INFECTION AND IMMUNITY, Sept. 1991, p. 3297–3300
0019-9567/91/093297-04$02.00/0

Vol. 59, No. 9

Pathogenicity of *Candida albicans* Auxotrophic Mutants in Experimental Infections

DONALD R. KIRSCH[1]†* AND RITA R. WHITNEY[2]

Department of Microbial Biochemistry and Genetics[1] and Department of Microbiology,[2] The Squibb Institute for Medical Research, Princeton, New Jersey 08540

Received 4 February 1991/Accepted 29 May 1991

Auxotrophic and prototrophic control strain pairs of *Candida albicans* constructed by molecular biology methodologies were evaluated for pathogenicity in a systemic mouse model. Mutants that were auxotrophic for adenine, uracil, and heme each showed a lowered level of pathogenicity relative to control strains. It can be concluded from these experiments that decreased pathogenicity in each case is due to the auxotrophic mutation, because mutant and control strains were constructed so as to differ at a single locus. These observations suggest that new therapeutic agents for *Candida* infections might be designed based upon the inhibition of biosynthetic pathways that, in some cases, might be absent from the host.

Candida albicans is a commensal human microbe that under certain conditions can produce superficial and disseminated infections. The development of safe and efficacious treatments for *Candida* infections remains a major challenge for medical research. One approach to the discovery of novel anticandidal agents is through the identification of new differential targets for antifungal action. Virulence mechanisms constitute one class of novel antifungal targets. The ability to produce a disease state distinguishes *C. albicans* and several related species from a large number of yeast species that show an otherwise broad similarity to *C. albicans*. The specific nature of this difference has been the subject of a number of studies that have sought to identify virulence determinants in *C. albicans*. These reports have largely focused on the characterization of toxins (3, 4, 9), secreted hydrolytic enzymes (3, 5, 19, 22, 23), tissue adhesion (2, 26), and the dimorphic growth response of *C. albicans* (27, 29). A second approach to the discovery of novel antifungal agents is to consider as targets aspects of *C. albicans* physiology that are essential for pathogenicity and that are absent from the host.

In three prior studies, nutrient auxotrophy was examined as a potential modifier of pathogenicity (16, 18, 27). These reports suggested that auxotrophy for adenine, lysine, serine, and potentially proline and uracil produced a marked decrease in virulence. This is surprising in view of the fact that mammalian serum contains significant levels of many metabolites. The concentrations of amino acids in serum range from 40 to 660 mM in adults (8). This compares favorably with levels of supplementation required for optimal growth of auxotrophic mutants of *Saccharomyces cerevisiae*, which require amino acid levels ranging from approximately 0.1 to 3.6 mM (28). Significant turnover and hepatic synthesis are observed for purines and pyrimidines, which should lead to high levels of these compounds in serum (6). However, high nucleoside levels in serum may not be adequate for auxotrophic supplementation, since *C. albicans* auxotrophs cannot effectively utilize exogenous adenosine (24). In addition, the identification of pyrimidine biosynthesis as a virulence factor is supported by the activity of flucytosine, which can be an effective treatment for systemic *Candida* infections and which acts on thymidylate synthase as one of its principal targets of action (25).

A definitive answer to the question of whether prototrophy is essential for pathogenicity could be important for the development of therapeutic agents. The biosynthesis of amino acids has recently been a focus of significant interest in the area of herbicide development. A number of novel commercial herbicides have recently been developed that act via the inhibition of various steps in amino acid biosynthesis (reviewed in reference 17). These herbicides inhibit enzymes in the pathways for the biosynthesis of aromatic amino acids, branched-chain amino acids, histidine, and glutamine. The efficacy of these agents stems at least in part from the fact that amino acid synthesis is essential in plants, which have no efficient mechanism or opportunity for the uptake of exogenous amino acids. The toxicity profile for these compounds is generally extremely favorable, because they inhibit enzymes for essential amino acid pathways that do not occur in animal cells; this has led to their rapid regulatory approval.

