA was prepared by a standard fungal bead bash protocol (4). DNA quality was checked on a 0.8% agarose gel and quantified by $A_{260/280}$ readings. Restriction enzyme digestions were performed by cutting 3 μg of sample in a volume of 40 μl by using 20 U of EcoRI (New England BioLabs, Beverly, Mass.) at 37°C for 4 to 12 h. Samples were electrophoresed in a 0.8% agarose gel and blotted onto Hybond N^+ membranes (Amersham Biosciences, Inc., Piscataway, N.J.).

PCR and hybridizations. Approximately 20 kb of genomic sequence from the TIGR database (http://www.tigr.org/tdb/e2k1/aful/) containing the *pyrG* gene (16) was used to design primer pairs that were predicted to yield PCR products of various sizes. Long-distance PCR was performed with the Expand Long Template PCR system (Roche Diagnostics, Inc., Indianapolis, Ind.) according to the manufacturer's instructions. The primers and their sizes were as follows: Af.pyrg.far.F, (5′-CCTCAAACAATGCTCTTCACCC-3′) and Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′), 2.1 kb; flank.pyrg.F3 (5′-TATGGCTTCTCTCGGCTCAG-3′) and flank.pyrg.R3 (5′-CCACGGTTGTCTTCTTGGTGTAG-3′), 5.5 kb; and Af.pyrg.R (5′-CATTCCCTATCAACTCCCCCTC-3′), and flank.pyrg.F1 (5′-GCTCTATTCAACGGACTTCAACC-3′), 8.5 kb. The long terminal repeat of *Afut1* (AfutLTR) was used as a hybridization probe for fingerprinting a panel of *A. fumigatus* isolates digested with EcoRI as described previously (10). The 2.1-kb PCR product from primers Af.pyrg.far.F and Af.pyrg.R was used as a hybridization probe to compare the integrities of the DNA prepared by bead bashing and by our method. Hybridizations were performed in Rapid Hyb buffer (Amersham Biosciences, Inc.) according to the manufacturer's instructions. Membranes were visualized by autoradiography by using Kodak Biomax MS imaging film (Fisher Scientific).

DNA quality. Overnight growth (~16 h) in PD broth provided sufficient mycelia from all species. The procedure could be scaled down in order to facilitate a simpler approach by using 14-ml tubes with 3.0 ml of medium or screw-cap 50-ml tubes with 10 ml of medium. Each of these approaches yielded at least 200 mg of mycelia. For the nine strains of *A. fumigatus*, the yield of DNA per 200 mg of mycelial was 500 to 700 μg of DNA. The entire procedure took 4 to 6 h. Aliquots of these preparations were run on a gel to assess the quality of the DNA. Little shearing was observed, a finding which was confirmed by hybridization (Fig. 1A and B). Since species other than *A. fumigatus* occasionally show up as clinical isolates, we investigated the utility of the kit on some these species, including *Aspergillus terreus*, *Aspergillus versicolor*, *Aspergillus nidulans*, *A. flavus*, and *A. niger*. Our approach yielded satisfactory DNA for all species except *A. flavus* and *A. niger* (Fig. 1C). The procedure for *A. niger* was modified by reducing the growth period to 6 to 8 h, which was enough time to allow conidia to germinate but not to develop into profuse hyphae. Both *A. flavus* and *A. niger* required PCIA treatment alone or in addition to treatment with proteinase K to eliminate residual nuclease activity. Although phenol extraction is one of the steps

