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Protein and Enzyme Electrophoresis Profiles of Selected *Candida* Species

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The cellular protein profiles and malate dehydrogenases, superoxide dismutases, alkaline phosphatases, and esterases from whole cell extracts of *Candida* spp. were studied with polyacrylamide gel electrophoresis. We investigated isolates that differed in their ability to assimilate sucrose as the sole carbon source. The protein and enzyme patterns of *Candida tropicalis* and its sucrose-negative variant "*Candida paratropicalis* Baker, Salkin, Pincus et D'Amato" were indistinguishable. Although the cellular protein and superoxide dismutase patterns of *Candida albicans* and its sucrose-negative variant "*Candida stellatoidea*" were quite similar, differences were noted in the profiles of the other enzymes studied. In addition, the *C. stellatoidea* isolates were found to be separable, on the basis of their enzyme profiles, into the same two types that have been reported by Kwon-Chung et al. (K. J. Kwon-Chung, B. L. Wickes, and W. G. Merz, Infect. Immun. 56:1814–1819, 1988).

The anamorph genus *Candida* includes a heterogeneous group of yeastlike organisms composed of the anamorphs of both ascomycetous and basidiomycetous fungi (14). Species identification is based on a number of phenotypic characteristics, of which carbohydrate assimilation is of primary importance. However, because there is a relatively low number of such phenotypic characters, species may be separated, in some instances, on the basis of a single character. We have studied *Candida albicans* (Robin) Berkhout and *Candida tropicalis* (Castellani) Berkhout; each of these species can be subdivided into two groups based on an ability or inability to utilize sucrose as the sole carbon source. Although the sucrose-negative isolates have been given species status in the past, "*Candida stellatoidea* (Jones et Martin) Langeron et Guerra" and "*Candida paratropicalis* Baker, Salkin, Pincus et D'Amato" are now considered to be variants of *C. albicans* and *C. tropicalis*, respectively, rather than being distinct species (4, 14). This decision has been based on both morphologic and physiologic studies including, in the case of *C. stellatoidea*, DNA reassociation techniques (1, 4, 13, 14). However, for the sake of clarity, we have chosen to use the older species designations throughout this communication. That a question exists concerning the taxonomic relationship of the sucrose-negative and sucrose-positive strains of *Candida* species has been reemphasized in the recent report of two types of *C. stellatoidea* (10). *C. stellatoidea* type II was described as being closer to *C. albicans* than was *C. stellatoidea* type I, based in part on pathogenicity for mice and similarity in chromosomal migration patterns.

We report here the cellular protein profiles and enzyme patterns of these four *Candida* species as determined by using polyacrylamide gel electrophoresis (PAGE). The profiles show a close relationship between *C. tropicalis* and *C. paratropicalis*; these were distinct from *C. albicans*. *C. stellatoidea* would appear to be separable, as has been reported, into two types (10).

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MATERIALS AND METHODS

Test organisms. Test organisms are listed in Table 1. They were obtained as subcultures of fresh clinical isolates and of stocks in the collections of one of the authors (I.F.S.) and of K. J. Kwon-Chung (10), B. B. Magee (11), and W. S. Riggsby (12). All were identified by standard morphologic and physiologic procedures (2, 14). Isolates of *C. paratropicalis* were identified as described previously (2, 3). The identification of *C. stellatoidea* isolates as type I or II was made or confirmed by using extracellular proteinase production tests as described by Kwon-Chung et al. (10). Yeast strains were stored at −70°C in sterile glycerol solution (15 ml of glycerol, 85 ml of water). For the experiments described here, the yeast cells were maintained on glucose-neopeptone agar (D-glucose, 20 g; neopeptone, 10 g; agar, 15 g; distilled water, 1 liter). Petri dish cultures were subcultured every 2 to 3 weeks.

Preparation of extracts and PAGE. For protein extraction, one or two colonies of test organisms were inoculated from 2-day-old subcultures into 50 ml of nutrient broth (yeast extract, 10 g; peptone, 20 g; D-glucose, 20 g; distilled water, 1 liter) in 250-ml Erlenmeyer flasks. The flasks were then placed onto a reciprocal shaker and incubated for 20 to 26 h at 100 rpm and 30°C. The cell suspensions from each flask were then harvested by centrifugation at 2,000 × *g* for 10 min and washed twice in 0.1 M Tris hydrochloride buffer (pH 8.0). The yeast cells were broken with glass beads. The procedure, based on that described for *C. albicans* by Hazen and Cutler (9), involved mixing 3 to 4 ml of glass beads (0.45 to 0.50 mm diameter; Biospec, Bartlesville, Okla.), which had been cleaned previously with chromic-sulfuric acid cleaning solution (Fisher Scientific Co., Pittsburgh, Pa.), with an equal volume of a 50% (vol/vol) suspension of yeast cells in the Tris hydrochloride buffer. The mixture, which was held in a 50-ml polypropylene screw-top tube, was agitated vigorously four times (7,000 rpm for 20 s each time, room temperature) with a high-speed vortex mixer (Wizard Mixer; Rees Scientific, Lambertsville, N.J.). Before each agitation, the tubes were held in an ice bucket for at least 1

