

Association of Electrophoretic Karyotype of *Candida stellatoidea* with Virulence for Mice

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Seven isolates of *Candida stellatoidea* were studied for their electrophoretic karyotype, virulence for mice, sensitivity to UV radiation, growth rate in vitro, reaction on cycloheximide-indicator medium, and proteinase activity. The isolates exhibited one of two distinct electrophoretic karyotypes as determined by orthogonal field alternating gel electrophoresis (OFAGE). Four isolates, including the type culture of *C. stellatoidea*, belonged to electrophoretic karyotype type I by OFAGE, showing eight to nine bands of which at least two bands were less than 1,000 kilobases in size as estimated by comparison with the DNA bands of *Saccharomyces cerevisiae*. These isolates failed to produce fatal infection in mice within 20 days when 5×10^5 cells were injected intravenously. The yeasts were cleared from the kidneys of two of three mice tested by day 30. Type I showed proteinase activity on bovine serum albumin agar at pH 3.8 and produced a negative reaction on cycloheximide-bromocresol green medium within 48 h. The three grouped in type II by OFAGE showed banding patterns similar to those of a well-characterized isolate of *Candida albicans*. The isolates of type II had an electrophoretic karyotype of six to seven bands approximately 1,200 kilobases or greater in size. All three type II isolates were highly virulent for mice, producing fatality curves similar to those of a previously studied *C. albicans* isolate. From 80 to 90% of the mice injected with 5×10^5 cells intravenously died within 20 days. The type II isolates produced a positive reaction on cycloheximide-bromocresol green agar and showed no proteinase activity on bovine serum albumin agar at the low pH. In addition, the type II isolates grew faster and were significantly more resistant to UV irradiation than the type I isolates. These results indicated that type II, but not type I, isolates can be considered simply as sucrose-negative *C. albicans*.

Candida stellatoidea is presently treated as a synonym for *Candida albicans* (16). The two yeasts are indistinguishable in morphology of yeast cells, germ tubes, and chlamydo-spores. The two yeasts share over 90% homology in their total DNA (15) and show similar carbon assimilation spectra except for sucrose. *C. albicans* assimilates sucrose, whereas the isolates of *C. stellatoidea* fail to utilize the sugar. They are both diploid organisms (8, 23) and contain similar amounts of DNA (8). Molecular genetic studies have shown differences as well as similarities between the two species. Mason et al. (12) showed that the flanking restriction sites of the highly conserved actin gene were identical in the two species and different from other medically important *Candida* species. Magee and co-workers showed that the karyotypes determined by orthogonal field alternation gel electrophoresis (OFAGE) and the restriction fragment patterns of ribosomal DNA are distinguishable between the two species (9, 10).

One of the reasons earlier workers considered *C. stellatoidea* a separate species (5, 11) was the difference in the pathogenicity of the two species. Martin et al. (11) observed that the yeast cells of *C. stellatoidea* failed to produce any evidence of infection in rabbits, while isolates of *C. albicans* produced fatal infection with an equivalent dose. The reports on virulence of *C. stellatoidea* for mice are mixed; while most isolates of *C. stellatoidea* show lower virulence than *C. albicans*, some isolates of *C. stellatoidea* were more virulent than the low-virulence isolates of *C. albicans* (5).

We report, in this paper, the occurrence of two distinct types of *C. stellatoidea* which are separable by their electrophoretic karyotypes. The karyotypes were associated

with differences in virulence for mice, sensitivity to UV light, growth rate in vitro, and proteinase activity on bovine serum albumin (BSA) agar at pH 3.8.