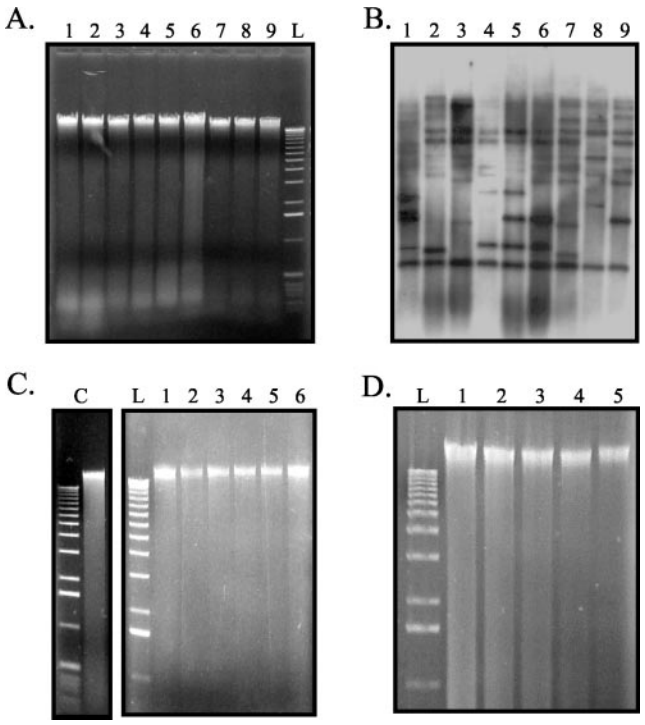

FIG. 1. Genomic DNA isolation. (A) *A. fumigatus* DNA isolation. Lanes 1 to 9, strains WSA-450, WSA-270, WSA-271, WSA-621, WSA-622, WSA-623, WSA-624, WSA-625, and WSA-626, respectively. L, 1.0-kb ladder. (B) Hybridization to Southern blots of the strains in panel A by use of *Afut1*. (C) DNA isolation from different species of *Aspergillus*. C, bead bash control. Lanes 1 to 6, *A. fumigatus* WSA-450, *A. terreus* WSA-174, *A. versicolor* WSA-175, *A. nidulans* WSA-176, *A. flavus* WSA-269, and *A. niger*, WSA-272, respectively. (D) Isolation of DNA from conidia. Lanes 1 to 5, PDA plate age of 3, 5, 7, 9, and 11 days, respectively.

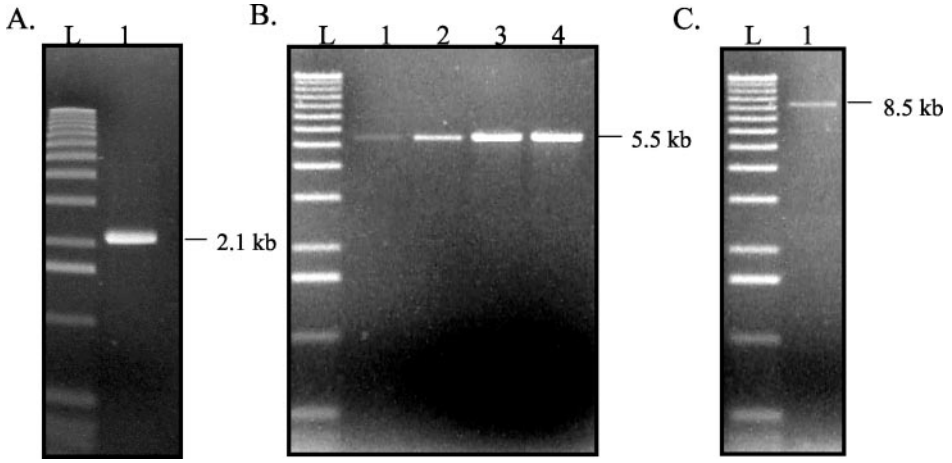

FIG. 2. PCR amplification of *A. fumigatus* DNA. (A) PCR product of 2.1 kb amplified with primers Af.pyrg.far.F and Af.pyrg.R. (B) PCR product of 5.5 kb amplified with primers flank.pyrg.F3 and flank.pyrg.R3. Lanes 1 to 4, standard DNA preparation (no PCIA extraction, no proteinase K treatment); proteinase K treatment alone; PCIA extraction alone; and PCIA extraction and proteinase K treatment, respectively. (C) PCR product of 8.5 kb amplified with primers flank.pyrg.F1 and Af.pyrg.R from genomic DNA treated with proteinase K and by PCIA extraction.

that would be preferable to omit during nucleic acid isolation, it was used only in small amounts due to the microcentrifuge-based volumes used in our protocol. We have on occasion seen strains of *A. fumigatus* or *A. nidulans* that yielded DNA that degraded, so in cases where many species of *Aspergillus* are likely to be encountered, it may be prudent to include PCIA extraction in the standard protocol.

