Isoenzyme Changes in *Candida albicans* during Domestication

PAUL F. LEHMANN,^{1*} LUNG-CHI WU,¹ AND DONALD W. R. MACKENZIE²

Department of Microbiology, Medical College of Ohio, P.O. Box 10008, Toledo, Ohio 43699-0008,¹ and Mycological Reference Laboratory, Central Public Health Laboratory, London NW9 5HT, United Kingdom²

Received 29 April 1991/Accepted 31 July 1991

Isoenzyme analysis was performed on multiple strains of two commonly used reference cultures of *Candida albicans* (B311 and NCPF3153). Whereas strains originating from *C. albicans* B311 showed no variation in isoenzyme profiles, some strains derived from NCPF3153 were identical to B311 strains but others showed variation in their glucose-6-phosphate dehydrogenase isoenzymes. The results are compared with those from previous analyses with these strains and show that *C. albicans* can undergo genetic alterations during prolonged maintenance in laboratories.

The development of methods for delineating strains of fungi is important for epidemiologic analyses of mycoses and for taxonomy. To test the value of isoenzyme profiles in distinguishing known strains of *Candida albicans* (Robin) Berkhout, we have studied a panel of strains obtained from several laboratories (7). These strains were derived from *C. albicans* 207 and B311, which originally had been used to define the serotype A of *C. albicans* (1). So that nomenclature may be standardized between this and previous communications concerning this panel (7, 9), *C. albicans* 207 will be referred to by its familiar accession number in the National Collection of Pathogenic Fungi (NCPF3153). Here we report that isoenzyme profiles placed laboratory strains derived from *C. albicans* B311 into a single type; however, the strains that had been derived from *C. albicans* NCPF3153 showed two patterns of glucose-6-phosphate dehydrogenase (G6PDH) isoenzymes. Though isoenzyme variation allowed the discrimination of additional strains that were not part of the panel, it did not prove useful in identifying the origin of strains derived from B311 and NCPF3153.

Fungi. Most of the strains were studied only after they had been assigned codes to disguise their origin. Only the unrelated strains NCPF3324 (National Collection of Pathogenic Fungi, Central Public Health Laboratory, London, United Kingdom) and MCO50 (5) were not disguised. The origins of the strains are given in Table 1 and have been discussed elsewhere in depth (7). To avoid confusion, we have used the term strain to refer to any laboratory culture derived from the original isolates.

Isoenzyme profiles. The preparation of cell extracts followed very closely the methods that have been described previously (5, 6). Each strain was grown for 3 days at 30°C on Sabouraud agar and then inoculated into a 250-ml Erlenmeyer flask containing 100 ml of yeast nitrogen base without amino acids-mannitol-sucrose broth (6). After incubation (2 days, 30°C, 200 rpm in a gyratory shaker), the yeast cells were harvested; then whole-cell extracts were prepared and subjected to polyacrylamide gel electrophoresis (5). Unless stated otherwise, an extract containing 350 µg of protein was loaded into each lane of a 7.5% (wt/vol) polyacrylamide gel. A minimum of two separate preparations of extract were studied; each was prepared on a different occasion.

The staining procedures for G6PDH (200 µg of protein per lane), sorbitol dehydrogenase (5% polyacrylamide gel), malate dehydrogenase (MDH), and alkaline phosphatase have been described elsewhere (6). Esterases were detected by using either 4-methylumbelliferyl acetate or α -naphthyl acetate with Fast Blue RR (4). Glycosidases, α -glucosidase (α -GLU; 200 µg of protein per lane), β -glucosidase (5% polyacrylamide gel), and α -mannosidase (5% polyacrylamide gel), were detected with 4-methylumbelliferyl esters (4). Catalase (5% polyacrylamide gel, 100 µg of protein per lane) and superoxide dismutase (SOD; 200 µg of protein per lane) were detected as described previously (4). For SOD, one set of gels was stained with reagents containing 50 mM potassium cyanide to show the cyanide-sensitive enzyme bands.

