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Isolation of a Variant of *Candida albicans*

HELEN R. BUCKLEY,* MARGARET R. PRICE,† AND LOLITA DANEO-MOORE

Department of Microbiology and Immunology, Temple University School of Medicine, Philadelphia, Pennsylvania 19140

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During the course of *Candida albicans* antigen production, a variant of this organism was encountered which did not produce hyphae at 37°C. Presented here are some of the characteristics of this variant. It produces hyphae at 25°C on cornmeal agar and synthetic medium plus *N*-acetylglucosamine and Tween 80. At 37°C, it does not produce hyphae on these media, although *C. albicans* normally does produce hyphae under these circumstances. In liquid synthetic medium, this variant does not produce hyphae at 37°C. The variant strain was analyzed for DNA, RNA, protein content, and particle size. After 50 to 70 h in balanced exponential-phase growth, particle size distribution was narrow, and there were no differences in the DNA, RNA, or protein content per particle in the two strains. When balanced exponential-phase cultures were brought into stationary phase, both strains contained the same amount of DNA per cell.

Candida albicans is a yeast which has the capability of producing hyphae under certain conditions at both 25 and 37°C. At 25°C, cornmeal agar and rice infusion agar containing 1% Tween 80 (1) favor the formation of hyphae and are used in many clinical diagnostic laboratories for the identification of species of *Candida*. At 37°C, serum and serum substitutes (17), egg white (2), various peptone media (6, 9), Sabouraud medium adjusted to pH 7.5 (5), *N*-acetylglucosamine (13, 14), and a synthetic medium (10) all favor the production of hyphae. The production of hyphae from blastospores has been considered by some as a major factor in pathogenesis (18), but this has been disputed by others (15).

During the course of mycelial antigen production in synthetic medium (16), a variant form of *C. albicans* has been isolated which produces hyphae at 25°C but is unable to do so at 37°C in a variety of substrates. This variant also was nonpathogenic for mice inoculated with 10^5 and 10^6 organisms intravenously and for rabbits inoculated with 3×10^6 organisms.

Here we also examine the DNA, RNA, and protein content per particle of this variant grown at 23 to 25°C. This *C. albicans* variant, which is temperature sensitive for the production of hyphae, provides a tool for the study of morphogenetic requirements.

† Present address: Baylor College of Medicine, Houston, TX 77030.

MATERIALS AND METHODS

C. albicans strain B311 (Hasenclever, National Institutes of Health) was streaked out for purity on Sabouraud medium. A total of 100 colonies was isolated and tested for their ability to produce germ tubes in fetal calf serum at 37°C. Three fresh clinical isolates were also examined in the same manner. The colonies were classified as variants if they did not produce germ tubes at 37°C.

The studies presented here were done with a colony isolated which produced 100% germ tubes in fetal calf serum (B311-10) and one which produced no germ tubes in fetal calf serum (B311V6).

The ability to produce hyphae at 25 and 37°C was tested in liquid and solid media. The liquid media were synthetic medium (10), synthetic medium plus 1% Tween 80, and synthetic medium plus *N*-acetylglucosamine (2.5 mM). Solid media were the same three media with the addition of 1.5% agar and cornmeal agar plus 1% Tween 80. The media were inoculated with a cut streak and covered with a glass cover slip. The liquid media growth was examined after 4, 6, 12, and 24 h of growth, and the solid media growth was examined after 48 h.

Two groups of 50 Swiss-Webster female mice (25 to 30 g) were inoculated intravenously with 10^5 and 10^6 cells, respectively, of both the variant and the germ tube producer, and two New Zealand rabbits (2.5 kg) each were inoculated with 3×10^6 organisms. Hematoxylin and eosin stain and periodic acid-Schiff stain were done on representative rabbit and mice kidneys, lungs, and spleens, and these tissues were cultured on Emmons' modifications of Sabouraud medium.

C. albicans B311-10 and B311V6 grown for 24 h on Sabouraud agar slants were inoculated directly into a chemically defined liquid medium (SMC, 10) and were incubated with shaking on a gyrotary shaker (200 rpm) at temperatures from 23 to 26°C (Table 1). Growth was

TABLE 1. Morphological changes in liquid media after 24 h of growth

Medium	B311-10		B311V6	
	25°C	37°C	25°C	37°C
SMC	Budding yeast cells	Germ tubes and clumps of hyphae	Budding yeast cells	Budding yeast cells
SMC and Tween 80 (1%)	Budding yeast cells	Germ tubes and clumps of hyphae	Budding yeast cells	Budding yeast cells
SMC and *N*-acetylglucosamine (2.5 mM)	Budding yeast cells	Germ tubes and clumps of hyphae	Budding yeast cells	Budding yeast cells

monitored in a Coleman 14 spectrophotometer (Coleman Instrument Corp., Maywood, Ill.) by determining the absorbance at 675 nm (A_{675}) of cultures below 0.5 absorbance unit or by making appropriate dilutions in SMC before absorbance determinations. Under these conditions, a linear relationship could be obtained upon dilution. Dry weight determinations gave 0.5 ± 0.05 mg/ml per absorbance unit.