We next wanted to determine if *A. fumigatus* conidia har-

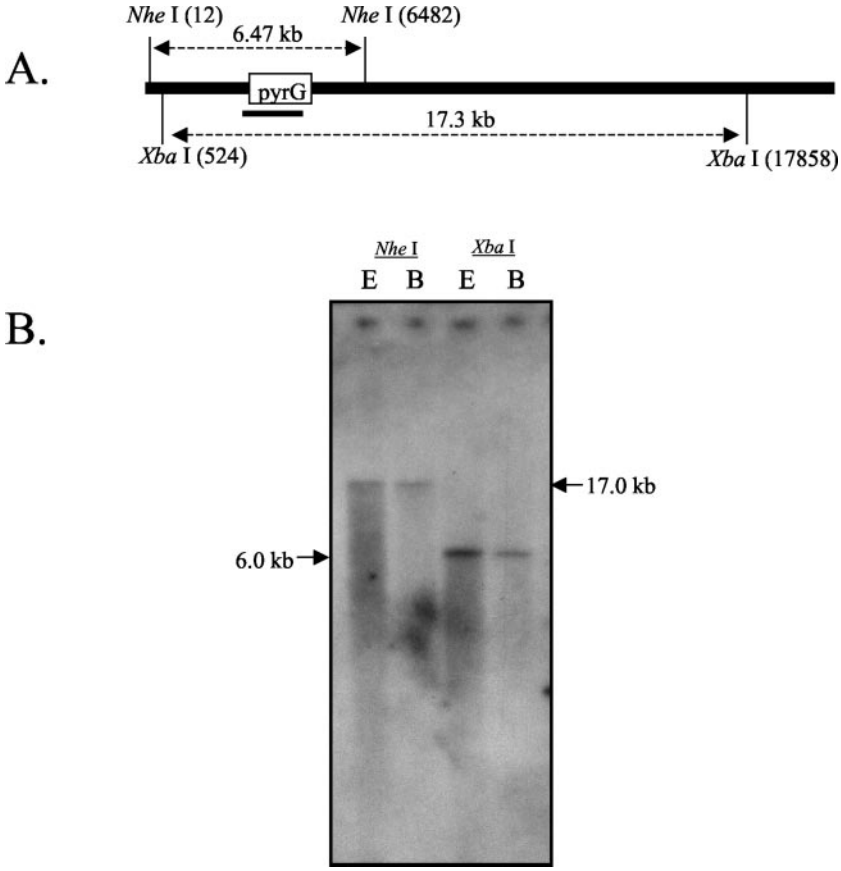

FIG. 3. Comparison of DNA qualities by Southern hybridization. (A) Restriction sites were mapped on an ~20-kb *pyrG*-containing fragment, and two sites (NheI and XbaI) that gave different-size fragments after digestion were selected. (B) DNA was prepared by our protocol (lanes E) or bead bashing (lanes B) and then digested with each enzyme. Digests were separated on a 0.8% gel, subjected to Southern blotting, and hybridized with a probe derived from the *pyrG* coding sequence (bar in panel A).

vested directly from plates could serve as suitable sources of genomic DNA. We observed that DNA could be recovered from conidia as soon as they appeared (~3 days) as well as from conidia grown for 5, 7, 9, and 11 days (Fig. 1D). Yields for the different days ranged from a total of 185 μg for 3-day-old conidia to 96.5 μg for the 11-day-old conidia, with a range of 10^{10} to 10^{12} conidia harvested per plate. Since the *A. fumigatus* genome sequence is nearing completion, most genes will be recovered as PCR products. Consequently, DNA preparations need to be sufficiently pure to ensure that even the largest genes can be recovered by PCR. Long-distance PCR revealed that genomic DNA obtained by an Epicentre kit could serve as a template for PCR products as long as 8.5 kb (Fig. 2), although both a proteinase K step and a PCIA extraction step were needed. Finally, a comparison of our method to bead bashing was made in order to determine how much shearing resulted from the two methods when recovery of larger fragments was needed. Figure 3 demonstrates that fragments with sizes up to approximately 17 kb are relatively free from shearing when our method is used.

Our laboratory conducts a number of studies of a diverse variety of fungi. As a result, we utilize a wide variety of DNA extraction strategies, which differ depending on the species of fungus and the subsequent use of the DNA. Grinding frozen mycelia (or lyophilized cultures) and bead beating work very well for unknown or poorly studied fungi and generally involve universal protocols. However, these processes are labor-intensive and may not be suitable for multiple samples. Since we have found the Epicentre kit easy to use for DNA isolation from *Candida* species and *Cryptococcus neoformans* (with PCIA extraction), which do not require grinding or bead beating, we thought the kit would have greater utility if it could be used without a physical cell breakage step. We found that the grinding step could indeed be omitted as long as the weight of the mycelia was <200 mg. Perhaps more significant, the use of mycelia was optional, as our procedure worked fine with conidia, regardless of age. Finally, although we have not used the kit for a wide variety of non-*Aspergillus* fungi, we have tried it for a few other fungi (e.g., a *Scedosporium* sp.) and have been successful in recovering similar levels of DNA that is sufficiently pure for PCR amplification. These results suggest that the technique is robust and probably applicable to a wider variety of fungi and samples than we have tested.

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