C. albicans is an asexual diploid; as a consequence, strains carrying single, defined genetic lesions cannot be obtained readily (14). Therefore, one general weakness of prior studies on the effect of auxotrophy on virulence has been the inability to selectively determine, through the comparison of strains differing by a single mutation, the contribution of specific mutations to virulence. Evidence from prior studies is compatible with the hypothesis that mutational blocks in the biosynthetic pathway for certain metabolites, potentially combined with an inefficient system for the uptake of the pathway end product, could result in significant decreases in in vivo growth rate and/or pathogenicity. Inefficient transport of certain metabolites in *C. albicans* is suggested by observations of the slow growth of auxotrophic strains constructed with molecular biology methodologies on normally supplemented media (10, 11). However, it is also possible that the strains used in studies of pathogenicity carried mutations in addition to the auxotrophic markers and that these other mutations were responsible for the decreases in pathogenicity.

We were stimulated to reexamine the question of whether mutations in biosynthetic pathways block pathogenicity for

* Corresponding author.

† Present address: Department of Animal Industry Discovery, American Cyanamid, Agricultural Research Division, Princeton, NJ 08540.

TABLE 1. *C. albicans* auxotrophic strains used in this study

Strain	Genotype	Reference or source
SC5314	Wild type	7
SGY16[a]	*leu/leu*	This study
SGY129	*ade2/ade2 pro/pro MET/met*	11
A81-Pu	*ade2/ade2*	16
SGY652[b]	*ADE2/ade2*	This study
SGY135[c]	*ade2/ade2*, pMC2	12
SGY298[d]	*ade2/ade2*, p56	13
SGY269	*ura3/ura3*	11
SGY276[e]	*ura3/ura3*, pET18	11
SGY567	*hem3/hem3*	15
SGY595[f]	*hem3/hem3*, pMK34	15

[a] Derived from strain SC5314 by UV and nitrous acid mutagenesis.
[b] *ADE2*$^+$ spontaneous revertant of A81Pu.
[c] Strain A81Pu carrying an integrated vector with the *ADE2*$^+$ gene.
[d] Strain A81Pu carrying a multicopy vector with the *ADE2*$^+$ gene.
[e] Strain SGY269 carrying an integrated vector with the *URA3*$^+$ gene.
[f] Strain SGY567 carrying an integrated vector with the *HEM3*$^+$ gene.

TABLE 2. Virulence of *C. albicans* in systemic infections

Strain	Description	LD_{50} (CFU)	
		Normal mice	Immunosuppressed mice
SC5314	Wild type	4.2×10^4	$<1 \times 10^4$
SGY16	*leu*		$<2 \times 10^4$
SGY129	*ade2 pro*	$>9.8 \times 10^6$	
A81-Pu	*ade2*	$>1.6 \times 10^7$	2.5×10^6
SGY652	A81-Pu revertant (*ADE*$^+$)	6.3×10^4	
SGY135	A81-Pu(pMC2 (*ADE*$^+$)	1.4×10^5	$<1.4 \times 10^4$
SGY298	A81-Pu(p56) (*ADE*$^+$)	4.0×10^4	
SGY269	*ura3*		3.2×10^6
SGY276	SGY269(pET18) (*URA*$^+$)		1.5×10^5
SGY567	*hem3*	$>6.7 \times 10^6$	
SGY595	SGY567(pMK34) (*HEM*$^+$)	2.7×10^6	
		3.5×10^6	

two reasons. First, a number of new auxotrophic mutants of *C. albicans* have become available as a result of the development of new methods for the molecular genetic manipulation of that species (14). These mutants were constructed by molecular biology methodologies and therefore are more likely to carry single, defined mutations. Second, as described above, the discovery, development, and regulatory approval of a number of inhibitors of amino acid biosynthesis for use as herbicides raise the possibility of developing analogous compounds for use as anticandidal agents.

C. albicans auxotrophic strains were selected for use in this study largely on the basis of availability. These strains had been recovered in experiments that were directed toward the development of methods for the molecular genetic manipulation of *C. albicans*. Therefore, these strains do not necessarily present a broad representation of auxotrophic markers, although they do represent a broad variety of the *C. albicans* auxotrophs that have been constructed by molecular biology methodologies (Table 1). These strains include auxotrophs for the biosynthesis of purine (phosphoribosylaminoimidazole carboxylase; *ade2*), pyrimidine (orotidine-5′-phosphate decarboxylase; *ura3*), and heme (uroporphyrin I synthase; *hem3*). In addition, a leucine-auxotrophic strain that had been isolated by classical genetic methods was included in this study for comparative purposes.

