DNA Probe for the Identification of *Histoplasma capsulatum*

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A 1.85-kilobase *Hind*III nuclear DNA probe from *Histoplasma capsulatum* G217B detected polymorphic restriction fragments within whole-cell DNA from different clinical isolates of *H. capsulatum*, consistent with the previous system of classification. The probe failed to hybridize to DNA from *Blastomyces dermatitidis*, *Candida* spp., *Saccharomyces cerevisiae*, *Sepedonium chrysospermum*, and *Chrysosporium keratinophilum* under low-stringency conditions and therefore may have value as a diagnostic reagent to identify *H. capsulatum*.

We have recently cloned and characterized several yeast-phase-specific genes of the dimorphic fungal pathogen *Histoplasma capsulatum* by differential hybridization. We believe that one of these clones, yps-3, might be important in pathogenicity, since variable expression of this nuclear gene was observed (5) in several *H. capsulatum* strains differing in thermotolerance and virulence (7). To determine whether this gene might function as a diagnostic probe, we screened a panel of pathogenic and nonpathogenic fungal DNAs with the yps-3 sequence. We report here that the yps-3 gene may be a valuable diagnostic reagent, since it fails to hybridize to *Blastomyces dermatitidis*, *Candida* spp., *Sepedonium chrysospermum*, *Chrysosporium keratinophilum*, and *Saccharomyces cerevisiae* DNA under low-stringency conditions.

Initial experiments were designed to detect the yps-3 homolog in the dimorphic fungus *B. dermatitidis* and in the yeast *Candida albicans*. DNA from all isolates was prepared by breaking washed cells under liquid nitrogen with a mortar and pestle (1) and processing as previously described (5).

Genomic DNA from five *H. capsulatum* strains (Downs, ATCC 38904; G184B, ATCC 26028; G186B, ATCC 26030; G217B, ATCC 26032; and G222B, ATCC 26034), from avirulent CR and virulent FW *B. dermatitidis* strains (19), and from *C. albicans* 1012A (Washington University Mycology Center Collection) were digested with *Ava*I and *Hind*III, transferred by Southern blot (13) to Nytran (Schleicher & Schuell, Inc.), and hybridized with the nick-translated (9) 1.85-kilobase (kb) *Hind*III yps-3 probe at 40°C (Fig. 1A). The aqueous hybridization mixture (3) contained 7% sodium dodecyl sulfate, 1% bovine serum albumin, 0.5 M sodium phosphate (pH 7.2), 1 mM EDTA, and 10⁶ cpm of probe per ml; blots were washed in 5× sodium citrate-sodium chloride buffer (SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate]) (6) containing 0.1% sodium dodecyl sulfate at 30°C.

The nuclear yps-3 gene probe detected polymorphic restriction fragments in all *H. capsulatum* strains assayed (Fig. 1A); the predicted *Ava*I-*Hind*III fragments of 1.12, 0.47, and 0.25 kb were obtained with G217B (5). The major differences in hybridization profiles observed among Downs (class 1), G184B/G186B (class 3), and G217B/G222B (class 2) were consistent with the previously described *H. capsulatum* classification scheme based on mitochondrial restriction

fragment length polymorphisms (16). Minor differences in the *Bgl*II restriction patterns of strains within each class were also present (5) and may be useful in identifying individual strains. Southern blot analyses performed on 32 clinical and soil isolates (14, 16) of *H. capsulatum* with the yps-3 gene probe confirmed the classification in all cases (data not shown). The yps-3 gene probe also hybridized to DNA prepared from *H. capsulatum* var. *farcinosum* (ATCC 28798), an epizootic *H. capsulatum* variant (11, 15) producing macroconidia in vitro and disease in horses and mules. Although the pattern obtained with this organism was very different from those observed with the *H. capsulatum* strains (data not shown), these findings provide molecular support for the classification scheme advanced by Weeks and co-workers (17). To determine whether the polymorphic restriction profile obtained with the yps-3 gene probe was unique, the blot was stripped and rehybridized with a ³²P-labeled 1.1-kb *Bam*HI-*Sal*I probe containing most of the single G217B *H. capsulatum* beta tubulin gene (G. S. Harris, E. J. Keath, and J. Medoff, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, I2, p. 172). The nuclear tubulin gene probe hybridized to common 0.65- and 2.15-kb *Ava*I-*Hind*III fragments in both CR and FW strains under identical aqueous hybridization conditions (Fig. 1B) at 40°C. A single 3.4-kb *Ava*I-*Hind*III fragment was detected in all *Histoplasma* strains tested with the homologous beta tubulin probe. Therefore, the yps-3 gene probe clearly differentiated among *H. capsulatum* isolates, while the probe representing the well-conserved beta tubulin gene failed to discriminate among *H. capsulatum* or *B. dermatitidis* strains. Moreover, no significant cross-species hybridization to the *H. capsulatum* beta tubulin probe was observed with *C. albicans* 1012A.