Morphology was monitored by observing fixed cells in a Zeiss microscope. Particle number and volumes were obtained from cells fixed for 1 to 48 h in 9.7% formaldehyde and then analyzed in an Electrozone/Celloscope particle counter (Particle Data Corp., Elmhurst, Ill.). Mean cell volumes were derived from a printout of mean cell diameters, assuming a spherical shape. The instrument was calibrated by using 2.03-μm diameter polystyrene beads, and counts were made in 0.5% NaCl.

For long-term experiments, cultures were maintained in balanced exponential growth by repeated dilutions at A_{675} below 0.01. The absence of bacterial growth was checked by plating portions of cultures on blood agar.

DNA content was determined using the diphenylamine reaction (3) with deoxyribose as a standard. Alternatively, cultures were grown in the presence of [^{14}C]uracil for at least six mass doublings (0.5 μCi/ml with 50 μg/ml of carrier uracil). Samples (0.5 ml) were precipitated in 5 ml of 10% trichloroacetic acid and were extracted with alkali by the method of Hatzfield (8). The residue from alkali treatment could be solubilized totally by hydrolysis in 10% trichloroacetic acid for 1 h at 90°C. Approximately 6% of the total trichloroacetic acid-precipitable counts were alkali resistant.

RNA (plus DNA) and protein contents were estimated by using [^{14}C]uracil and [^{3}H]leucine incorporation into 10% trichloroacetic acid-precipitable material. The residue from a pronase digest of the [^{3}H]leucine incorporation product was less than 12% and the residue from an acid hydrolysis of the [^{14}C]-uracil incorporation product was less than 2.4% trichloroacetic acid-precipitable after hot-acid hydrolysis.

All radioisotopes were obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

Upon purification of the original B311 strain, there were 2 colonies from the 100 picked that did not produce germ tubes in fetal calf serum. We present data on one of these colonies (B311V6). This colony was cloned in the same manner, and no germ tube producer colonies were found. A colony which was picked as a 100% germ tube producer (B311-10) did not yield any nongerm tube producers when cloned. Studies on three clinical isolates did not reveal a variant population. The confirmation of the identification of this B311V6 strain as *C. albicans* was done by David Yarrow at the Centraal-

TABLE 2. Morphological changes in solid media after 48 h of growth

Medium	B311-10		B311V6	
	25°C	37°C	25°C	37°C
SMC	Chains of budding yeast cells	Hyphae	Short chains of budding yeast cells	Short chains of budding yeast cells
SMC and Tween 80 (1%)	Hyphae	Hyphae	Hyphae	Short chains of budding yeast cells
SMC and *N*-acetylglucosamine (2.5 mM)	Hyphae	Hyphae	Hyphae	Short chains of budding yeast cells
Cornmeal agar	Hyphae	Hyphae	Hyphae	Short chains of budding yeast cells