MATERIALS AND METHODS

Isolates. The seven isolates of *C. stellatoidea* used for the study were received either from the American Type Culture Collection or S. Riggsby: B-4252 (ATCC 11006), B-4257 (ATCC 36232), B-4365 (ATCC 20408), B-4403 (SR1114), B-4404 (SR1145), B-4405 (SR1144), and B-4406 (SR1146). The four isolates received from Riggsby were originally either from Spain (B-4403) or from the Centers for Disease Control in Atlanta, Ga. (B-4404 through B-4406). Upon receipt of the cultures, clonal isolations were made on yeast extract-peptone-glucose (YEPD) agar (yeast extract, 10 g; peptone, 20 g; glucose, 20 g; agar, 20 g; 1 liter of distilled water) medium. The purified isolates were tested for their germ tube formation, chlamydo-spore formation, and carbon assimilation profile. All produced germ tubes and chlamydo-spores and were identified as *C. stellatoidea* according to carbon assimilation spectra tested with the API 20C system. Their inability to assimilate sucrose was reconfirmed by the absence of growth on yeast nitrogen base plus 2% sucrose (YNBS) agar plates. The isolates were maintained on YEPD as well as malt extract agar slants.

Morphology. To study the colony morphology, yeast cells were streaked (thick single streaks) on malt extract and YEPD agar plates. The color, texture, and presence or absence of mycelial fringes of the colonies were examined after 1 week of incubation at 30°C. Cell morphology was studied with Dalmau plate cultures on corn meal agar, as well as on malt extract agar, after 48 h at 30°C.

OFAGE. An OFAGE apparatus with specifications of

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Carle and Olson (2) was used. Chromosome-sized DNA was prepared according to the modifications of Merz et al. (14) of the embedded agarose procedure of Schwartz and Cantor (20). OFAGE was performed as described by Carle and Olson (2). The circulating buffer was $0.5 \times$ TBE (44 mM Tris, 44 mM boric acid, 2 mM EDTA, pH 8.3) maintained at 10°C , and electrophoresis was carried out using 1.0% agarose in $0.5 \times$ TBE with 5-min alternating pulses of 120 V for 48 h. Gels were stained with ethidium bromide ($1 \mu\text{g}/\text{ml}$ in distilled water) for 10 to 15 min and destained for 30 min to 1 h in water. *Saccharomyces cerevisiae* YP-49 (courtesy of Philip Hieter) and *C. albicans* B-4201 (strain C9 in reference 7) were used as standards.

Determination of virulence. The yeast cells from 48-h malt extract agar cultures were suspended in sterile saline, and serial dilutions were prepared. Dilutions containing 2.5×10^6 cells per ml, as determined by hemacytometer and plate count, were used as inocula. Ten female general-purpose white mice, each weighing 20 to 24 g, were used for each isolate. Mice were injected with 0.2 ml of inoculum (5×10^5 cells) into the lateral tail vein and observed for up to 20 days to determine survival rates. The CFU in the kidneys were counted according to the method previously described (7).

UV irradiation. The cell suspensions prepared from 48-h cultures on malt extract agar were diluted, counted by hemacytometer, and plated on malt extract agar plates (150 to 250 cells per plate) immediately. Seeded plates were irradiated for 5, 10, 15, 20, and 25 s with a Sylvania germicidal lamp (type G, 30T8). The dose rate at the agar surface was $32 \text{ ergs}/\text{mm}^2 \text{ per s}$ as measured with an intensity meter (model J225; Ultraviolet Products Inc., San Gabriel, Calif.). The plates were incubated at 30°C for 48 h before colonies were counted. Percent survival for each isolate was computed, and UV doses (based on exposure time) required for 50% death (UV LD_{50} s) were compared using least-square analysis. Statistical significance for the difference in the UV sensitivity between two subtypes was analyzed by Student *t* test.

Cycloheximide indicator medium. Salkin developed a cycloheximide-containing diagnostic medium for the differentiation of *C. stellatoidea* from *C. albicans* (19). The medium contained 100 mg of cycloheximide and 28 ml of 0.08% bromocresol green (indicator) per liter of modified Sabouraud agar (3). The control plates used were the same except that the cycloheximide was omitted. Cell suspensions of the seven *C. stellatoidea* isolates and one *C. albicans* strain (B-4201) were prepared and plated on the medium by the method of Salkin (19) and incubated at 30°C for 48 h. In another series, the cell suspensions were diluted to contain 1×10^3 to 2×10^3 cells per ml. Samples of 0.1 ml were plated on the medium and incubated for 48 h at 30°C .

