Fast DNA Isolation from *Histoplasma capsulatum*: Methodology for Arbitrary Primer Polymerase Chain Reaction-Based Epidemiological and Clinical Studies

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The arbitrary primer polymerase chain reaction (also called random amplified polymorphic DNA, or RAPD) is a DNA fingerprinting method that provides an efficient, sensitive way of discriminating between independent isolates of *Histoplasma capsulatum*, but its widespread application has been hampered by the arduous 2-day procedure traditionally used to extract DNA from *H. capsulatum*. We present here a quick (~2-h) extraction method and show that the resultant DNA is suitable for sensitive and reproducible identification of individual strains of this pathogenic fungus.

The fungus *Histoplasma capsulatum* infects humans and other mammals when they inhale its airborne conidia and hyphal fragments. It often causes mild respiratory infections that are generally suppressed by the immune system and can result in long-term latency. A subsequent decline in immune function due, for example, to advancing age or AIDS can result in reactivation of the fungus and systemic infection (1, 9). Efficient methods for distinguishing different isolates of *H. capsulatum* are needed for analyses of the epidemiology and mechanisms of infection, latency, and reactivation of this organism. Restriction fragment length polymorphisms (RFLPs) in mitochondrial DNA and in ribosomal and *yps* nuclear genes have allowed *H. capsulatum* isolates to be divided into about six different classes that correlate with geographic distribution and levels of virulence. In particular, organisms of RFLP class 2 are often isolated from patients in the American Midwest, and apparently less virulent RFLP class 1 strains can often be isolated from AIDS patients (3, 5–7). Our use of this RFLP fingerprinting method has been limited by its relatively low sensitivity (failure to distinguish individual isolates in these RFLP classes) and also by the special efforts needed to prepare *Histoplasma* DNA that can be cleaved completely by standard restriction endonucleases. The standard DNA preparation methods require at least 2 days and involve grinding mycelia in liquid nitrogen or enzymatic spheroplasting of yeast-form cells and then multiple protease and RNase treatments, organic extractions, ethanol precipitations, and sometimes cesium chloride density gradient centrifugation (4, 6, 11).

We have recently found that use of the arbitrary primer (also called random amplified polymorphic DNA, or RAPD) polymerase chain reaction (PCR) method allows independent isolates within a single RFLP class to be distinguished with sensitivity, ease, and reproducibility (4). In this method, arbitrarily chosen oligonucleotides prime DNA synthesis from sites throughout the target genome that they fortuitously match or almost match, resulting in strain-

specific arrays of DNA fragments (8, 10). Our initial RAPD experiments had used *H. capsulatum* DNAs purified by the 2-day procedures outlined above. We report here a simple 2-h method for preparing DNA suitable for RAPD analysis from mycelial or yeast-phase *H. capsulatum* cultures. This simple method should greatly increase the efficiency of RAPD-based epidemiological studies of *H. capsulatum* infection.

H. capsulatum isolates of several RFLP classes were grown as mycelia (at room temperature) or as yeasts (at 37°C) in HMM broth or on HMM-agarose plates (12). Five-milliliter late-log- or stationary-phase broth cultures of yeasts or mycelia, or water suspensions of yeast cells from solid medium (approximately 3×10^9 cells), were pelleted by centrifugation and washed with distilled deionized water. The pellet was suspended in a 0.5-ml volume of 0.25 M Tris (pH 8.0)–1.5% sodium dodecyl sulfate and incubated for 30 min in a boiling water bath. The suspension was vortexed for 2 min, and then the DNA was extracted with an equal volume of phenol-chloroform-isoamyl alcohol (volume ratio, 25:24:1) and precipitated from the aqueous phase with 1/10 volume of 3 M sodium acetate (pH 5.2) and 2 volumes of ethanol. The DNA pellet was resuspended in 75 μ l of water, and the DNA concentration was estimated by electrophoresis of a 5- μ l sample in an agarose gel. Aliquots ranging from 1 to 15 μ l, depending on the estimated DNA concentration, were added to the standard RAPD PCR mixes (25- μ l final reaction volume), and DNAs were amplified as described previously (4).

Figure 1 shows representative results from our RAPD analysis and allows comparison of arrays of amplification products obtained with DNAs purified by the 2-day extraction procedures (lanes D) and DNAs extracted by the present quick method from yeast and mycelial forms (lanes R, S, and E; see below). We used three lineages (G184A [class 3], UCLA 531 [class 1], and *H. capsulatum* and var. *duboisii* RV26821 [not RFLP classified]) and three variants of each lineage (parental wild-type rough [R] colony morphology strains and two smooth [S and E] colony morphology variants, which lack α -1,3-glucan, a major cell wall constituent of R strains [2, 4]). Both short (10-nucleotide) (Fig. 1A and B) and long (27-nucleotide) (Fig. 1C and D) arbitrary primers were tested by using appropriate temperatures in the annealing step of PCR (36°C and 45°C, respec-

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FIG. 1. Comparison of arbitrary primer PCR profiles obtained with DNAs prepared by the quick method described here (lanes R, S, and E) and those obtained by the traditional (11) 2-day method (lanes D). G184A, UCLA, and Dub (*H. capsulatum* var. *duboisii*) refer to strains from three different RFLP classes, as described in the text. R, wild-type rough colony morphology; S and E, smooth colony morphology variants, which lack α -1,3-glucan, a major cell wall constituent, as described in the text. The primers used are 5'-AACGCGCAAC (primer 1281) and 5'-GGCCATAGAGTCTTGCAGACAACTGC (primer 10730). The conditions for amplification were as described in reference 4. The 1-kb ladder (GIBCO/BRL) provided DNA markers (lane M); fragment sizes (in base pairs) are indicated on the left.

tively; see reference 4). The results show essentially identical arrays of amplified fragments for any combination of lineage and primer, independent of whether the template DNAs were from either yeast- or mycelial-phase organisms of R, S, or E variants.

All preparations were boiled for 30 min to kill both mycelial- and yeast-phase cells. We have not investigated whether this boiling step could be shortened or omitted, because intact double-stranded DNA is not needed for arbitrary primer PCR, and it is convenient to use killed cells, especially when many samples of a pathogen are to be processed. Omission of any subsequent step—vortexing, organic extraction, or ethanol precipitation—resulted in

DNA samples that did not yield such consistent and reliable patterns, especially for DNA fragments more than 1.5 kb in size. In further tests, small differences in intensities of fragments amplified from yeast- and mycelium-derived DNAs were occasionally observed, especially at limiting template DNA concentrations and for fragments larger than 2 kb (data not shown). Thus, we recommend that comparisons among isolates be carried out with organisms of the same morphotype and with similar nonlimiting amounts of DNA.

In conclusion, arbitrary primer PCR allows independent *H. capsulatum* isolates to be distinguished much more sensitively than has been possible by RFLP analysis, and with equivalent reliability. The simple, fast DNA preparation method presented here, in combination with arbitrary primer PCR analysis, should allow cost-effective analyses of numerous clinical, environmental, and experimental isolates and result in significant new insights into the epidemiology and infection mechanisms of this respiratory and systemic pathogen.

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