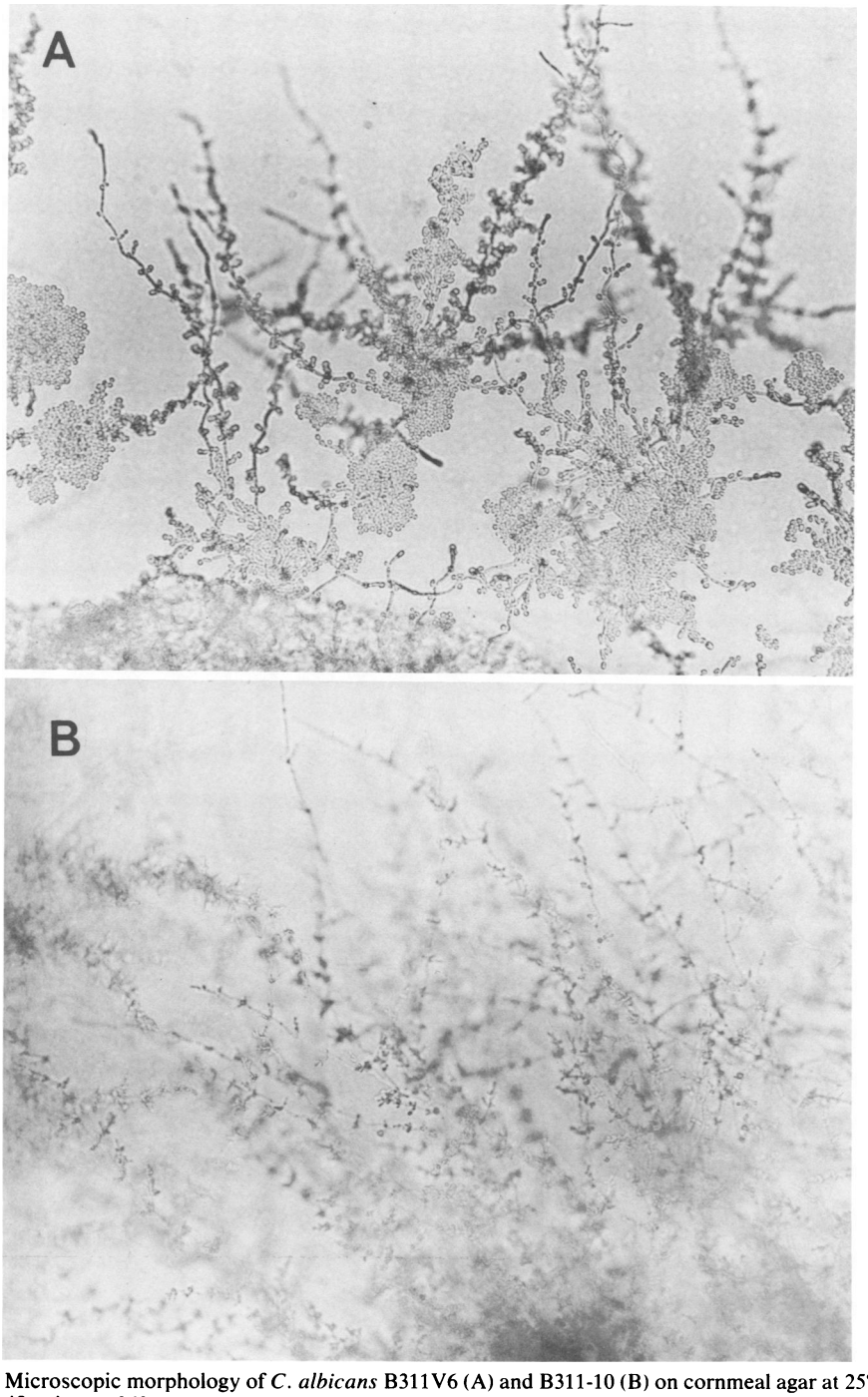

FIG. 1. Microscopic morphology of *C. albicans* B311V6 (A) and B311-10 (B) on cornmeal agar at 25°C after 3 days. Magnification, ×960.

bureau voor Schimmelcultures, Delft, the Netherlands.

B311-10 and B311V6 were examined after various periods of growth in the liquid media. Table 1 shows the morphological changes seen in liquid media after 24 h of growth, and Table 2 shows the changes seen in solid media after 48 h. B311V6 is identical to B311-10 except that it is unable to produce hyphae at 37°C (Tables 1 and 2). Figure 1 shows the microscopic morphology