The above strains were tested for virulence in a systemic mouse infection model as follows. *Candida* cells were suspended in 0.85% saline and injected intravenously into the lateral tail veins of female Swiss-Webster mice at concentrations ranging from 10^4 to 10^7 CFU per mouse. Ten mice were injected at each inoculum level and observed for 21 days for mortality or morbidity resulting from the *Candida* challenge. In some experiments, mice were immunosuppressed by cyclophosphamide treatment before the challenge. Mice were made neutropenic (leukopenic) by the intraperitoneal injection of 100 mg of cyclophosphamide per kg 4 days and 1 day before infection. This procedure decreased the total leukocyte count to below 1,000 cells per mm^3 and the polymorphonuclear count to below 1% of the total count. Injections of cyclophosphamide (100 mg/kg) were required every 3 or 4 days for the duration of the experiment to maintain this leukopenic state. In most cases where deaths were observed, the kidneys were excised and cultured to provide evidence that death had resulted from *Candida* infection. After 21 days the lethal doses for 50% of mice (LD_{50}s) were calculated and the relative virulence was determined based upon comparison with that of control strains.

In this infection model, the wild-type control strain SC5314 showed a high level of virulence and consistently produced LD_{50}s of approximately 4×10^4 CFU (3.8×10^4 and 4.5×10^4 CFU in independent replicate determinations; Table 2). Not surprisingly, the apparent virulence of this strain increased in the immunosuppressed animals, and lethality was observed at the lowest inoculum levels tested (LD_{50}, $<1 \times 10^4$ CFU). The inoculum was not further diluted to obtain a specific LD_{50}. This observation demonstrates that the immunosuppressive treatment employed in this study was efficacious, since the immunosuppressed host animals were not able to survive levels of pathogen treatment that were inactive against the untreated controls (Table 2).

As an initial experiment to characterize the system, adenine auxotrophs, prototrophic transformants, and a prototrophic revertant were tested in the mouse virulence model. Adenine auxotrophic strains SGY129 and A81-Pu (*ade2*) were the first *C. albicans* strains employed in transformation experiments (12). These two strains showed extremely low virulence in the animal model with LD_{50}s of $>9.8 \times 10^6$ and $>1.6 \times 10^7$ CFU, respectively, or almost 3 orders of magnitude lower in virulence than the wild-type strain (Table 2). Superficially, this result would seem to suggest that adenine prototrophy is important for virulence. However, the virulence of the prototrophic and auxotrophic strains cannot be accurately compared, because SC5314 is not genetically related to either SGY129 or A81-Pu and may differ from these strains in a number of factors that could affect virulence. Strain A81-Pu was derived by a single round of mutagenesis from wild-type strain A81 (16). However, strain A81 was subsequently lost and therefore could not be included in our experiments. As a substitute, strain SGY652 was isolated as a spontaneous prototrophic revertant of strain A81-Pu. Strain SGY652 had an LD_{50} of 6.3×10^4 CFU, which is reasonably close to the value obtained for strain SC5314 (Table 2). Since A81-Pu and SGY652 are likely to differ at a single locus, this result suggests that adenine auxotrophy as a single factor can decrease virulence by almost 3 orders of magnitude.

To confirm this, we tested two A81-Pu transformants: strain SGY135, which is prototrophic and carries an integrated *ADE2* gene, and strain SGY298, which is prototrophic and carries the *ADE2* gene on a high-copy-number autono-

mously replicating vector. Both transformants showed an increase in virulence to levels approaching that seen for the wild-type control (LD_{50}s of 1.4×10^5 and 4.0×10^4 CFU, respectively). This result confirms that adenine prototrophy is an important virulence factor in *C. albicans*. One interpretation of all of these results is that the measured differences in virulence may be directly due to differences in growth rate between these strains. However, this experiment indicates that strain SGY298 is slightly more virulent than strain SGY135. Because of plasmid instability, strain SGY298 has a slightly lower growth rate than strain SGY135 (13), suggesting that observed differences in virulence cannot be due to growth rate alone. Although strain SGY135 showed a dramatic increase in virulence, it appeared to be slightly less virulent than the two other prototrophic strains. To further investigate this, strains A81-Pu and SGY135 were tested for virulence in immunocompromised mice. Similar to the result obtained with wild-type strain SC5314, strain A81-Pu showed increased virulence when tested in the immunocompromised mouse model (Table 2). However, strain A81-Pu had lower virulence when compared with the wild type in this study (LD_{50} of 2.5×10^6 CFU versus $<1 \times 10^4$ CFU for the wild type). In the immunocompromised animals, the auxotrophic transformant SGY135 showed high virulence (LD_{50}, $<1.4 \times 10^4$ CFU), indicating that transformation had restored a high degree of virulence. In this experiment, no animals survived even in the group receiving the lowest inoculum.

