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# Characterization of a Tetraploid Derivative of *Candida albicans* ATCC 10261

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**A morphometric analysis of *Candida albicans* yeast cells utilizing scanning electron microscopy showed that the cell volume and the DNA content of a tetraploid strain (derived by cell fusion) were 2.4 to 3.0 and 2.0 times, respectively, those of the progenitor diploid strain, ATCC 10261. The pathogenicities of both strains were similar.**

The dimorphic fungus *Candida albicans*, an opportunistic human pathogen, is generally considered to exist naturally only in the asexual state (1). The recent development of a procedure for spheroplast fusion has permitted parasexual genetic analysis of this organism (3, 4, 6). In these studies we observed that a stable prototrophic fusion product, dOG 8 (for derivation of strains, see reference 3), was considerably larger than the auxotrophic strains, hOG 2 and hOG 7, from which it was derived. Measurements from photomicrographs indicated average lengths of 6.5, 4, and 4 μm for dOG 8, hOG 2, and hOG 7, respectively (3). Recombination analyses indicated that *C. albicans* ATCC 10261 and auxotrophic derivatives (e.g., hOG 2 and hOG 7) are diploid and that the fusion products, such as dOG 8, are tetraploid (4). A further 10 stable tetraploid fusion products (isolated from a variety of strains in addition to hOG 2 and hOG 7 [3]) exhibited a similar increase in cell length over the parental strains when examined by light microscopy. There was, however, a wide variation in cell size and shape within each tetraploid strain.

Sarachek et al. (6) found that two *C. albicans* fusion products had about twice the cell volume and DNA content of the parental strains, whereas the cell volumes and DNA contents of the five other fusion products showed irregular and disproportionate increases. Their data were obtained by phase-contrast light microscopy with strains of uncertain ploidy, and it was concluded that two strains were diploid and the remaining strains were aneuploids. There is, however, a wide margin of error associated with the measurement of cell dimensions by this method because of the complex and variable cell shape and the difficulty of delineating the cell perimeter through low resolution and cell-cell overlap. Accordingly, the present morphometric analysis, utilizing strains of known ploidy and the high resolution of scanning electron microscopy (SEM), was undertaken to ascertain in more detail the size relationship between diploid and tetraploid *C. albicans* yeast cells. The DNA content and the toxicity to mice of dOG 8 were also investigated.

Prototrophic dOG 8 was isolated after fusion of hOG 2 and hOG 7 and was cultured as described previously (3). Strains hOG 2 ($Ade^-$ $Pro^-$) and hOG 7 ($Ura^-$ $Lys^-$) are auxotrophic derivatives of wild-type (prototrophic) *C. albicans* ATCC 10261. For SEM, yeast cells were suspended from yeast extract-peptone-agar slopes and washed in cacodylate buffer (0.1 M cacodylate, pH 7.2) by centrifugation and resuspension. A drop of the washed-cell suspension was spread over a glass cover slip attached to an aluminum SEM stub and then fixed by the addition of an equal volume of modified Karnovsky fixative (2% formaldehyde plus 3% glutaraldehyde in cacodylate buffer). After fixation for 60 min at room temperature, the cells were postfixed for 30 min with osmium tetroxide (2% in cacodylate buffer) and then dehydrated to absolute acetone and critical point dried from liquid carbon dioxide in a Polaron apparatus. Dried specimens were sputter-coated with gold in an argon atmosphere by using a Polaron diode unit and then examined in a Siemens Autoscan scanning electron microscope with primary acceleration of 15 to 20 kV. Electron micrographs were exposed with the specimen at a tilt angle of 0° to optimize the cross-sectional profile. Sampling was randomized by taking serial micrographs in a traverse of the cover slip through a region of suitable cell density. Micrographs were printed to a standardized magnification (×20,000) on resin-coated paper (Ilfospeed). All cell forms present in the micrographs were cut out and weighed with the exception of those that (i) were only partially represented in the field; (ii) had collapsed during preparation; or (iii) were obscured by overlying cells. Taken together, these three classes of exclusion represented about 5% of the cell population. Cell forms weighed in multiple prints obtained from a single micrograph and in multiple micrographs of the same field indicated reproducibility of greater than 98%.