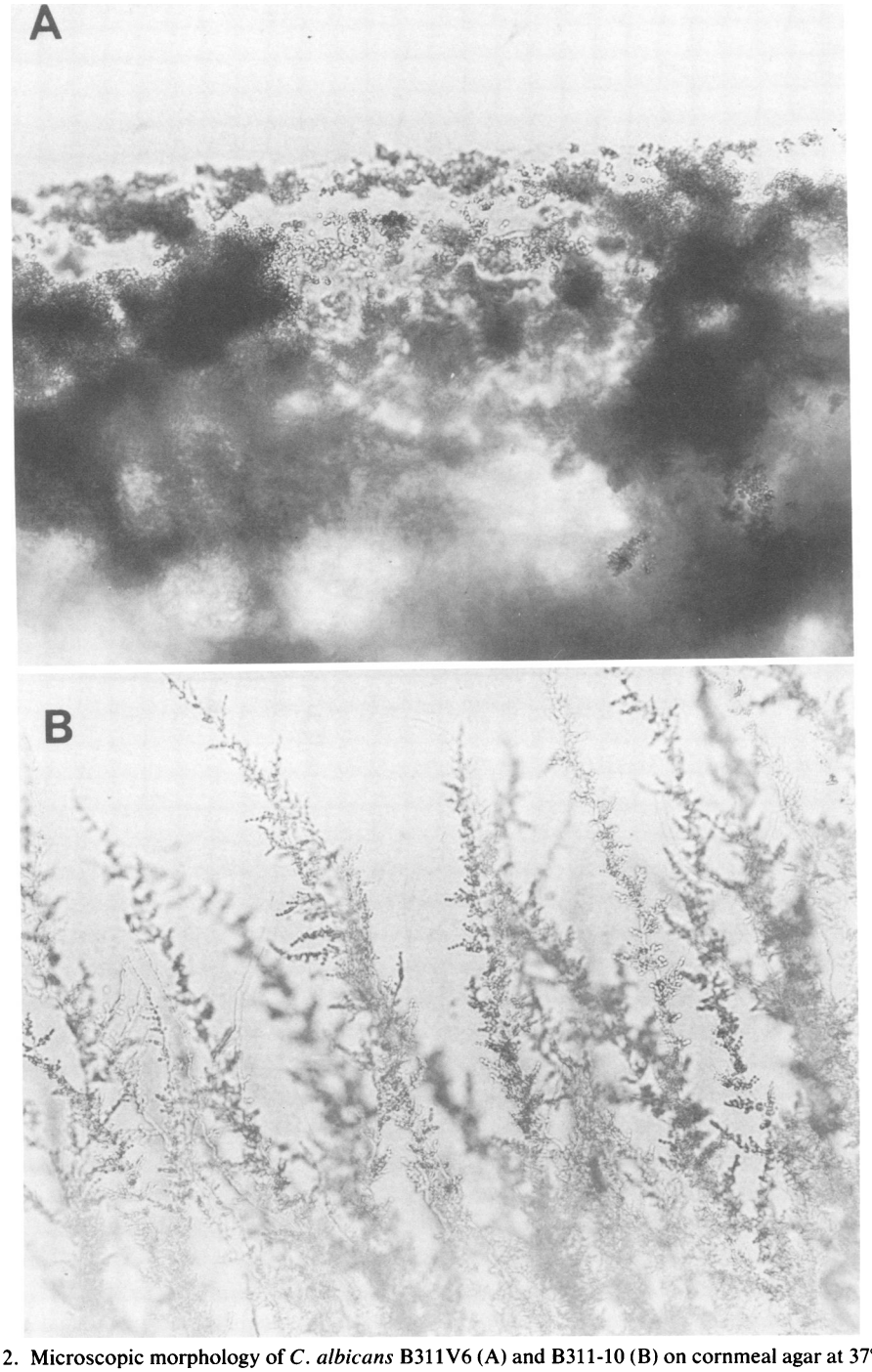

FIG. 2. Microscopic morphology of *C. albicans* B311V6 (A) and B311-10 (B) on cornmeal agar at 37°C after 3 days. Magnification, ×960.

of both B311V6 and B311-10 on cornmeal agar with Tween 80 at 25°C. Both strains produced chlamydospores at 25°C. It can be seen that B311V6 can produce hyphae at 25°C. Figure 2 shows these same two strains grown on the same medium at 37°C. As can be seen, B311V6 does not show hyphae production under these conditions at 37°C.

TABLE 3. Effect of temperature on cell separation of B311-10 and B311V6[a]

Strain	Temperature[b] (°C)	Generation time (min)	Singlets, doublets, or triplets (%)	More than four in chains (%)	More than four in clusters (%)
B311V6	23	132	30.7	44.8	24.5
B311-10	23	132	57.0	15.7	27.3
B311V6	24	144	70.8	20.2	9.0
B311-10	24	144	54.9	18.5	26.6
B311V6	27	120	70.4	20.2	9.2
B311-10	27	120	44.7	19.8	35.4

[a] The time in exponential-growth phase was 36 h in all cases.
[b] Temperature was maintained at ±0.5°C throughout the experiments.

All mice and rabbits inoculated with B311-10 died within 4 to 10 days and showed the pathological features associated with *C. albicans* infections. Kidneys and lungs showed the typical hyphae and yeast forms of the organisms; and *C. albicans* was isolated from these organs after plating on Sabouraud medium. In no case did an animal succumb to infection with B311V6. The