TABLE 1. Yeast strains used[a]

Strain (synonym[s])	Source
C. albicans (30 strains used)	
MCO 43 (C9[b])	
MCO 50 (3181A[d])	
MCO 351 (A9[e])	Oral swab
C. stellatoidea (type I)	
MCO 265 (CPA5Am5[f])	
MCO 276 (B452,[g] ATCC 11006[h])	
MCO 277 (B4257,[g] ATCC 36232[h])	
MCO 279 (B4404[g])	
MCO 280 (B4406[g])	
MCO 281 (121[c])	
MCO 282 (233-84[c])	Peritoneal fluid
MCO 283 (234-84[c])	Vaginitis
MCO 284 (114[c])	Prostatic fluid
MCO 285 (115[c])	
MCO 286 (124[c])	
MCO 287 (632[c])	
MCO 288 (671[c])	
MCO 289 (683[c])	
MCO 290 (697[c])	
C. tropicalis (15 strains used)	
MCO 291 (451-85[c])	Sputum
MCO 292 (464-85[c])	Urine
MCO 293 (622-85[c])	Sputum
C. stellatoidea (type II)	
MCO 311 (B4403[g])	
MCO 368 (B4365,[g] ATCC 20408[h])	
MCO 370 (B4405)	
C. paratropicalis	
MCO 301 (974-85[c])	Lung aspirate
MCO 302 (1506-85[c])	Bronchial wash
MCO 303 (841-85[c])	Bronchial wash
MCO 304 (710-85[c])	Sputum
MCO 305 (636-85[c])	Blood
MCO 306 (635-85[c])	Blood
MCO 307 (622-85[c])	Bronchial wash
MCO 308 (571-85[c])	Sputum
MCO 309 (464-85[c])	Urine
MCO 310 (451-85[c])	Sputum

[a] Only the strains of *C. albicans* and *C. tropicalis* shown in Fig. 1 through 4 are included.
[b] From P. T. Magee, Michigan State University, East Lansing.
[c] Mycology Laboratory, New York Department of Health, Albany, N.Y.
[d] Serotype A strain from E. Reiss, Centers for Disease Control, Atlanta, Ga.
[e] Serotype B isolate from W. L. Whelan, National Institutes of Health, Bethesda, Md.
[f] From Magee and Magee (11).
[g] Synonymous strain names listed in Kwon-Chung et al. (10) and Mason et al. (12).
[h] American Type Culture Collection.

min to chill the suspension. After homogenization, the glass beads were allowed to settle, and the supernatant was collected. The soluble extracts were obtained after centrifugation (13,000 × *g*, 2 min, 25°C) to remove cellular debris and were held in an ice bath. The protein concentrations of the extracts were determined by the Bradford (6) method with bovine serum albumin as a standard. The extracts were then diluted in Tris hydrochloride buffer (0.1 M, pH 8.0) to 5 mg of protein per ml, and 100-μl samples of each were loaded onto native discontinuous polyacrylamide gels (1.5-mm-thick slab). The extracts were subjected to electrophoresis in Tris hydrochloride-glycine discontinuous buffer (separation at pH 9.5, 7.5% [wt/vol] acrylamide in resolving gel, 4°C, 5 to 6 V/cm, approximately 15 h) under nondissociating conditions (7). During the electrophoresis, proteins passed through a stacking gel (2.5% [wt/vol] acrylamide) and then into the resolving gel (13 to 14 cm high) in the direction of the anode.