Enzyme variation. When any two strains were compared, the majority of bands of enzyme activity were identical. This finding is consistent with our experience of comparing strains derived from a single yeast species; other *Candida* species, for example, *C. tropicalis* (6), show marked differences in their enzyme profiles relative to *C. albicans*. Within the complete set of *C. albicans* strains studied here, distinctive variation in the positions of enzymes was found in gels stained for α -GLU, G6PDH, and MDH. Examples are shown in Fig. 1. When the comparison was restricted to strains derived from *C. albicans* B311 and NCPF3153, the sole enzyme heterogeneity seen was in G6PDH. The strains derived from *C. albicans* B311 had identical isoenzyme profiles; however, strains that had been derived from *C. albicans* NCPF3153 formed two types associated with different positions for G6PDH. Therefore, the isoenzyme typing system failed to discriminate between the majority of the *C. albicans* NCPF3153-derived strains and the strains derived from *C. albicans* B311 (Table 1).

The SODs, catalase, esterases, alkaline phosphatase, and sorbitol dehydrogenase showed no heterogeneity in migration (Fig. 1). The faster-moving SOD was cyanide sensitive and therefore likely to be the copper-zinc form. Staining for esterase was poor when α -naphthyl acetate was employed, a finding that had been noticed previously with *Trichosporon* strains (4), but it improved when 4-methylumbelliferyl acetate was used as a substrate. The minor differences seen in the migration of the α -mannosidases and β -glucosidases were too marginal for reliable scoring. Faint and poorly resolved bands were seen after staining for some enzymes; however, since they were not found consistently, they were ignored in the analysis (see legend to Fig. 1).

* Corresponding author. Electronic mail address: Lehmann%opus@mcclarc.

TABLE 1. Types of *C. albicans* strains based on isoenzymes

Strain	Origin	Original designation ^a	Type ^b
C1	NCPF3153	3153	α -GLU ₁ , G6PDH ₂ , MDH ₃
C10	NCPF3153	3153	α -GLU ₁ , G6PDH ₂ , MDH ₃
C16	NCPF3153	3153	α -GLU ₁ , G6PDH ₂ , MDH ₃
C2	B311	CBS 5982	α -GLU ₁ , G6PDH ₃ , MDH ₃
C4	B311	IP 995.69	α -GLU ₁ , G6PDH ₃ , MDH ₃
C6	B311	ATCC 32354	α -GLU ₁ , G6PDH ₃ , MDH ₃
C9	B311	IP 995.69	α -GLU ₁ , G6PDH ₃ , MDH ₃
C13	B311	UCLA 335	α -GLU ₁ , G6PDH ₃ , MDH ₃
C15	B311	B311	α -GLU ₁ , G6PDH ₃ , MDH ₃
C3 ^c	B311	IP 884.65	α -GLU ₁ , G6PDH ₃ , MDH ₃
C5	NCPF3153	IP 992.69	α -GLU ₁ , G6PDH ₃ , MDH ₃
C7	NCPF3153	IP 884.65	α -GLU ₁ , G6PDH ₃ , MDH ₃
C8	NCPF3153	IP 992.69	α -GLU ₁ , G6PDH ₃ , MDH ₃
C12	NCPF3153	UCLA 300	α -GLU ₁ , G6PDH ₃ , MDH ₃
C14	NCPF3153	3153A	α -GLU ₁ , G6PDH ₃ , MDH ₃
C11	Unrelated		α -GLU ₁ , G6PDH ₁ , MDH ₂
MCO50	Unrelated	CDC 3181A	α -GLU ₂ , G6PDH ₁ , MDH ₃
NCPF	Unrelated	NCPF3324	α -GLU ₁ , G6PDH ₁ , MDH ₁

^a CDC, Centers for Disease Control, Atlanta, Ga.; full details for remaining sources were given previously (7).

^b Type for each enzyme based on the patterns shown in Fig. 1.

^c The likely derivation of strain C3 from *C. albicans* B311, rather than from *C. albicans* NCPF3153, is based on its restriction fragment length polymorphism profile, 5-fluorocytosine resistance, and resistogram results (7).