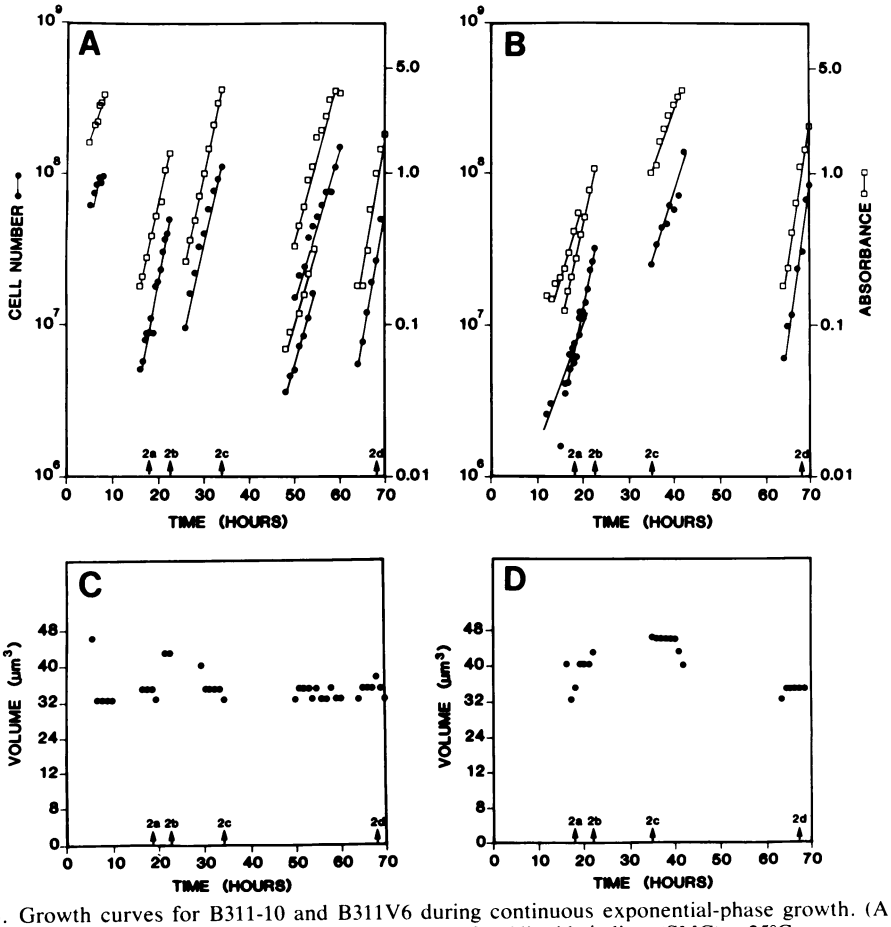

FIG. 3. Growth curves for B311-10 and B311V6 during continuous exponential-phase growth. (A) Absorbance (□) and cell numbers (●) of B311-10 in chemically defined liquid medium (SMC) at 25°C over a period of 70 h. Dilutions were made at the top of each growth curve to maintain the cultures in the exponential phase of growth. Arrows indicate the times when samples were taken for the volume distributions. (B) Same data for B311V6 in SMC at 25°C over a period of 70 h. (C and D) Mean cell volumes over the same period of 70 h for B311-10 and B311V6, respectively.

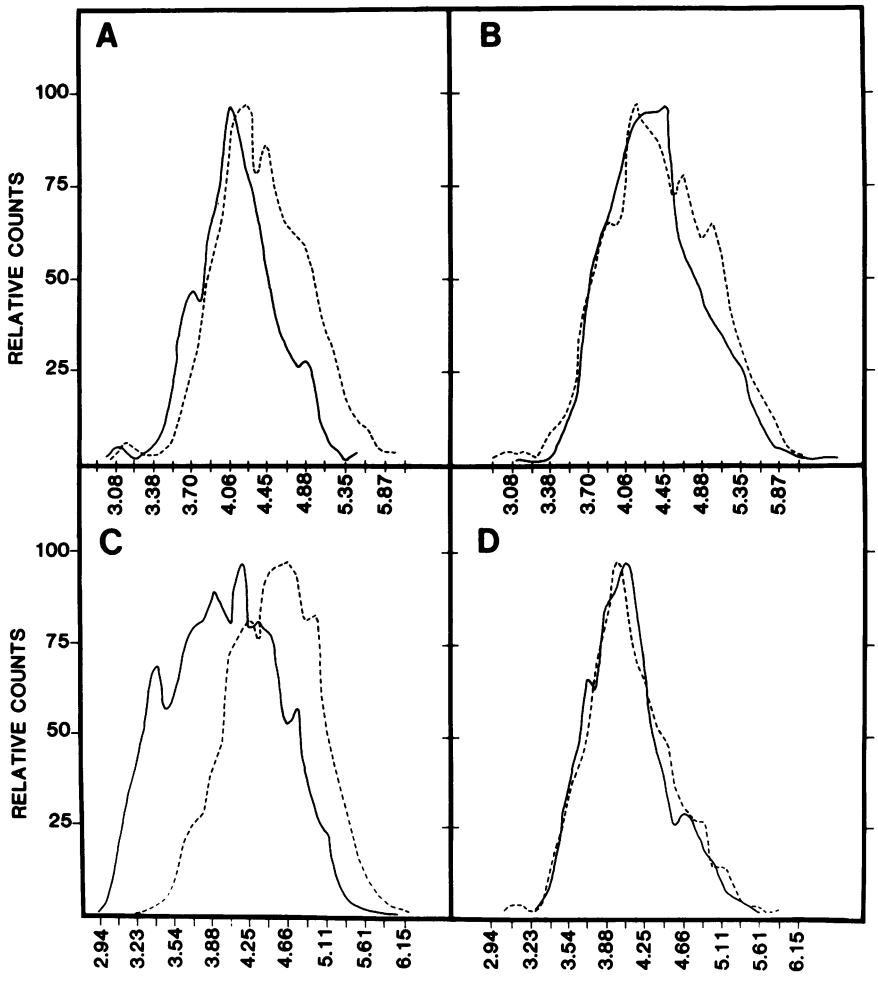

FIG. 4. Distribution of diameters (in microns) of B311-10 (——) and B311V6 (---). Symbols are for samples taken at the times indicated by the arrows in Fig. 3.

mice and rabbits were sacrificed after 23 days, and no organisms could be isolated from, or seen in, pathological sections.

The effect of incubation temperature on the doubling time and morphology of the two strains of *C. albicans* are provided in Table 3, which represents a typical experiment. It is obvious that an incubation temperature of 24°C was optimal for cultivation of both organisms as single cells, doublets, or triplets. At temperatures higher than 24°C, a substantial proportion of the B311-10 cells was found in clusters or clumps, whereas at temperatures below 24°C, the B311V6 grew in short chains (Table 3).