Protein and enzyme detection. Proteins were stained with Coomassie brilliant blue G250 (400 mg per liter of 3.5% [vol/vol] perchloric acid) (15). Enzymes were detected after washing the gel two times with buffers appropriate for enzyme activity and prepared as previously described (8). Alkaline phosphatases were detected with a cocktail of α-naphthyl phosphate (30 mg), $CaCl_2$ (1 mM), $MgCl_2$ (1 mM), and $MnCl_2$ (1 mM) in Tris hydrochloride (100 ml, 25 mM, pH 8.0). Malate dehydrogenase (MDH) and superoxide dismutate (SOD) were stained with L-malic acid (300 mg), NAD (10 mg), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (5 mg), and phenazine methosulfate (5 mg) in Tris hydrochloride buffer (25 ml, 25 mM, pH 8.0). The MDH was detected by a dark purple formazan band, whereas unstained areas formed during exposure to light were indicative of SOD, since these enzymes can oxidize the formazan. Esterases were detected with a cocktail of α-naphthyl acetate (30 mg) and Fast Blue RR (100 mg) in Tris hydrochloride buffer (100 ml, 25 mM, pH 8.0).

Reagents. Agar (Bacto-Agar), neopeptone, peptone, and yeast extract were obtained from Difco Laboratories (Detroit, Mich.). *N-N'*-methylene-bisacrylamide, Tris, glycine, NAD, α-naphthyl phosphate, α-naphthyl acetate, L-malic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, phenazine methosulfate, and Fast Blue RR were obtained from Sigma Chemical Co. (St. Louis, Mo.). Coomassie brilliant blue G250 was obtained from Eastman Kodak Co. (Rochester, N.Y.). Acrylamide (electrophoretic grade) was obtained from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). Other reagents were analytical reagent or American Chemical Society grades.

RESULTS

The cellular protein and enzyme gel patterns are shown in Fig. 1 through 4. Although only three isolates of each species are shown in each figure, the patterns are typical of all isolates studied. However, it should be noted that in this portion of the investigation, all isolates of *C. stellatoidea* were type I (10).

The protein (Fig. 1) and enzyme patterns (Fig. 2 through 4) clearly distinguish the *C. albicans-C. stellatoidea* pair from the *C. tropicalis-C. paratropicalis* pair. No major differences were found between *C. tropicalis* and *C. paratropicalis* in any of the gels. An isozymal variant of alkaline phosphatase was detected in 1 of the 10 *C. tropicalis* strains (Fig. 3). The stronger upper esterase band of *C. tropicalis* in Fig. 4 was not consistently found and reflects strain variability.

In contrast, although the total protein and SOD patterns for *C. albicans* and *C. stellatoidea* type I strains were very similar, the MDH profiles were distinct. Three MDH bands migrated close together in *C. stellatoidea* as compared with

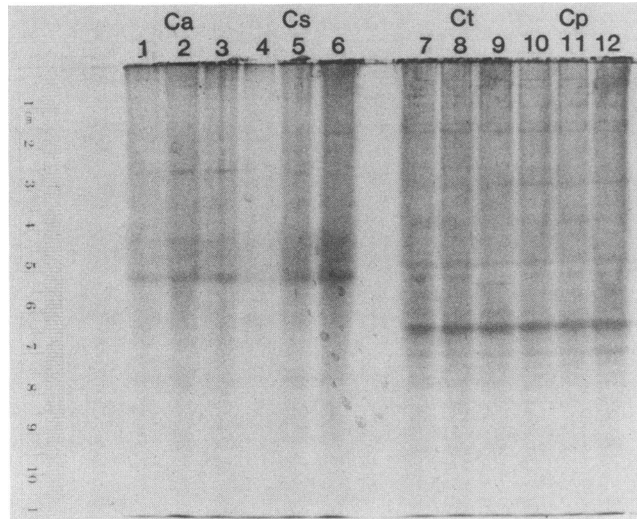

FIG. 1. Protein bands stained with Coomassie brilliant blue G250 after PAGE of cell extracts of *Candida* spp. The anode is at base of figure. Lanes 1 through 3: *C. albicans* (Ca) MCO 50, MCO 351, and MCO 43, respectively. Lanes 4 through 6: *C. stellatoidea* (Cs) MCO 281, MCO 282, and MCO 283, respectively (all were type I strains). Lanes 7 through 9: *C. tropicalis* (Ct) MCO 291, MCO 292, and MCO 294, respectively. Lanes 10 through 12: *C. paratropicalis* (Cp) MCO 301, MCO 302, and MCO 303, respectively.

the two widely separate MDH bands in *C. albicans* (Fig. 2). However, the most motile MDH band migrated the same distance in preparations of *C. albicans* and *C. stellatoidea* isolates. Differences in alkaline phosphatase production were also found; *C. albicans* produced a single band, but no detectable activity was noted with isolates of *C. stellatoidea* (Fig. 3). Finally, esterase bands were distinct, with a faint band present in *C. albicans* preparations but absent in those of *C. stellatoidea* (Fig. 4).