The high degree of sensitivity to *Candida* infection observed in the immunocompromised mice raised the concern that these animals might be so sensitive to *Candida* infection that any mutation would result in a loss in virulence in this model. It would be useful to have a mutant that retained virulence in the immunosuppressed animals to verify that the model was able to distinguish genetic changes that specifically affected virulence. We therefore decided to determine the virulence in immunocompromised animals of strain SGY16, a rare leucine-auxotrophic strain which had been isolated after UV mutagenesis of strain SC5314 by the method of Whelan and coworkers (30). This experiment indicated no difference in virulence between the auxotrophic mutant strain and the prototrophic parent strain, indicating that this leucine auxotrophy mutation did not effect virulence in this model. This experiment serves as a control to demonstrate that the immunosuppressed model could be used to distinguish auxotrophic mutations that affect virulence from auxotrophies that do not. The virulence of auxotrophic mutants has also been observed by Manning and coworkers (18), who reported that two different auxotrophies in their study did not result in a loss of virulence.

At this point, it seemed possible that the observed effect of adenine auxotrophy on virulence might have been due to a specific interaction between an auxotrophic mutation and other factors specific to the A81 background rather than to the *ade2* mutation alone. Therefore strain SGY129, a genetically unrelated *ade2* auxotroph, was also tested for virulence. This strain was virtually avirulent in this mouse infection model, indicating that auxotrophy in this genetic background also produces a loss of virulence. However, strain SGY129 was derived from wild-type strain ATCC 22114 by multiple rounds of mutagenesis and is homozygous or heterozygous for at least three loci that affect amino acid biosynthesis (21). It is possible that the loss of virulence resulted from either the adenine or proline auxotrophy or from other genetic factors. It therefore did not appear worthwhile to compare the virulence of SGY129 with that of ATCC 22114, since, if differences were seen, no conclusion could be formed as to the identity of the virulence factor. We can at least conclude from this experiment that lack of virulence in this model can be observed with *ade2* auxotrophs from more than one clinical isolate.

A second set of experiments was performed on a strain that is auxotrophic for uracil (SGY269 [*ura3*]) in order to extend, with a pyrimidine auxotroph, the results obtained with a purine auxotroph (*ade2*). Strain SGY269 is a derivative of strain A81-Pu and carries a homozygous disruption of the *URA3* locus produced by inserting the *C. albicans ADE2* gene into the *URA3* gene (11). As a control, prototrophic strain SGY276 was constructed by transforming strain SGY269 with plasmid pET18, an integrating vector carrying the *URA3* gene. To determine the contribution of uracil auxotrophy to virulence, strains SGY269 and SGY276 were tested in immunosuppressed animals (Table 2). Immunosuppressed animals were chosen for this experiment to amplify any effects that the *ura3* mutation might have on virulence.

Strain SGY269, similar to the *ade2* auxotroph, showed greatly reduced virulence in this test, producing an LD_{50} of 3.2×10^6 CFU, which is 2 orders of magnitude higher than the LD_{50} seen in experiments with prototrophic strains. However, when the prototrophic control transformant was tested in this model, virulence was restored (LD_{50}, 1.5×10^5 CFU), but not to the level previously observed for wild-type strains. The increase in virulence by a factor of greater than 1 order of magnitude demonstrates that uracil auxotrophy is a virulence factor. However, the relatively lower level of resistance seen in the prototrophic transformant suggests that other factors may be influencing virulence in strain SGY269. Strain SGY269 was derived from strain A81-Pu via transformation to obtain a heterozygous disruption, and UV mutagenesis was then employed to induce mitotic recombination to generate a homozygous auxotroph (11). It is possible that additional mutations that affect virulence were introduced into strain SGY269 during its construction. However, the use of prototrophic strain SGY276, which would also carry these mutations, as a control demonstrates that uracil auxotrophy by itself affects virulence.