SEM of ATCC 10261, hOG 2, hOG 7, and dOG 8 revealed heterogeneous populations of ovoid cells. No differences in morphology were discerned between wild type (ATCC 10261) and the auxotrophic derivatives (hOG 2 and hOG 7), and so ATCC 10261 was used in subsequent morphometric analyses. Figures 1A and B are typical fields of ATCC 10261 and dOG 8, respectively, illustrating the similarity in gross morphology but marked size difference between these strains. Linear measurements made from SEM micrographs indicated an average dimension of 4.0 μm (long axis) by 3.0 μm (short axis) for ATCC 10261 and 6.3 by 3.8 μm for dOG 8. Using the formula of Lörincz and Carter (2) for calculating the volume of yeast cells from the short and long axis measurements, the dOG 8/ATCC 10261 cell volume ratio was 2.44. The difference in size was also quantitated by weighing cell forms cut from micrographs of each strain (Fig. 2). The mean weight of dOG 8 profiles (72.9 mg; standard

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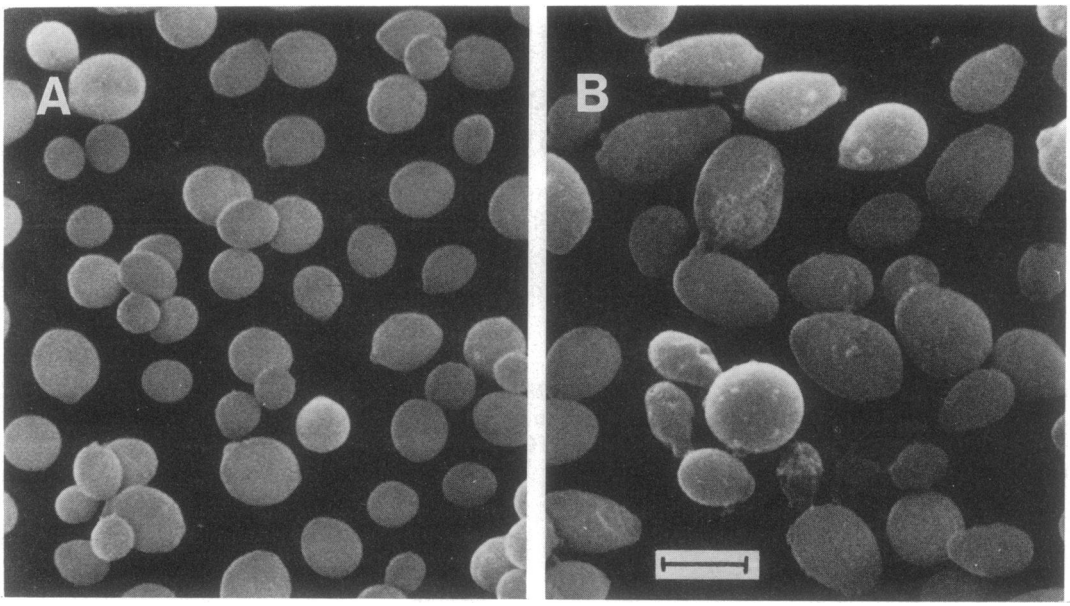


FIG. 1. Scanning electron micrographs of *C. albicans* yeast cells. (A) Wild-type diploid ATCC 10261. (B) Tetraploid fusion product, dOG 8. The two cell populations exhibit similar gross morphology but differ markedly in size. Marker bar, 5 μm.

deviation = 24.59, $n$ = 523) was 2.17 times that of ATCC 10261 profiles (33.8 mg; standard deviation = 12.07, $n$ = 442). The wide range of shape complicates assessments of volume, but by simply considering the cells as spheres, a twofold increase in cross-sectional area would result in a threefold increase in cell volume.

In recent recombination studies, we showed that *C. albicans* is naturally diploid and that fusion products, such as dOG 8, are tetraploid (4). Using a modified diphenylamine procedure (reference 7, method B), we found that the DNA content of *C. albicans* ATCC 10261 (37 ± 2.5 fg per cell) was the same as that of diploid, and twice that of haploid, *Saccharomyces cerevisiae* reference strains (38 ± 5.6 and 20 ± 3.3 fg per cell, respectively). The fusion product, dOG 8, had a DNA content (78 ± 1.5 fg per cell) twice that of ATCC 10261, consistent with the proposed tetraploid state. The ATCC 10261 value is in close agreement with data obtained from other *C. albicans* strains (5, 6).