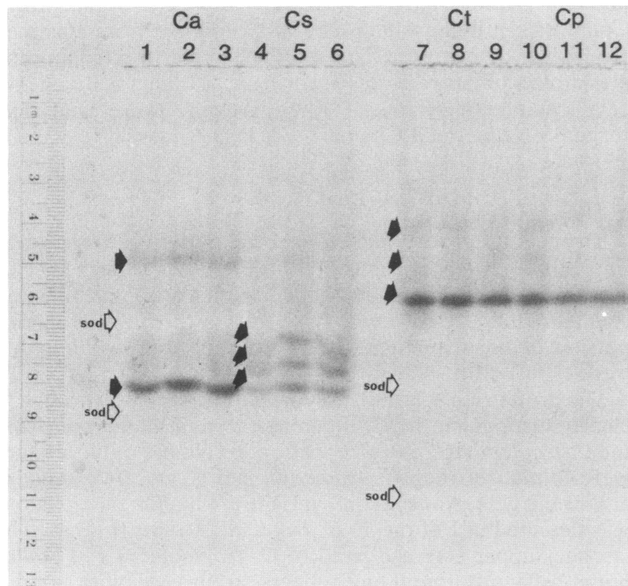

FIG. 2. MDH and SOD activity stain shown after PAGE of cell extracts of *Candida* spp. Lanes are as described in the legend to Fig. 1. Filled arrows show MDH bands, and open arrows show SOD bands not shown clearly in the photograph.

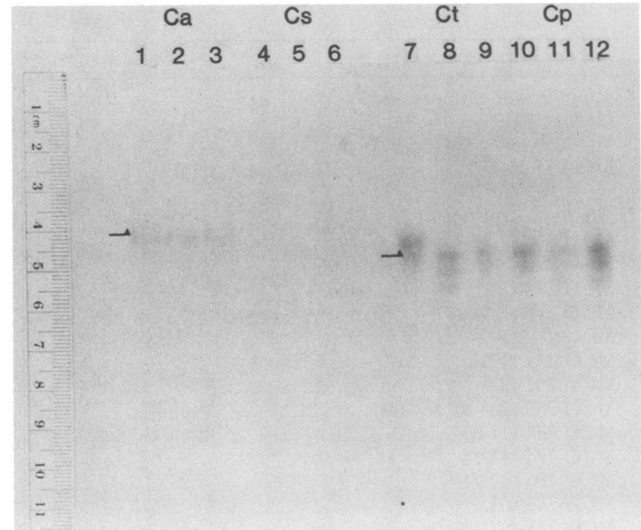

FIG. 3. Alkaline phosphatase activity stain after PAGE of extracts of *Candida* spp. Lanes are as described in the legend to Fig. 1. The left-hand arrow shows enzyme activity in lanes 1 through 3 but not in lanes 4 through 6. The right-hand arrow shows isozymal variant pattern in MCO 291.

In comparative studies of type I and type II *C. stellatoidea* isolates, type II isolates were found to have enzyme patterns more characteristic of *C. albicans* than those of type I (Fig. 5). Indeed, type II isolates were indistinguishable from *C. albicans* with our PAGE tests. For example, a faint esterase band was observed only in type II preparations.

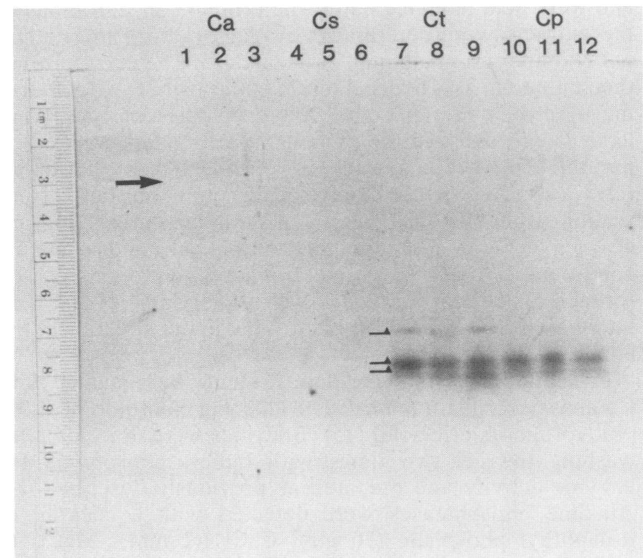

FIG. 4. Esterase activity stain after PAGE of extracts of *Candida* spp. Lanes are as described in the legend to Fig. 1. The left-hand arrow points to the position of an extremely faint band of activity seen in lanes 1 through 3 but not in lanes 4 through 6. Central arrows show bands with esterase activity in *C. tropicalis* and *C. paratropicalis*. Because the reaction was stopped at a time appropriate for the development of bands in lanes 7 through 12, the band in lanes 1 through 3 was not dense enough to be detected in this photograph. It is equivalent to the faint band seen in lanes 2 through 4 in Fig. 5, bottom right panel.