When an inoculum of *C. albicans* B311-10 was transferred from Sabouraud slants to SMC, the cells grew with a mean mass doubling time of about 140 min (Fig. 3A). Mass doubling time was estimated from measurements of A_{675} using appropriate dilutions as described above. The cultures were transferred serially several times at low cell densities, with no consistent change in mass doubling time (Fig. 3A). Particle counts and volumes were also determined during these experiments (Fig. 3C and D).

After the first few transfers, the mean particle volume approached a minimal value of 32.8 to 35.2 μm^3, the particle diameter was 4.0 to 4.2 μm, and the mean mass per particle averaged 17.7 pg. Examples of typical size distributions are given in Fig. 4A to D for samples taken at 18, 24.5, 34, and 68 h, respectively. In several such experiments, it appeared that the distribution of particle sizes fluctuated during early transfers, but approached uniformity after about 50 h of continuous exponential growth. No consistent relationship could be observed between mean particle volume and culture density, indicating

TABLE 4. Mean cell volume and DNA content of B311-10 and B311V6 in different growth phases

Growth phase	Time in culture (h)	Strain	A_{675}	Cells per ml[a]	Cell vol (μm^3)	DNA per cell ($\mu g \times 10^{-9}$)
Expt 1:	48	B311-10	1.0	3.6×10^7	46.5	22.3 ± 0.283
exponential	48	B311V6	1.0	3.5×10^7	43.4	20.3 ± 0.283
	50	B311-10	2.0	7.2×10^7	46.5	21.0 ± 1.061
	50	B311V6	2.0	6.6×10^7	43.4	22.3 ± 0.283
Expt 2:	68	B311-10	1.0	3.2×10^7	35.3	24.9 ± 0.778
exponential	68	B311V6	1.0	3.2×10^7	35.3	22.4 ± 0.0
Expt 3: 12 h in	29	B311-10	9.5	1.7×10^8	ND[b]	15.4 ± 1.464
stationary	29	B311V6	7.5	2.0×10^8	ND	13.5 ± 1.195
Expt 4: 36 h in	120	B311-10	13.5	10.9×10^8	13.3	6.6 ± 0.058
stationary	120	B311V6	8.0	9.4×10^8	10.1	5.8 ± 0.208

[a] Values are the means of five determinations within 5%.
[b] ND, Not determined.

that mean particle volume was not affected by high or low cell densities in the range of 0.2 to 3.0 A_{675}. Examination of culture samples during early and late transfers indicated that during early transfers the cells tended to remain in pairs or in short chains, whereas during late passages, single yeast cells and budding yeast cells predominated.

The mass doubling time of B311V6 was not significantly different from that of B311-10 (Fig. 3B). During early transfers the cultures appeared to contain particles which were somewhat larger in volume than those of B311-10. The increased mean particle volumes are shown in Fig. 3D, and the volume distributions of B311V6 compared with those of B311-10 are given in Fig. 4A to D. After 64 to 70 h in culture, the mean particle volume, particle diameter, and the size distribution of B311V6 were 32.8 to 35.2 μm^3, 4.0 to 4.2 μm (Fig. 4D), and 17.1 pg per particle, respectively, which are indistinguishable from those of B311-10 (compare Fig. 3C and D and Fig. 4D). During early transfers, the yeast cells were predominantly in pairs and chains of four as seen in the phase microscope. Late transfers contained primarily budding yeast cells.

The DNA content of B311-10 and B311V6 was determined after 29 to 120 h of culture in SMC (Table 4). In these experiments the DNA content per particle of exponential-phase cells did not appear to change significantly with time in exponential-phase growth. B311-10 and B311V6 did not exhibit a significant difference in DNA content per particle. In an experiment performed at 68 h, when both B311-10 and variant had the same mean cell size, the DNA content per particle was also the same (Table 4). After 12 to 36 h in stationary-phase growth (Table 4, experiments 3 and 4), the DNA content per cell was reduced, as would be expected for nondividing cells. The two strains contained an equal amount of DNA.

In one of two experiments performed with [^{14}C]uracil as a DNA precursor, after 12 h of exponential-phase growth the DNA content per particle was substantially greater for B311V6 than for B311-10 (Fig. 5A, Table 5). This discrepancy could be accounted for in part by a slightly larger mean cell size for the variant than for B311-10. Actually, B311V6 had a somewhat higher DNA content per A_{675} as well (Fig. 5B), suggesting slight differences in the timing of cell division cycle events in the two cell populations. The data shown in Fig. 5A and B illustrate some of the possible fallacies in the analysis of DNA content in cultures where the samples are taken during the early stages of exponential growth, when the cell size distribution has not approached that expected from a population in a steady state of exponential growth.