Determination of growth rate. Growth rates of the isolates in YEPD broth at 30°C were determined according to the method described previously (7). Cells collected from 48-h malt agar slants were transferred into 10 ml of YEPD broth and incubated for 15 h on a shaker (200 rpm). At the end of 15 h of incubation, 150 μl of the culture was transferred into fresh medium (100 ml) and absorbancy at 600 nm (Gilford 2600 spectrophotometer) was measured at 1-h intervals. The initial optical density was approximately 0.05.

Detection of proteinase activity. A small loopful of yeast cells from 48-h YEPD agar cultures was patched onto bovine hemoglobin agar at pH 5.5 (7) and BSA agar at pH 3.8 (17). The plates were incubated at 30 or 37°C for 1 week.

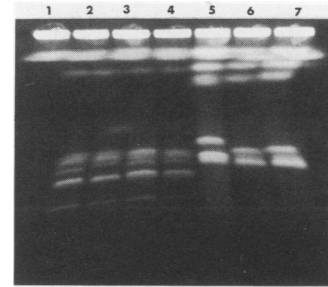


FIG. 1. Chromosome-sized DNA bands of seven *C. stellatoidea* isolates employed in the study. Lanes: 1, B-4404; 2, B-4406; 3, B-4252; 4, B-4257; 5, B-4365; 6, B-4403; 7, B-4405.

RESULTS

Electrophoretic karyotype. The chromosome-sized DNA-banding patterns of seven isolates of *C. stellatoidea* are shown in Fig. 1. The isolates were largely divided into two distinct types according to the number and migration patterns of their DNA bands. Four isolates, B-4404 (Fig. 1, lane 1), B-4406 (lane 2), B-4252 (lane 3), and B-4257 (lane 4), showed at least eight ethidium bromide-stained DNA bands. These isolates were designated as type I *C. stellatoidea*. The remaining three isolates, B-4365 (lane 5), B-4403 (lane 6), and B-4405 (lane 7), had no more than six to seven resolved bands. These isolates were designated as type II *C. stellatoidea*.

The comparison of bands in type I isolates with those of *C. albicans* and *S. cerevisiae* is presented in Fig. 2. There were eight discernible bands in *C. albicans* (lane 1). Since *C. albicans* is a diploid yeast which has been reported to have six chromosomes (22), the homologs of two small chromosomes appeared to have been resolved to show eight bands. Although isolate B-4406 (Fig. 2, lane 3) showed an extra band with very low intensity, the migration pattern of the bands in the four type I isolates were nearly identical. In all four isolates, there were at least two bands less than 1,000 kilobases in size as estimated by comparison with the bands of *S. cerevisiae* (Fig. 2, lane 6). Banding patterns of type II isolates were compared with those of *C. albicans* (Fig. 3, lane 1) and *S. cerevisiae* (Fig. 3, lane 5). The number and migration patterns of the DNA bands from the three type II isolates (Fig. 3, lanes 2 through 4) were remarkably similar to those of *C. albicans* (lane 1). No small bands were observed in type I isolates, and all bands in type II appeared to be 1,200 kilobases or greater in size.

Virulence for mice. The survival rate of mice inoculated intravenously with 5×10^5 *C. stellatoidea* cells is shown in

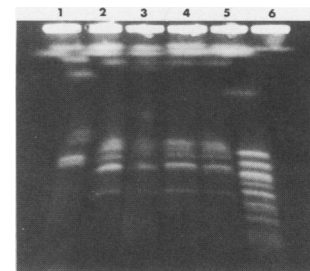


FIG. 2. Chromosome-sized DNA bands of *C. albicans* B-4201 (lane 1), type I isolates of *C. stellatoidea* (lanes 2 through 5), and *S. cerevisiae* YP-49 (lane 6).