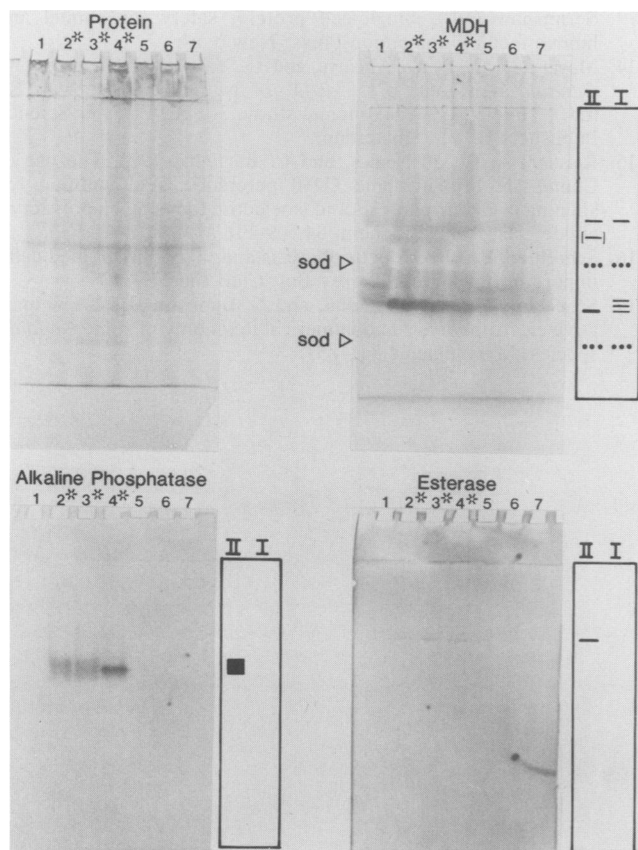

FIG. 5. Protein and enzyme activity stains after PAGE of extracts of *C. stellatoidea* types I and II. A schematic diagram has been included beside the gels. Lanes 1 through 7: MCO 371, MCO 370, MCO 311, MCO 368, MCO 276, MCO 277, and MCO 280, respectively. Type II strains are identified with an asterisk (*). Proteins were stained with Coomassie brilliant blue G250. The patterns of SOD bands (●●●) are similar for both type I and type II strains.

DISCUSSION

The identification of fungal form genera and species is based on a body of morphologic and physiologic characters. Variability in these characters is expected and accepted by most mycological taxonomists. For example, although *C. albicans* is defined, in part, by its ability to form germ tubes under specific environmental conditions, a small percentage of isolates of this yeastlike fungus may fail to form these distinctive structures. In addition, almost all *Candida* species vary in their ability to assimilate one or more carbohydrate sources. Consequently, such variability in the characters used to differentiate these species does not in itself negate the entire body of information accumulated for individual taxa, nor does it invalidate the taxonomic status of a given species.

We have studied the cellular protein and enzyme electrophoresis patterns of two pairs of closely related *Candida* species. Although similar investigations have been used to a limited extent to distinguish *Candida* species (5, 16, 17), this is the first investigation to use this tool in studies of the recently reported *C. stellatoidea* types (10). Cellular protein and SOD profiles were found to be quite similar in *C. albicans* and *C. stellatoidea*; however, differences were noted in the migration of the MDHs and the availability of alkaline phosphatases and esterases in these species. In addition, similar differences were found in the same enzymes in the *C. stellatoidea* preparations. Whereas the MDH, alkaline phosphatase, and esterase profiles of the type II *C. stellatoidea* isolates described by Kwon-Chung et al. were indistinguishable from those of *C. albicans*, those of type I isolates were clearly different. Thus, in agreement with the findings of Kwon-Chung et al., who studied different characters, we find *C. stellatoidea* type I isolates to be readily distinguishable from *C. albicans* and *C. stellatoidea* type II isolates. However, although the differences may be viewed as being significant taxonomically and could suggest that the *C. stellatoidea* type I isolates belong to a distinct species or variety rather than being variants of *C. albicans*, it must be noted that the characters that differ represent only a part of the descriptions of the organisms. Indeed, DNA homology would seem to argue for a close similarity between *C. stellatoidea* and *C. albicans*. Meyer (13) reported that an isolate derived from the same material used to prepare the nomenclatural type of *C. stellatoidea* (a type I sensu Kwon-Chung et al.) showed a very close DNA homology with *C. albicans*. Furthermore, our own studies show that the protein and SOD profiles of the *C. stellatoidea* type I isolates are not observably different from those of other *C. albicans* isolates, yet the profiles associated with another species, in this case *C. tropicalis*, are clearly distinct.