To extend these studies, genomic DNA from additional *C. albicans* strains, B311 (ATCC 32354) and H317 (10), as well as from other *Candida* species, was prepared by the method of Willems et al. (18), digested in agarose blocks with *Hind*III, and purified by phenol extraction. The ethidium bromide-stained gel (data not shown) showed similar *Hind*III mitochondrial DNA digestion profiles for all *C. albicans* strains (1012A, B311, and H317), but variable patterns were observed in the other *Candida* species. The gel was transferred (13) to Nytran and UV cross-linked. As seen in Fig. 2A, the 1.85-kb *Hind*III probe from yps-3 detected polymorphic *Hind*III restriction fragments in the tested *H. capsula-*

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FIG. 1. Southern blot analysis of pathogenic fungal DNAs. Whole-cell DNAs from *B. dermatitidis* (strains CR and FW), *C. albicans* B1012A, and *H. capsulatum* (strains Downs, G184B, G186B, G217B, and G222B) were digested with *Ava*I and *Hind*III, electrophoresed in 0.9% agarose, and transferred to Nytran. In panel A, the sizes of the polymorphic restriction fragments detected with the 1.85-kb *Hind*III yps-3 probe (5) under aqueous hybridization conditions at 40°C are indicated in kilobases. A partial 1.59-kb *Ava*I-*Hind*III fragment is detectable in G184B but is absent in complete digests of G186B. In panel B, the Nytran blot was stripped and rehybridized with the 1.1-kb *Bam*HI-*Sal*I probe containing most of the *H. capsulatum* beta tubulin gene under identical hybridization conditions.

tum strains but failed to hybridize at 40°C under aqueous conditions to *Candida* spp. or *S. cerevisiae* DNA. To obtain an appropriate positive control, the Nytran blot was stripped and rehybridized with a cloned 8.3-kb *Not*I ribosomal DNA repeat from *H. capsulatum* G217B (14). Due to the high degree of evolutionary conservation of ribosomal DNA sequences (8), this ribosomal probe (Fig. 2B) hybridized to each *H. capsulatum*, *S. cerevisiae*, and *Candida* spp. DNA assayed under identical low-stringency hybridization and washing conditions. Previous studies using this cloned probe in Southern blots of *Eco*RI-digested class 1 (Downs) and class 2 (G222B) DNA have differentiated these strains on the basis of ribosomal DNA polymorphisms (14). However, since *Hind*III cleaves outside of the ribosomal DNA locus in both strains, no size differences between class 1 and class 2 *H. capsulatum* isolates were observed (Fig. 2B).

To determine the sensitivity of this assay, DNAs from *H. capsulatum* strains, *C. albicans* 1012A, and *B. dermatitidis* CR were serially diluted in 5× SSC and dot blotted on Nytran filters. Two molds which morphologically resemble the mycelial phase of *H. capsulatum*, *S. chrysospermum* (ATCC 16084) and *C. keratinophilum* (ATCC 14803), were also analyzed. The results (Fig. 3A) suggested that as little as 60 to 120 ng of *Histoplasma* DNA was detected under conditions where 2 µg of other fungal DNAs failed to hybridize. Control experiments with the labeled 8.3-kb *Not*I *H. capsulatum* ribosomal DNA probe hybridized with all

fungal DNAs tested and confirmed the transfer efficiency of the dot blot procedure (Fig. 3B).

This study extends our previous characterization of the nuclear yeast-phase-specific gene, yps-3 (5). Polymorphic restriction fragments detected with the yps-3 probe in Southern blots with *Hind*III (Fig. 2A), *Ava*I-*Hind*III (Fig. 1A), and *Bgl*II (5) digests of genomic DNAs can be used to group *H. capsulatum* strains into three classes. These results are in agreement with previous reports (16).

The observation that the 1.85-kb *Hind*III yps-3 sequence is apparently *Histoplasma*-specific suggests that the protein encoded by this nuclear gene may be a novel virulence determinant which in some manner is related to thermotolerance and pathogenicity. Recent studies analyzing DNA enzymatically amplified by the polymerase chain reaction technique (12) suggest that this in vitro method produces a 10⁵-fold increase in the amount of target sequence (4), making the analysis of *H. capsulatum* in crude cell lysates from biological fluids feasible by using oligonucleotide probes derived from the yps-3 sequence. Application of these in vitro techniques could dramatically reduce to several days the time currently required for diagnosis of histoplasmosis while permitting *Histoplasma* typing for epidemiologic analyses; further studies are clearly warranted by these observations. Identification of the DNA sequence of the yps-3 gene should not only provide insight into the nature of its protein product but could also extend the application of

FIG. 2. Southern blot analysis of *Candida* species. Cells from *H. capsulatum* strains (Downs, G186B, and G222B), *S. cerevisiae* AB972 (2), and various *Candida* species, including *Candida lusitanae* (ATCC 45090), *Candida pseudotropicalis* (ATCC 4135), *Candida stellatoidea* (ATCC 36232), and *Candida tropicalis* (ATCC 750) were embedded in agarose blocks, digested with *Hind*III, and electrophoresed in 0.75% agarose. In panel A, the Nytran-transferred gel was hybridized at 40°C with the 1.85-kb *Hind*III yps-3 probe. In panel B, the Nytran blot was rehybridized under identical aqueous conditions with a cloned 8.3-kb *Not*I ribosomal DNA probe isolated from G217B. Fragment sizes are indicated in kilobases.

FIG. 3. Dot blot hybridization of serially diluted fungal DNAs. DNAs from *C. albicans*, *B. dermatitidis*, *S. chrysospermum*, *C. keratinophilum*, and various *H. capsulatum* strains were serially diluted in 5× SSC and blotted to Nytran. In panel A, the blot was hybridized to the 1.85-kb *Hind*III yps-3 probe to determine the limits of detection. In panel B, the blot was stripped and rehybridized with the control cloned 8.3-kb *Not*I ribosomal DNA probe.

the probe as a sensitive tool in the identification and classification of *H. capsulatum* strains in clinical specimens.

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