A final set of experiments was performed on a strain (SGY567) which is auxotrophic for heme. Heme auxotrophs carry mutations in one of the enzymes of the porphyrin biosynthesis pathway and also compose one class of mutants that are resistant to polyene antibiotics (e.g., nystatin, amphotericin B) (1). In *C. albicans*, resistance to polyene antibiotics is not seen in clinical settings, although polyene-resistant strains of *C. albicans* are readily isolated in the laboratory (20). One possible explanation for this is that mutations leading to polyene antibiotic resistance produce a concomitant decrease in virulence so that such mutants are never recovered from patients. The availability of a genetically engineered *hem3* mutant in *C. albicans* made it possible to test this hypothesis. When the heme auxotrophic strain SGY567 was tested in the mouse model, virulence was as low as that in any strain examined in this study (LD_{50}, $>6.7 \times 10^6$ CFU). Strain SGY595 (strain SGY567 transformed to heme prototrophy with plasmid pMK34) was tested as a control and was found to be more virulent than the heme auxotroph (LD_{50}s, 2.7×10^6 and 3.5×10^6 CFU in replicate determinations). This demonstrates that heme auxotrophy can decrease virulence.

However, the control strain was considerably less virulent than other tested prototrophs. The heme auxotroph and control strains were both derived from the uracil auxotrophic strain SGY243 after multiple rounds of transforma-

tion and mutagenesis. It is therefore possible that additional mutations affecting virulence were introduced during the construction of the heme auxotrophic strain SGY567. However, since the control auxotrophic strain SGY595 showed increased virulence relative to the heme auxotroph, one can state that heme auxotrophy is necessary for high-level virulence.

In conclusion, each of three biosynthetic mutations analyzed via molecular genetic strain construction was shown to contribute to virulence in *C. albicans*. This is not true for all pathways, since in this study a leucine-auxotrophic strain produced by conventional methods showed no decrease in virulence. However, these experiments, taken together with the results of prior studies (18, 27), suggest that many biosynthetic pathways are necessary to achieve a high level of virulence in *C. albicans*. It may therefore be possible to develop new pharmaceutical agents for the treatment of *Candida* infections through a judicious selection of biosynthetic pathway and target enzymes and the application of screening and/or pharmaceutical chemistry to develop inhibitors.

We thank M. Kurtz and R. Kelly for strains and W. Scott, D. Bonner, and A. K. Field for their guidance. Carol Bollinger is acknowledged for high-quality secretarial assistance.