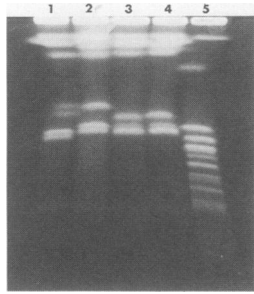


FIG. 3. Chromosome-sized DNA bands of *C. albicans* B-4201 (lane 1), type II isolates of *C. stellatoidea* (lanes 2, 3, and 4, B-4365, B-4403, and B-4405, respectively), and *S. cerevisiae* (lane 5).

Fig. 4. All three isolates of type II *C. stellatoidea* produced 80 to 90% mortality within 20 days. The average survival time for these fatally infected mice varied from 8.6 to 11 days, depending on the isolate. One of the surviving mice from each group was sacrificed on day 21, and CFU in the kidneys were counted (7). Counts were 6.2×10^6 CFU/g of tissue for the mouse infected with B-4365 and 1.4×10^7 and 5.1×10^7 for the mice infected with B-4403 and B-4405, respectively. Nearly 1,000 colonies each from these mice were replicated on sucrose agar to test for their ability to assimilate sucrose; the results showed that they were sucrose negative.

The four isolates of type I cultures failed to produce fatal infection in mice within 20 days. All 40 mice appeared normal and gained weight during the experimental period. One animal each from the groups of mice receiving B-4252, B-4404, and B-4406 was sacrificed on day 30 to count CFU in the kidneys. Yeasts were not recovered from the mice injected with B-4252 and B-4406. One mouse receiving cells of B-4404, however, had 1.9×10^4 CFU/g of kidney.

Colony and cell morphology. The colonies of type I and type II isolates grown on malt extract agar for 1 week were indistinguishable. All isolates produced smooth, cream-colored colonies with or without mycelial margins. On YEPD agar, however, the colony morphology of the two types could be differentiated by 48 to 72 h, although the degree of differences diminished as time went on. The isolates of type I produced cream-colored colonies with highly cerebriform membranous surfaces. Mycelial margins were not detectable in the type I isolates until the colonies were 1 week old. Two of the four isolates failed to produce mycelial margins. The colonies of type II were smooth on the surface until 48 h, but two of the three isolates produced rough to wrinkled centers

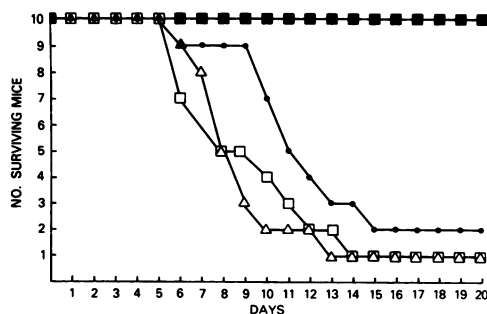


FIG. 4. Survival of mice infected with 5×10^5 cells. Symbols: ■, all four type I strains, B-4252, B-4257, B-4404, and B-4406; ●, □, △, type II strains B-4365 (●), B-4403 (□), and B-4405 (△).

by 1 week. All three isolates produced colonies with smooth margins composed of yeast cells and fringes of mycelium (Fig. 5).

The morphology of cells on Dalmau plates of corn meal agar and malt extract agar cultures examined after 24 and 48 h was indistinguishable between the two types. The cells were mostly oblong or elongated with single buds, but some globose large cells were found with multiple buds.

Growth rate. The colonies of type I isolates were always smaller than those of type II isolates on various agar media. The colony size of the isolates among the same type, however, was not significantly different. These observations suggested that the growth rate of type I isolates is slower than that of type II isolates. The isolates of type I grown in YEPD (pH 6.5) broth at 30°C had doubling times of 102 to 108 min, compared with 60 to 74 min for type II isolates. At pH 4.5, the doubling time of type I ranged from 114 to 132 min, as compared with 72 to 78 min for type II isolates.

Reaction on cycloheximide indicator medium. Yeast cells of type II and *C. albicans* B-4201 grew well on cycloheximide-bromocresol green agar medium, and the medium turned yellow in color within 48 h. The isolates of type I, however, showed only pinpoint-size colonies, and the medium remained greenish blue during the same period (Fig. 6). The same results were obtained on the indicator medium without cycloheximide. These results indicate that growth rate and not cycloheximide is responsible for the differentiation of the two types.