The relationships between relative protein content, RNA and DNA content, and absorbance were examined in cultures after 12 to 24 h of growth in SMC. The equations relating these parameters were linear and exhibited intercepts which were not significantly different from zero (Table 5). Thus, apparently balanced exponential growth could be obtained during relatively early stages of culture in SMC in terms of RNA, DNA, protein, and culture dry weight.

DISCUSSION

We have isolated a variant of *C. albicans* which is unable to produce hyphae at 37°C on media which support this morphological form. This variant is biochemically identical to *C. albicans*, as shown by its identification at the Centraalbureau voor Schimmelcultures. It appears that this is a temperature-sensitive variant

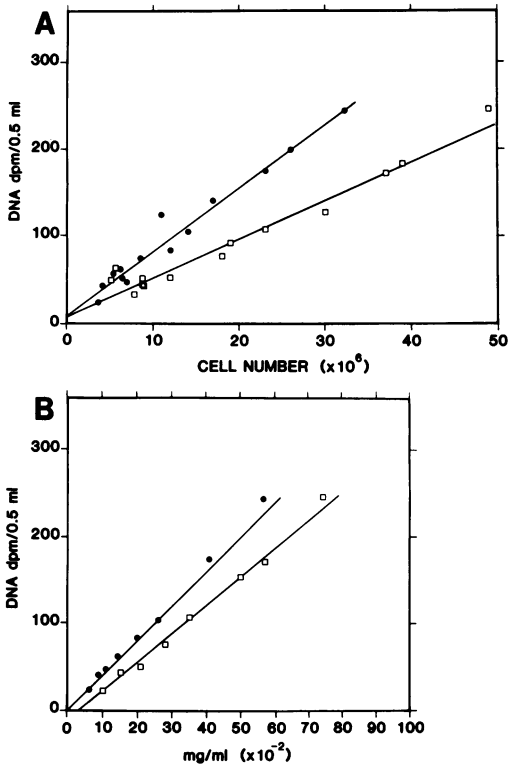

FIG. 5. (A) DNA content plotted against cell number for B311-10 and B311V6. DNA (in disintegrations per minute per 0.5 ml of culture) was determined as described in Materials and Methods. (B) DNA content (in disintegrations per minute per 0.5 ml of culture) was plotted against milligrams of dry weight. Symbols: ●, B311V6; □, B311-10.

for germ tube and hyphae formation at 37°C. Other variants of *C. albicans* (11) have been reported as being germ tube negative; however, when they were inoculated into mice they proved to be pathogenic. B311V6 differs in this respect, and this may be the result of its inability to produce hyphae at 37°C. This variant appeared to be nonpathogenic for mice and rabbits under the conditions stated (this is being further investigated).

As is the case with bacterial cultures, it appears that prolonged periods of incubation in exponential-phase growth are required to obtain single-cell populations of these two strains. The presence in cultures of clumps containing several cells was especially predominant during early transfers and at incubation temperatures between 26 and 27.5°C. The fluctuations in extent of chaining or clumping during early hours of exponential growth made it impossible to obtain reliable data. At 25°C or below, the cultures would grow only occasionally in chains of four cells. The analysis of volume distributions provided an objective measure of uniformity of cell populations. Once balanced exponential growth was established, there was no relationship between culture density and cell volume distribution. The volume distributions, cell diameters, and mean mass per particle were the same for B311-10 and B311V6.

The DNA content per particle was determined for both organisms by chemical and isotope methods. The values obtained for stationary-phase cultures were identical for the two strains and are comparable on a per-cell basis to values reported for *Saccharomyces cerevisiae* by Hartwell (7). Our data, obtained from balanced cultures whose cell sizes are comparable, are not in agreement with the results of Olaiya et al. (12) who obtained a nearly twofold difference in the DNA content of B311-10 and the B311V6 variant. Our values for B311-10 are somewhat lower than the 37 to 39 fg per cell reported by Riggsby et al. (12a) for other strains of *C. albicans*.

After several transfers in exponential-phase growth, there was a linear relationship between

TABLE 5. Equations expressing relationship between leucine and uracil incorporation and absorbance[a]

Equation	Correlation coefficient	SD	No. of determinations
B311-10 leucine = $3.48 \times 10^4 A_{675} + 3.03 \times 10^3$	0.99	1.54×10^4	9
B311V6 leucine = $3.05 \times 10^4 A_{675} + 2.18 \times 10^3$	0.97	1.26×10^4	8
B311-10 uracil = $1.53 \times 10^3 A_{675} + 2.19 \times 10^2$	0.97	6.88×10^2	8
B311V6 uracil = $1.41 \times 10^3 A_{675} + 2.19 \times 10^2$	0.96	5.88×10^2	8
B311-10 uracil (alkaline resistant) = $1.18 \times 10^2 A_{675} + 32.5$	0.82	25.00	13
B311V6 uracil (alkaline resistant) = $1.91 \times 10^2 A_{675} + 25.5$	0.91	24.83	13
B311-10 uracil (alkaline resistant)[b] = $0.98 \times 10^2 A_{675} + 21.06$	0.995	83.14	8
B311V6 uracil (alkaline resistant)[b] = $1.99 \times 10^2 A_{675} + 4.43$	0.994	29.39	8

[a] Data were subjected to linear regression of relative disintegrations per minute per 0.5 ml of isotope incorporated into TCA-precipitable material versus A_{675} obtained at various times of exponential growth. Consequently the equations represent the relative specific radioactivity per absorbance unit. The statistical parameters were obtained by using a standard statistical analysis program.