In addition to the G6PDH variation, heterogeneity among strains of the NCPF3153 set has now been observed for restriction fragment length polymorphisms produced after digestion of chromosomal DNA with *EcoRI*, morphotypes, biotypes, and resistotypes (7). Furthermore, compared with the B311 strains, the NCPF3153 strains showed greater heterogeneity when analyzed by pyrolysis mass spectrometry (9). In contrast to the strains derived from NCPF3153, the B311-derived strains showed no variation in their isoenzymes and, in general, appeared to be more homogeneous than the NCPF3153-derived strains. Although there was some heterogeneity found among their biotypes, all B311-derived strains were reported as having identical restriction fragment length polymorphism profiles, and they showed little variation when analyzed by resistotyping, morphotyping, and pyrolysis-mass spectrometry (7, 9).

Among potential causes of the strain-to-strain variation found within the *C. albicans* NCPF3153 set is the possibility of contamination of the cultures during their storage. The three strains (C1, C10, and C16 from London, Leeds and Villeneuve d'Ascq) have a discrete G6PDH type. These same strains can be placed in a subgroup based both on their resistotype and on their morphotype (wrinkled surface). However, these particular phenotypes do not exclude all the remaining strains in the NCPF3153 set, and restriction fragment length polymorphisms show that strain C1 differs from the other strains of the set (7). Because this subgroup of NCPF3153 strains (i.e., C1, C10, and C16) neither is homogeneous nor differs remarkably from all other NCPF3153-derived strains, it is unlikely to have arisen from a single incident of cross-contamination in the laboratory.

FIG. 1. Enzyme profiles of *C. albicans* strains. EST, esterase stained with 4-methylumbelliferyl acetate; SOD, SOD stained in the absence of cyanide. α -MAN, α -mannosidase. MDH, G6PDH, and α -GLU showed obvious variants. Note that the upper bands in the gel stained for SOD (especially in lanes 1 and 2) and the lower faint esterase bands (all lanes) were neither well resolved nor present in replicate gels performed with the same strains. Lanes 1 through 10 contained strains NCPF, C11, MCO50, C1, C10, C2, C14, C12, C6, and C13, respectively. *, strain derived from *C. albicans* NCPF3153; +, strain derived from *C. albicans* B311.

Although contamination of some NCPF3153 cultures remains a possibility, it is possible to explain their heterogeneity by predicting NCPF3153 to be a strain that is more liable to fix mutations than is B311. Such a property could have led to significant alterations in its genotype during its domestication in laboratories over the last 30 or more years. In this regard, strain C12 (Table 1) and other strains derived from *C. albicans* NCPF3153 have been reported to generate nongerminating variants with fairly high frequency even in the absence of deliberate mutagenesis (reviewed in reference 2).

It would have been preferable to have tested the organisms with a larger panel of isoenzyme assays that were capable of discriminating between strains, but we did not discover other enzymes that were useful. Such testing might have helped both in the separation of the B311 group from the NCPF3153 group of strains and in assessing the chances for a new type of *C. albicans* NCPF3153 to have arisen by mutation or through contamination during domestication. This distinction is of importance in the epidemiological analysis of *C. albicans* types, because mutations may accumulate during an infection and during examination of the yeast strains in culture. Such mutations could be expected to be unrelated to the changes that are associated with phenotypic switching (14). These changes were first observed for a derivative of the isolate from which NCPF3153 was obtained. Although alterations in the electrophoretic karyotype have been observed, specific mutations in genes leading to switching in colony phenotype have not been described (11).

Other methods have been developed for genetic fingerprinting of *C. albicans* (3, 10). They include the use of nucleic acid probes that bind to multiple midrepeat sequences (12, 13) and the digestion of *C. albicans* DNA with restriction enzymes allowing strain characterization based on the lengths of DNA fragments bearing genes that are detectable by hybridization (8). Probes for midrepeat sequences can be powerful tools for detecting mutations in the absence of strong selective pressure; for instance, Scherer and Stevens commented on the detection of a change in one of four undefined strains of *C. albicans* that had been grown in rich media for ~300 generations (12). These probes appear to have great discriminatory power, and they should also be useful in monitoring mutations occurring during the long-term maintenance of cultures.