Sensitivity to UV radiation. A comparison of the survival rates of post-UV-irradiated cells showed a significant difference in sensitivity to UV irradiation between type I and type II cultures (Table 1). Figure 7 shows the mean survival curve of the two types. In both types, there were initial shoulders followed by exponential death rate, and the initial shoulder was more pronounced in type II than in type I. The mean UV LD₅₀ for type I isolates was 9.3 s (297.6 erg/mm²) versus 15.2 s (486.4 erg/mm²) for type II isolates. Statistical analysis by Student *t* test showed that the sensitivity to UV irradiation was significantly different ($P < 0.01$) between the two types.

Proteinase. The colonies of type I isolates produced clear halos on BSA agar (Fig. 8), but not on bovine hemoglobin agar, within 1 week. The clear zones produced around the colonies on BSA agar were wider at 37°C than those at 30°C. The colonies of type II isolates failed to produce clear zones on bovine hemoglobin agar or BSA agar within 1 week at either temperature.

DISCUSSION

C. stellatoidea is one of the infrequently reported yeasts from clinical laboratories. Of 9,479 yeasts recovered from 1977 to 1983 at the North Carolina Memorial Hospital (13), only 9 (0.09%) isolations of *C. stellatoidea* were recorded, versus 6,351 isolations of *C. albicans* (67%). In their 1966 report, Fell and Meyer (4) stated that of 2,432 yeasts obtained from routine clinical examination, 0.8% were *C. stellatoidea* in contrast to 52% *C. albicans*. It is not possible to obtain more recent data, since major clinical laboratories (including the National Institutes of Health, Bethesda, Md., and Johns Hopkins Hospital, Baltimore, Md.) no longer attempt to separate the two species as long as they produce germ tubes. *C. stellatoidea* was first isolated from vaginal tracts of women by Jones and Martin in 1937 (6) and was described as a new species by Martin et al. in that year (11). They described the yeast as a species distinct from *C. albicans* on the basis of its inability to assimilate sucrose and its stellate colony morphology on blood agar (11).