In contrast, the cellular protein profile and all of the enzyme profiles of *C. tropicalis* and *C. paratropicalis* were found to be virtually indistinguishable. Such similarities are to be expected with PAGE when closely related organisms are compared; these results would support the suggestions of some authors to reduce *C. paratropicalis* to synonymy with *C. tropicalis* (4, 14). However, taking the total body of data that have accumulated concerning the isolates of this sucrose-negative variant, there remain several characters by which these may be distinguished from the more typical isolates of *C. tropicalis* (2, 3).

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LITERATURE CITED

1. **Ahearn, D. G., S. A. Meyer, G. Mitchell, M. A. Nicholson, and A. I. Ibrahim.** 1977. Sucrose-negative variants of *Candida tropicalis*. J. Clin. Microbiol. **5**:494–496.
2. **Baker, J. G., I. F. Salkin, D. H. Pincus, and R. F. D'Amato.** 1981. Diagnostic characters of an atypical *Candida*. J. Clin. Microbiol. **13**:199–203.
3. **Baker, J. G., I. F. Salkin, D. H. Pincus, and R. F. D'Amato.** 1981. *Candida paratropicalis*, a new species of *Candida*. Mycotaxon **13**:115–119.
4. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 1983. Yeasts: characteristics and identification. Cambridge University Press, Cambridge.
5. **Berchev, K., and I. Izmirov.** 1967. Isoenzymes of some oxidoreductases in the *Candida* genus as a basis of species identification after electrophoresis. Experientia **23**:961–962.
6. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
7. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 404–427.
8. **Harris, H., and D. A. Hopkinson.** 1976. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Co., Amsterdam.

9. **Hazen, K. C., and J. E. Cutler.** 1982. Optimal conditions for breaking medically important yeasts by an inexpensive and simple method. Mycopathologia **80:**113–116.
10. **Kwon-Chung, K. J., B. L. Wickes, and W. G. Merz.** 1988. Association of electrophoretic karyotype of *Candida stellatoidea* with virulence for mice. Infect. Immun. **56:**1814–1819.
11. **Magee, B. B., and P. T. Magee.** 1987. Electrophoretic karyotypes and chromosome numbers in *Candida* species. J. Gen. Microbiol. **133:**425–430.
12. **Mason, M. M., B. A. Lasker, and W. S. Riggsby.** 1987. Molecular probe for identification of medically important *Candida* species and *Torulopsis glabrata*. J. Clin. Microbiol. **25:**563–566.
13. **Meyer, S. A.** 1979. DNA relatedness between physiologically similar strains and species of yeasts of medical and industrial importance, p. 13–19. *In* S. Garattini, S. Paglialunga, and N. S. Scrimshaw (ed.), Single-cell protein: safety for animal and human feeding. Pergamon Press, New York.
14. **Meyer, S. A., D. G. Ahearn, and D. Yarrow.** 1984. Genus 4. *Candida* Berkhout, p. 585–844. *In* N. J. W. Kreger-van Rij (ed.), The yeasts. A taxonomic study, 3rd ed. Elsevier Science Publishers B. V., Amsterdam.
15. **Reisner, A. H., P. Nemes, and C. Bucholtz.** 1975. The use of Coomassie brilliant blue G250 perchloric acid solution for staining in electrophoresis and isoelectric focusing on polyacrylamide gels. Anal. Biochem. **64:**509–516.
16. **Schechter, Y.** 1973. Electrophoresis and taxonomy of medically important fungi. Bull. Torrey Bot. Club **100:**277–287.
17. **Schechter, Y., J. W. Landau, and N. Dabrowa.** 1972. Comparative electrophoresis and numerical taxonomy of some *Candida* species. Mycologia **64:**841–853.