REFERENCES

1. **Bard, M., R. A. Woods, and J. M. Haslam.** 1974. Porphyrin mutants of *Saccharomyces cerevisiae*: correlated lesions in sterol and fatty acid biosynthesis. Biochim. Biophys. Acta **56**:324–330.
2. **Calderone, R. A., R. L. Cihlar, D. Dong-Soo Lee, K. Hoberg, and W. M. Scheld.** 1985. Yeast adhesion in the pathogenesis of endocarditis due to *Candida albicans*: studies with adherence negative mutants. J. Infect. Dis. **152**:710–715.
3. **Chattaway, F. W., F. C. Odds, and J. E. Barlow.** 1971. An examination of the production of hydrolytic enzymes and toxins by pathogenic strains of *Candida albicans*. J. Gen. Microbiol. **67**:255–263.
4. **Cutler, J. E., L. Friedman, and K. C. Milner.** 1972. Biological and chemical characterization of toxic substances from *Candida albicans*. Infect. Immun. **6**:616–627.
5. **Douglas, L. J.** 1988. Candida proteinases and candidosis. Crit. Rev. Biotechnol. **8**:121–129.
6. **Ganong, W. F.** 1979. Review of medical physiology, 9th ed., p. 220–231. Lange Medical Publications, Los Altos, Calif.
7. **Gillum, A. M., E. Y. H. Tsay, and D. R. Kirsch.** 1984. Isolation of the *Candida albicans* gene for orotidine-5′-phosphate decarboxylase by complementation of *S. cerevisiae* ura3 and *E. coli* pyrF mutations. Mol. Gen. Genet. **198**:179–182.
8. **Harper, H. A., V. W. Rodwell, and P. A. Mayes.** 1977. Review of physiological chemistry, 16th ed., p. 341. Lange Medical Publications, Los Altos, Calif.
9. **Iwata, K.** 1977. Toxins produced by *Candida albicans*. Contrib. Microbiol. Immunol. **4**:77–85.
10. **Kelly, R., S. M. Miller, and M. B. Kurtz.** 1988. One-step gene disruption by cotransformation to isolate double auxotrophs in *Candida albicans*. Mol. Gen. Genet. **214**:24–31.
11. **Kelly, R., S. M. Miller, M. B. Kurtz, and D. R. Kirsch.** 1987. Directed mutagenesis in *Candida albicans*: one-step gene disruption to isolate *ura3* mutants. Mol. Cell. Biol. **7**:199–207.
12. **Kurtz, M. B., M. W. Cortelyou, and D. R. Kirsch.** 1986. Integrative transformation of *Candida albicans*, using a cloned *Candida ADE2* gene. Mol. Cell. Biol. **6**:142–149.
13. **Kurtz, M. B., M. W. Cortelyou, S. M. Miller, M. Lai, and D. R. Kirsch.** 1987. Development of autonomously replicating plasmids for *Candida albicans*. Mol. Cell. Biol. **7**:209–217.
14. **Kurtz, M. B., D. R. Kirsch, and R. Kelly.** 1988. The molecular genetics of *Candida albicans*. Microbiol. Sci. **5**:58–63.
15. **Kurtz, M. B., and J. Marrinan.** 1989. Isolation of hem3 mutants from *Candida albicans* by sequential gene disruption. Mol. Gen. Genet. **217**:47–52.
16. **Kwon-Chung, K. J., and W. B. Hill.** 1970. Studies on the pink adenine-deficient strains of *Candida albicans*. I. Cultural and morphological characteristics. Sabouraudia **8**:48–59.
17. **LaRossa, R. A., and S. C. Falco.** 1984. Amino acid biosynthetic enzymes as targets of herbicide action. Trends Biotechnol. **2**:158–161.
18. **Manning, M., C. B. Snoddy, and R. A. Fromtling.** 1984. Comparative pathogenicity of auxotrophic mutants of *Candida albicans*. Can. J. Microbiol. **30**:31–35.
19. **Odds, F. C.** 1985. *Candida albicans* proteinase as a virulence factor in the pathogenesis of Candida infections. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **260**:539–542.
20. **Pesti, M., and L. Ferenzy.** 1982. Protoplast fusion hybrids of *Candida albicans* sterol mutants differing in nystatin resistance. J. Gen. Microbiol. **128**:123–128.
21. **Poulter, R. T. M., and E. H. A. Rikkerink.** 1983. Genetic analysis of red, adenine-requiring mutants of *Candida albicans*. J. Bacteriol. **156**:1066–1077.
22. **Price, M. F., and R. A. Cawson.** 1977. Phospholipase activity in *Candida albicans*. Sabouraudia **15**:179–185.
23. **Pugh, D., and R. A. Cawson.** 1975. Cytochemical localization of phospholipase A and lysophospholipase in *Candida albicans*. Sabouraudia **13**:110–115.
24. **Sarachek, A.** 1964. Promotion or retardation of growth of adenine auxotrophs of *Candida albicans* by purines, pyrimidines and nucleosides. Antonie van Leeuwenhoek J. Microbiol. Serol. **30**:289–302.
25. **Scholer, H. J.** 1980. Flucytosine, p. 35–106. *In* D. C. E. Speller (ed.), Antifungal chemotherapy. John Wiley & Sons, Inc., New York.
26. **Segal, E., A. Soroka, and A. Schecter.** 1984. Correlative relationship between adherence of *Candida albicans* to human vaginal epithelial cells in vitro and candida vaginitis. Sabouraudia **22**:191–200.
27. **Shepherd, M. G.** 1985. Pathogenicity of morphological and auxotrophic mutants of *Candida albicans* in experimental infections. Infect. Immun. **50**:541–544.
28. **Sherman, F., G. A. Fink, and C. W. Lawrence.** 1974. Methods in yeast genetics, p. 62. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Sobel, J. D., G. Muller, and H. R. Buckley.** 1984. Critical role of germ tube formation in the pathogenesis of *Candida* vaginitis. Infect. Immun. **44**:576–580.
30. **Whelan, W. L., R. M. Partridge, and P. T. Magee.** 1980. Heterozygosity and segregation in *Candida albicans*. Mol. Gen. Genet. **180**:107–113.