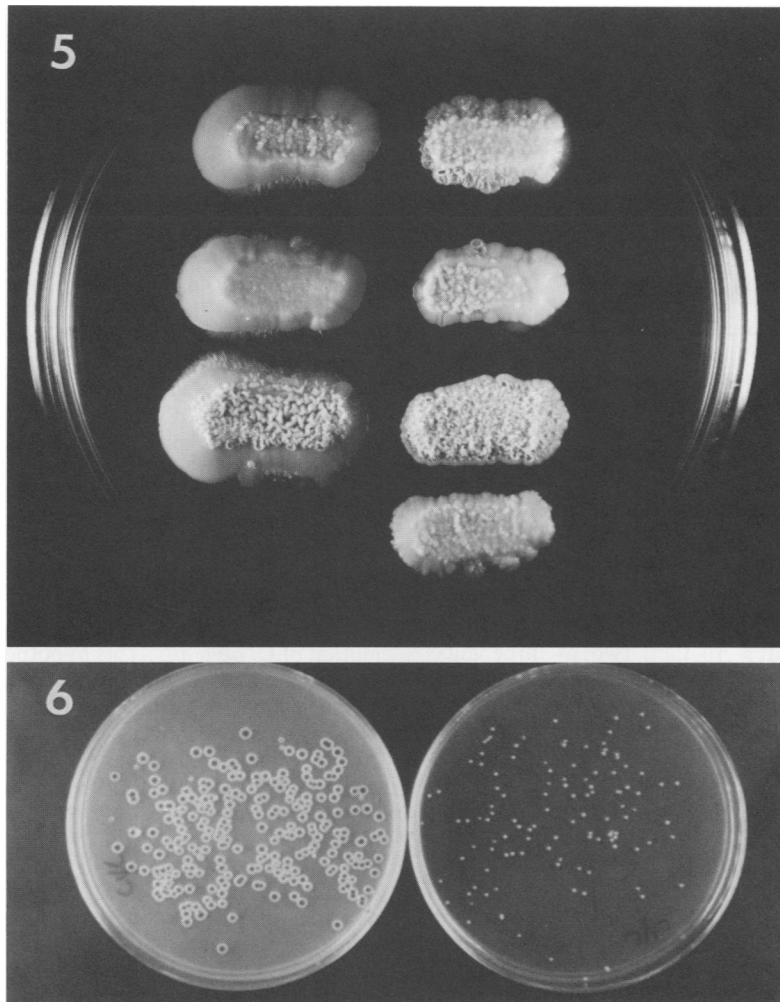


FIG. 5. Colony morphology of *C. stellatoidea* grown on YEPD agar for 1 week at 30°C. From top to bottom in the right column, type I strains B-4252, B-4257, B-4404, and B-4406; from top to bottom on the left, type II strains B-4365, B-4403, and B-4405.

FIG. 6. Growth of a type I isolate, B-4257 (right), and a type II isolate, B-4365 (left), on cycloheximide indicator medium.

One of the prevailing notions is that *C. stellatoidea* is generally less virulent than *C. albicans* (3). This belief is due to the fact that *C. stellatoidea* has been mainly reported from colonization of vaginal tracts (6, 13) rather than disseminated systemic disease, and that experimental infection in rabbits

and mice showed lower virulence than *C. albicans*. Martin et al. (11) found that all six isolates of *C. albicans* they tested produced fatal infection with kidney lesions in rabbits in 4 to 5 days. Three strains of *C. stellatoidea* in equivalent doses, however, did not cause death in any of the rabbits, and no lesions were found in an animal which was killed for examination on day 7 (11). The study by Hasenclever and Mitchell (5) showed that 10 of 11 *C. stellatoidea* isolates (90%) had LD₅₀s (in mice) between 10⁶ and 10⁸ yeast cells. On the other hand, 44 of 47 isolates (94%) of *Candida albicans* had LD₅₀s between 10⁴ and 10⁶ yeast cells (5).

The present study revealed three significant findings: (i) there are two distinct subtypes among *C. stellatoidea* in terms of their mouse virulence and other phenotypic markers; (ii) the phenotypic differences of the two groups are associated with their electrophoretic karyotypes; and (iii) type II isolates of *C. stellatoidea* are as virulent as *C. albicans* is for mice. The isolates equally as virulent as *C. albicans* had band patterns (type II) similar to those of *C. albicans* B-4201. All three isolates of type II *C. stellatoidea* produced 80 to 90% fatality in mice injected with 5 × 10⁵ cells within 20 days. The type I isolates of *C. stellatoidea*

TABLE 1. Survival of irradiated cells of seven *C. stellatoidea* isolates^a

OFAGE karyotype	Isolate	% Survival after UV dose (at 32 ergs/mm ² /s):					UV LD ₅₀ (s)
		5 s	10 s	15 s	20 s	25 s	
I	B-4252	92	61	10	4	0	10.9
	B-4257	82	35	3	0.5	0	8.3
	B-4404	83	36	5	1	0	8.6
	B-4406	91	43	3	2	0	9.4
II	B-4365	88	87	32	10	0.9	12.9
	B-4403	99.5	95	53	16	3.3	16.5
	B-4405	99.5	85	81	12	1.9	16.1

^a Mean UV LD₅₀ was 9.3 s (standard error, 0.58) for type I isolates and 15.2 s (standard error, 1.14) for type II isolates.

