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Ploidy and DNA Content of *Candida stellatoidea* Cells

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Three isolates of *Candida stellatoidea* contained approximately the same amount of DNA per blastospore (38.3 to 41.9 fg) as did a known diploid isolate of *Candida albicans* and about twice as much as did a haploid isolate of *Saccharomyces cerevisiae*. The diploidy of *C. stellatoidea* was supported by demonstration of mitotic segregation of an *ade* marker.

Candida stellatoidea is a pathogenic yeast which has been recovered from the vagina but rarely from any other part of the human body (7). Despite the morphological and biochemical similarities between *Candida albicans* and *C. stellatoidea*, the two species were considered distinct (7) until Meyer (3) showed that there is a significant degree of DNA relatedness (>90% homology) between the type strains of the two species. On the basis of DNA relatedness, Meyer et al. treated *C. stellatoidea* as a synonym for *C. albicans* (4). It is well established that *C. albicans* is a diploid organism and that it contains approximately twice the amount of DNA of haploid isolates of *Saccharomyces cerevisiae* (5, 6). However, the high degree of DNA relatedness between *C. albicans* and *C. stellatoidea* measured by DNA reassociation techniques does not necessarily indicate that *C. stellatoidea* is also diploid.

We measured DNA content in various isolates of *C. stellatoidea* and obtained genetic evidence of diploidy by using a pink, adenine-requiring mutant and its heterozygous derivatives. The results of these experiments support the hypothesis that *C. stellatoidea* is diploid.

The identities of three *C. stellatoidea* isolates, B-4254 (from S. Riggsby), B-4257 (ATCC 36232), and B-4365 (ATCC 20408), were confirmed by carbon assimilation profile, germ tube formation, and chlamydospore formation by the isolates. The DNA contents of blastospores were determined by the diphenylamine method of Riggsby et al. (6). A genetically characterized isolate of *C. albicans* (strain 208R1) (1) and a haploid strain of *S. cerevisiae* (XP300-37A; from T. R. Manney) were used for comparison (Table 1). The mean DNA values for *C. stellatoidea* isolates were approximately 2.3 times that for haploid *S. cerevisiae* and were indistinguishable from that for *C. albicans* 208R1.

To obtain a genetically marked derivative, exponentially growing cells (10^8 cells per isolate) of the three *C. stellatoidea* isolates were exposed to ethyl methanesulfonate (Sigma Chemical Co.) by the method of Lindegren et al. (2) except that the concentration of ethyl methanesulfonate was 100 μl/2 ml of cell suspension. The ethyl methanesulfonate-treated cells were plated on 50 malt extract agar plates (5×10^3 cells per plate), and 4 h later (at 30°C), plates were irradiated with 480 ergs of UV light (type G 30T8; Sylvania) per mm^2 consecutively for three times, 24 h apart. Plates were then incubated for 72 h in the dark at 30°C. A pink, adenine-requiring strain, B-4365-P, was isolated from isolate B-4365. To select for prototrophic derivatives, approximately 10^7 B-4365-P cells were plated on minimal agar (yeast nitrogen base without amino acid plus 2% glucose; Difco Laboratories) and incubated at 30°C for 72 h to isolate white, adenine-independent strains. White, adenine-independent colonies appeared at a frequency of 3×10^{-5}. Cells from several of these white colonies (B-4365-H1, B-4365-H2, and B-4365-H3) were irradiated with UV (480 ergs/mm^2) to induce mitotic segregation (8). The spontaneous revertants (B-4365-H1, B-4365-H2, and B-4365-H3) produced 3.8 to 5.3% pink, sectored colonies upon irradiation, indicating that these isolates are heterozygous for a gene that is required for synthesis of adenine (Table 2). Pink, adenine-requiring colonies (sectored or whole) were not observed among 5×10^3 colonies of unirradiated controls. Pink colonies were also absent from the irradiated parental isolate B-4365 (*ADE/ADE*) and the prototrophic sector B-4365-H1-H0 (*ADE/ADE*) derived from B-4365-H1 (*ADE/ade*).

TABLE 1. DNA content of *C. stellatoidea* and reference strains

Isolate or strain	No. of tests[a]	Mean amt (range) of DNA (fg/cell)
C. stellatoidea isolates		
B-4254	2	38.5 (35.6–40.4)
B-4257	3	38.3 (35.5–40.4)
B-4365	1	41.3
B-4365-P (*ade/ade*)	2	41.9 (40.1–43.7)
B-4365-H1 (*ADE/ade*)	2	38.3 (36.6–39.9)
B-4365-H2 (*ADE/ade*)	2	39.2 (38.7–39.7)
Reference strains		
S. cerevisiae XP300-37A	1	17.1
C. albicans 208R1	8	39.7 (37.1–41.4)

[a] Number of tests with different cultures grown from the same stock culture for each isolate.

TABLE 2. Evidence for *ADE/ade* heterozygosity[a]

Isolate	% Survival	% Pink-adenine-requiring colonies among survivors
B-4365 (*ADE/ADE*)	28	0
B-4365-H1 (*ADE/ade*)	31	4.7
B-4365-H2 (*ADE/ade*)	35	5.3
B-4365-H3 (*ADE/ade*)	40	3.8
B-4365-H1-H0 (*ADE/ADE*)	36	0

[a] Cells (5×10^3) irradiated with UV light at 480 ergs/mm^2. No pink, adenine-requiring colony or sector was observed among 5,000 colonies from unirradiated controls.

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Our results indicate that the DNA content of cells of *C. stellatoidea* is the same as that of *C. albicans* cells and that *C. stellatoidea* cells are diploid (8).

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