Although isoenzyme profiles were not found to be helpful in discriminating between derivatives from the two reference strains, this study emphasizes that mycologists should be cautious in their handling of reference cultures. As is true of other groups of microorganisms, fungi can undergo significant changes during cultivation in vitro. Persons responsible for maintaining reference cultures of fungi and those using these cultures in experiments, for example, as controls in antifungal screening assays or for studies on virulence, should plan strategies to minimize the number of passages in vitro that are needed for maintaining the strain. Given the consistent finding of phenotypic and genetic heterogeneity in the NCPF3153-derived set, strains of the *C. albicans* B311

pedigree may prove to be the better choice for situations where a replicable control is needed.

The panel of strains derived from NCPF3153 and B311 (ATCC 32354) is recommended to others who wish to determine the value and limitations of different methods used for strain delineation in *C. albicans*. It is maintained at the Mycological Reference Laboratory, Colindale, London.

This work was supported in part by Public Health Service grant AI 25679 (to P.F.L.) from the National Institutes of Health and by a Biomedical Research Support grant from the Medical College of Ohio.

REFERENCES

1. Hasenclever, H. F., and W. O. Mitchell. 1961. Antigenic studies of *Candida*. I. Observation of two antigenic groups in *Candida albicans*. *J. Bacteriol.* **82**:570-573.
2. Howard, D. H., M. L. Zeuthen, and N. Dabrowa. 1986. Phenotypic characteristics of a slow-growing, nongerminating variant of *Candida albicans*. *J. Gen. Microbiol.* **132**:2359-2366.
3. Hunter, P. R. 1991. A critical review of typing methods for *Candida albicans* and their applications. *Crit. Rev. Microbiol.* **17**:417-434.
4. Kemker, B. J., P. F. Lehmann, J. W. Lee, and T. J. Walsh. 1991. Distinction of deep versus superficial clinical and nonclinical isolates of *Trichosporon beigelii* by isoenzymes and restriction fragment length polymorphisms of rDNA generated by the polymerase chain reaction. *J. Clin. Microbiol.* **29**:1677-1683.
5. Lehmann, P. F., C.-B. Hsiao, and I. F. Salkin. 1989. Protein and enzyme electrophoresis profiles of selected *Candida* species. *J. Clin. Microbiol.* **27**:400-404.
6. Lehmann, P. F., B. J. Kemker, C.-B. Hsiao, and S. Dev. 1989. Isoenzyme biotypes of *Candida* species. *J. Clin. Microbiol.* **27**:2514-2521.
7. Mackenzie, D. W. R., and F. C. Odds. 1991. Non-identity and authentication of the two major reference strains of *Candida albicans*. *J. Med. Vet. Mycol.* **29**:255-261.
8. Magee, B. B., T. M. D'Souza, and P. T. Magee. 1987. Strain and species identification by restriction fragment length polymorphisms in the ribosomal DNA repeat of *Candida* species. *J. Bacteriol.* **169**:1639-1643.
9. Magee, J. T., J. M. Hindmarch, B. I. Duerden, and D. W. R. Mackenzie. 1988. Pyrolysis mass spectrometry as a method for inter-strain discrimination of *Candida albicans*. *J. Gen. Microbiol.* **134**:2841-2847.
10. Merz, W. G. 1990. *Candida albicans* strain delineation. *Clin. Microbiol. Rev.* **3**:321-334.
11. Rustchenko-Bulgac, E. P., F. Sherman, and J. B. Hicks. 1990. Chromosomal rearrangements associated with morphological mutants provide a means for genetic variation of *Candida albicans*. *J. Bacteriol.* **172**:1276-1283.
12. Scherer, S., and D. A. Stevens. 1988. A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. *Proc. Natl. Acad. Sci. USA* **85**:1452-1456.
13. Schmid, J., E. Voss, and D. R. Soll. 1990. Computer-assisted methods for assessing strain relatedness in *Candida albicans* by fingerprinting with the moderately repetitive sequence Ca3. *J. Clin. Microbiol.* **28**:1236-1243.
14. Soll, D. R., R. Galask, S. Isley, T. V. G. Rao, D. Stone, J. Hicks, J. Schmid, K. Mac, and C. Hanna. 1989. Switching of *Candida albicans* during successive episodes of recurrent vaginitis. *J. Clin. Microbiol.* **27**:681-690.