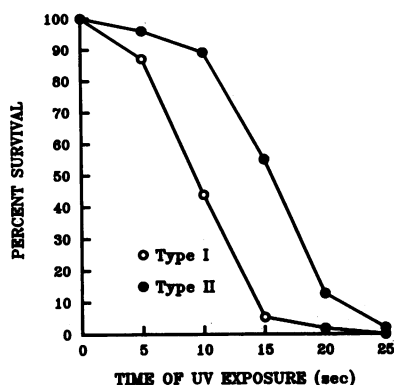


FIG. 7. Survival curves obtained for stationary-phase populations of *C. stellatoidea* post-UV irradiation. Symbols: ●, average survival of four isolates of type I; ○, average survival of three isolates of type II.

had very different OFAGE patterns from *C. albicans* B-4201. This type of band pattern was also observed by Magee and Magee in one isolate of *C. stellatoidea* they studied (10). None of four type I isolates caused death during the same period as the type II isolates. In fact, two of three mice receiving type I isolates showed no evidence of CFU in their kidneys by day 30. One animal showed 10^4 CFU/g of kidney, which is 10^2 to 10^3 fewer CFU than those found in the mice receiving type II cells.

The type strain of *C. stellatoidea* is B-4252 (ATCC 1066, CBS 1905), and it belonged to type I. It is almost certain that the strains used by Martin et al. (11) and the majority of the isolates studied by Hasenclever and Mitchell (5) were of type I.

It is significant that the electrophoretic karyotypes of the isolates we studied were also associated with increased sensitivity to UV irradiation, lower growth rate, different colony morphology on YEPD agar, and proteinase production at low pH. The isolates of type II were more resistant to UV irradiation, grew faster than type I isolates, and produced colonies indistinguishable from colonies of *C. albicans*. One of the reasons for type II isolates being more virulent than type I isolates may be their faster growth rate. The isolates of type I produced colonies with membranous surfaces on YEPD agar, due to extensive formation of hyphal cells. For this reason, homogeneous yeast cell suspensions could be prepared from 24- to 48-h malt extract agar cultures, but not from YEPD agar cultures. Although the type I colonies on YEPD agar somewhat resembled those of the "irregular wrinkle" type of *C. albicans* reported

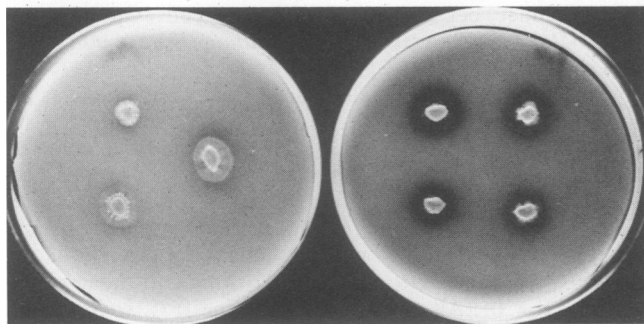


FIG. 8. Proteinase activity is shown by colonies of type I (right) but not by type II colonies (left) on BSA agar at 37°C after 1 week.

by Slutzky et al. (21), the folds on type I *C. stellatoidea* colonies were much more intricate and irregular.

Salkin's cycloheximide indicator agar medium can be used to distinguish type I from type II isolates, but not to distinguish *C. stellatoidea* from *C. albicans*. Since the indicator medium without cycloheximide produces exactly the same results as the medium with cycloheximide, the differentiation appears to be due to the faster growth rate of type II isolates. In fact, the isolates of type I also produced a positive reaction on the indicator medium by day 5.

Information on the clinical source (body sites) of the isolates used in this study is not available, except that the type culture B-4252 is almost certainly from the human vagina. The isolate B-4252 belonged to type I and produced a strong proteinase activity at pH 3.8, but not at pH 5.5. The human vagina is known to have a pH around 4.5 (18). The ability to produce proteinase at very low pH, but not at pH 5.5, by type I isolates may play a significant role for their restricted ecological niche in the human body.

C. stellatoidea was often referred to as an α -glucosidase-negative variant of *C. albicans* (1). There have not been, however, any enzymatic studies to support such a notion. In view of the identical karyotypes between type II isolates and a *C. albicans* strain, type II may be a sucrose-negative mutant of *C. albicans*. This hypothesis was supported by our experience that sucrose-positive clones can be isolated at a frequency of 10^{-7} (data not shown) from type II isolates. Sucrose-positive clones, however, were not isolated from 10^7 cells in type I isolates (data not shown). Since the type culture of *C. stellatoidea* belonged to type I karyotype and showed low virulence, only type I isolates should be referred to as *C. stellatoidea*. In any event, the present studies demonstrate that genetic heterogeneity exists among the isolates identified as *C. stellatoidea* and that the sucrose-negative phenotype, in itself, is not correlated with low virulence for mice.