To assess pathogenicity, 0.25-ml suspensions of yeast cells containing either $2 \times 10^7$ or $2 \times 10^8$ cells per ml of 0.85% (wt/vol) NaCl were injected into tail veins of mice restrained in a holder maintained at 40°C. The toxicity of dOG 8 was high and was similar to that of the wild-type strain, ATCC 10261, resulting in the death of all animals

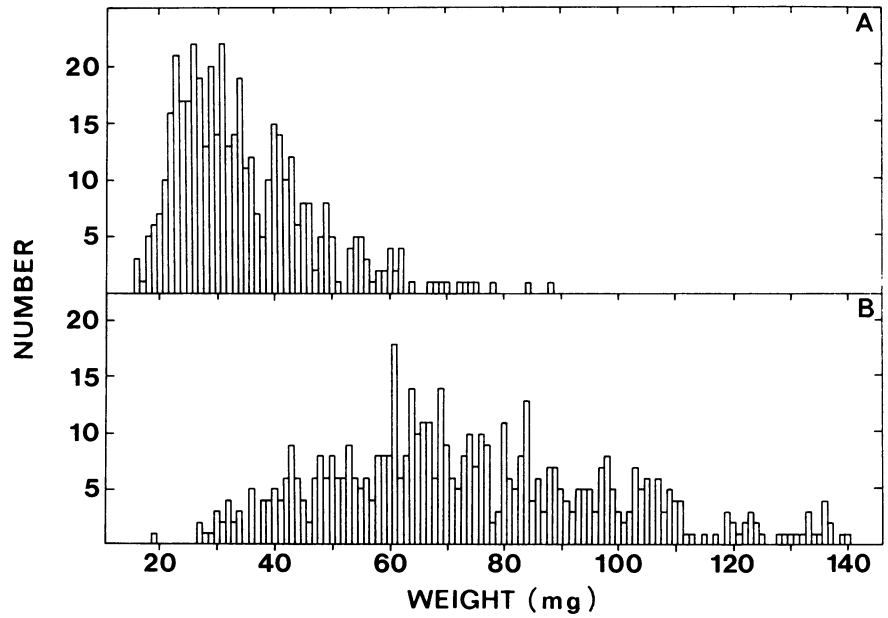


FIG. 2. Cell size distribution of diploid and tetraploid *C. albicans* strains. Cell size was measured by weighing cell forms cut from scanning electron micrographs of (A) ATCC 10261 and (B) dOG 8.

TABLE 1. Toxicity of *C. albicans* ATCC 10261 and derivatives after intravenous injection into mice[a]

| Strain[b] | Cells injected | No. of animals | No. of survivors after 15 days |
|---|---|---|---|
| ATCC 10261 | $5 \times 10^7$ | 14 | 0 |
| | $5 \times 10^6$ | 18 | 5 |
| dOG 8 | $5 \times 10^7$ | 16 | 0 |
| | $5 \times 10^6$ | 11 | 4 |
| hOG 2 | $5 \times 10^7$ | 11 | 11 |
| hOG 7 | $5 \times 10^7$ | 12 | 12 |

[a] Healthy adult random-bred laboratory mice fed on ad libitum pellet diet.
[b] For strain designations, see text and reference 3.

within 15 days (Table 1). In contrast, the auxotrophic derivatives, hOG 2 and hOG 7, were nontoxic when administered under identical conditions (Table 1). These data indicate that the tetraploid is similar to the parental diploid strain in pathogenicity, supporting the *Candida* nature of the strain. The construction of the tetraploid from nonpathogenic auxotroph strains implies that complementation is occurring and that some relationship between pathogenicity and prototrophy may exist.

In conclusion, this study has shown that a *C. albicans* tetraploid strain, dOG 8, has similar pathogenicity and twice the DNA content of the parental strain, ATCC 10261. The cell volume of the tetraploid, however, is 2.4 to 3 times that of the diploid, implying that cell volume, ploidy, and DNA content are not strictly interrelated in this organism.

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