[b] Data shown in Fig. 5B.

DNA, RNA, or protein and cell dry weight in both organisms (Table 5). The linearity of these relationships establishes that the cultures were in a balanced, exponential-phase growth, and that cell composition was constant during the experiments. The content of these macromolecules per cell was the same for both strains. Thus, in contrast to the results obtained by Dabrowa et al. (4) during early transfers, these cultures appeared to synthesize three major classes of macromolecules in a balanced manner. The data shown in Fig. 3C and D indicate that several transfers in exponential-phase growth are required to obtain constancy in cell volume distributions.

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LITERATURE CITED

1. **Beheshti, F., A. G. Smith, and G. W. Krause.** 1975. Germ tube and chlamydospore formation by *Candida albicans* in a new medium. J. Clin. Microbiol. **2**:345–348.
2. **Buckley, H. R., and N. van Uden.** 1963. The identification of *Candida albicans* within two hours by the use of an egg white slide preparation. Sabouraudia **2**:205–208.
3. **Burton, K.** 1956. A study of the conditions and mechanism of the dephenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem. J. **62**:315–323.
4. **Dabrowa, N., D. Howard, J. Landau, and Y. Shechter.** 1970. Synthesis of nucleic acids and proteins in dimorphic forms of *Candida albicans*. Sabouraudia **8**:163–169.
5. **Evans, E. G. V., F. C. Odds, M. D. Richardson, and K. T. Holland.** 1974. The effect of growth medium on filament production in *Candida albicans*. Sabouraudia **13**:148–153.
6. **Evans, E. G. V., M. D. Richardson, F. C. Odds, and K. T. Holland.** 1975. Optimum conditions for initiation of filamentation in *Candida albicans*. Can. J. Microbiol. **21**:338–342.
7. **Hartwell, L. H.** 1974. *Saccharomyces cerevisiae* cell cycle. Bacteriol. Rev. **38**:164–198.
8. **Hatzfield, J.** 1973. DNA labelling and its assay in yeast. Biochim. Biophys. Acta **299**:34–42.
9. **Joshi, K. R., J. B. Gavin, and D. A. Bremmer.** 1973. The formation of germ tubes by *Candida albicans* in various peptone media. Sabouraudia **11**:259–262.
10. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid medium for the development of mycelial and yeast forms of *Candida albicans*. Sabouraudia **13**:148–153.
11. **Ogletree, F., A. Abdelal, and D. Ahern.** 1978. Germ tube production by atypical strains of *Candida albicans*. Antonie van Leeuwenhoek J. Microbiol. Serol. **44**:15–24.
12. **Olaiya, A., J. Steed, and S. Sogin.** 1980. Deoxyribonucleic acid-deficient strains of *Candida albicans*. J. Bacteriol. **141**:1284–1290.

12a. **Riggsby, W. S., L. J. Torres-Bauzer, J. W. Wills, and T. M. Townes.** 1982. DNA content, kinetic complexity, and the ploidy question in *Candida albicans*. Mol. Cell. Biol. **2**:853–862.

13. **Shepherd, M. G., C. Y. Yin, S. P. Ram, and D. A. Sullivan.** 1980. Germ tube induction in *Candida albicans*. Can. J. Microbiol. **26**:21–26.
14. **Simonetti, N., V. Strippoli, and A. Cassone.** 1974. Yeast-mycelial conversion induced by *N*-acetyl-D-glucosamine in *Candida albicans*. Nature (London) **250**:344–346.
15. **Strippoli, V., and N. Simonetti.** 1973. Effect of tetracycline on the virulence of Y and M forms of *Candida albicans* in experimentally induced infections. Mycopathol. Mycol. Appl. **51**:65–73.
16. **Syverson, R. E., H. R. Buckley, and C. C. Campbell.** 1975. Cytoplasmic antigens unique to the mycelial or yeast phase of *Candida albicans*. Infect. Immun. **12**:1184–1188.
17. **Taschdjian, C. L., J. J. Burchall, and P. J. Kozinn.** 1960. Rapid identification of *Candida albicans* by filamentation on serum and serum substitutes. Am. J. Dis. Child. **99**:212–215.
18. **Taschdjian, C. L., and P. J. Kozinn.** 1957. Laboratory and clinical studies on candidiasis in the new-born infant. J. Pediatr. **50**:426–433.