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LITERATURE CITED

- 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.
- Carle, G. F., and M. V. Olson. 1984. Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. *Nucleic Acids Res.* 12:5647-5664.
- Emmons, C. W., C. H. Binford, J. P. Utz, and K. J. Kwon-Chung. 1977. *Medical mycology*, p. 535. Lea & Febiger, Philadelphia.
- Fell, J. W., and S. A. Meyer. 1967. Systematics of yeast species in the *Candida parapsilosis* group. *Mycopathol. Mycol. Appl.* 32:177-193.
- Hasenclever, H. F., and W. O. Mitchell. 1961. Antigenic studies of *Candida*. III. Comparative pathogenicity of *Candida albicans* group A, group B, and *Candida stellatoidea*. *J. Bacteriol.* 82:578-581.
- Jones, C. P., and D. S. Martin. 1938. Identification of yeast like organisms isolated from the vaginal tracts of pregnant and non-pregnant women. *Am. J. Obstet. Gynecol.* 35:98-106.
- Kwon-Chung, K. J., D. Lehman, C. Good, and P. T. Magee. 1985. Genetic evidence for role of extracellular proteinase in virulence of *Candida albicans*. *Infect. Immun.* 49:571-575.
- Kwon-Chung, K. J., B. L. Wickes, and W. L. Whelan. 1987. Ploidy and DNA content of the yeast cells of *Candida stellatoidea*. *Infect. Immun.* 55:3207-3208.

9. 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.
10. Magee, B. B., and P. T. Magee. 1987. Electrophoretic karyotypes and chromosome numbers in *Candida* species. *J. Gen. Microbiol.* **133**:425-430.
11. Martin, D. S., C. P. Jones, K. F. Yao, and L. E. Lee, Jr. 1937. A practical classification of the Monilias. *J. Bacteriol.* **34**:99-123.
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. McGinnis, M. R. 1986. Some yeasts of medical importance. VI International Conference on the Mycoses. PAHO Scientific Publication no. 479. Pan American Health Organization, Washington, D.C.
14. Merz, W. G., C. Connelly, and P. Hieter. Variation of electrophoretic karyotypes among clinical isolates of *Candida albicans*. *J. Clin. Microbiol.* **26**:842-845.
15. Meyer, S. A. 1979. DNA relatedness between physiologically similar strains and species of yeasts of medical and industrial importance, p. 13-19. *In* S. Garratini, S. Paglialunga, and N. S. Scrimshaw (ed.), *Investigation of single cell protein*. Pergamon Press, New York.
16. 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.
17. Odds, F. C., and A. B. Abbott. 1980. A simple system for the presumptive identification of *Candida albicans* and differentiation of strains within the species. *Sabouraudia* **18**:301-317.
18. Ryley, J. F. 1986. Pathogenicity of *Candida albicans* with particular reference to the vagina. *J. Med. Vet. Mycol.* **24**:5-22.
19. Salkin, I. F. 1979. New medium for differentiation of *Candida albicans* from *Candida stellatoidea*. *J. Clin. Microbiol.* **9**:551-553.
20. Schwartz, D. C., and C. R. Cantor. 1982. Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. *Cell* **37**:67-75.
21. Slutzky, B., J. Buffo, and D. R. Soll. 1985. High frequency switching of colony morphology in *Candida albicans*. *Science* **230**:666-669.
22. Snell, R. G., and R. J. Wilkins. 1986. Separation of chromosomal DNA molecules from *C. albicans* by pulsed field gel electrophoresis. *Nucleic Acids Res.* **14**:4401-4406.
23. Whelan, W. L., R. M. Partridge, and P. T. Magee. 1980. Heterozygosity and segregation in *Candida albicans*. *Mol. Gen. Genet.* **180**:107-113.