SHORT COMMUNICATION

Isolation of a Mycelial Mutant of *Candida albicans*

By RICHARD D. CANNON

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

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A mutant of *Candida albicans* strain MEN, which was unable to produce mycelia in SSV medium and in horse serum at 37 °C, was isolated by a physical separation procedure. The mutant was shown to be derived from the parental strain by growth and morphology studies, sugar uptake and fermentation patterns, and the presence of genetic markers.

INTRODUCTION

*Candida albicans* is a pathogenic dimorphic fungus existing in two major morphologies: yeast and mycelial. The interconversion of these forms is referred to as the dimorphic transition and can be induced by a number of factors (Odds, 1979). It is not certain which of the two forms is more pathogenic, but a study of dimorphism may lead to the development of an inhibitor of the dimorphic transition, which might help in treating patients suffering from invasive candidosis by limiting the growth and spread of the organism. An investigation of dimorphism may also yield important information on the nature of eukaryotic cellular differentiation.

The study of dimorphism is complicated by strain-dependent variations in stimuli inducing the transition. Once an induction stimulus has been given it is difficult to separate the causes of dimorphism from its effects. One way to overcome these problems is to study mutants defective in the dimorphic transition process, and to compare them with the parental strain. However, it is difficult to isolate morphological mutants, and this may be due to one or more factors. *C. albicans* is probably diploid (Olaiya & Sogin, 1979; Riggsby et al., 1982) and so double mutation events may be necessary to obtain the appropriate mutant. Also, it is not clear whether dimorphism is in fact genetically determined or whether it is a phenotypic response to a change in environmental conditions. If, however, dimorphism is genetically determined a mutation in a dimorphic-specific gene may be a lethal event.

Of those mutants that have been reported in the literature (Pomes et al., 1985; Mardon et al., 1969; Torosantucci & Cassone 1983; Hubbard et al., 1986) none are wholly satisfactory as they either show intermediate morphology such as pseudomycelia, have been subjected to high doses of mutagens or are no longer typical *C. albicans* strains. The objective of this study was to obtain a mutant defective in mycelium production from *C. albicans* strain MEN. *C. albicans* MEN was chosen because it is heterozygous for 5-fluorocytosine resistance and a methionine requirement. Using these markers it was possible to demonstrate that the mutant obtained was unequivocally derived from the parental strain.

METHODS

Organisms. *C. albicans* MEN was obtained from Dr W. L. Whelan (Whelan & Magee, 1981). Mutant MM2002 was obtained from *C. albicans* MEN by cyclic selection in SSV medium as described in Results and Discussion. *C. albicans* 6406 was obtained from the Mycological Reference Library, London School of Hygiene and Tropical Medicine, UK.
Media. The mycelial form was grown in starch, salts and vitamins (SSV) medium (Marriott, 1975) at 37 °C, and the yeast form was grown in Yeast Nitrogen Base (Difco) containing 1% (w/v) glucose (YNBG) at 37 °C.

Strain identification. Sugar utilization patterns were determined with an API 20C AUX gallery (API system) according to the manufacturer's instructions. Sugar fermentation was detected using an inverted Durham tube in a 10 ml culture of strain MEN or strain MM2002 in YNB containing sugar. Germ tube tests were done in inactivated horse serum (Mackenzie, 1966). Chlamydospore formation was studied on corn meal agar containing 1% (v/v) Tween 80 after incubation at 25 °C for 5 d. Heterozygosity for 5-fluorocytosine resistance was demonstrated by growing approximately 10^6 cells on MIN agar [minimal agar medium: 0.17% (w/v) YNB without amino acids, 0.5% (w/v) (NH_4)_2SO_4, 1% (w/v) glucose, 2% (w/v) agar] in the presence and absence of 5-fluorocytosine (50 μg ml^-1). Plates were incubated at 37 °C for 5 d (Whelan et al., 1981). Heterozygosity for methionine requirement was demonstrated by measuring the frequency of auxotrophic survivors after UV-induced mitotic segregation. BiGGY agar (Difco) plates were seeded with approximately 300 cells of C. albicans strain MEN, MM2002 or 6406 and subjected to 640 erg mm^-2 UV light from a Philips UV 15 W germicidal lamp. Plates were incubated for 72 h at 37 °C and the numbers of methionine auxotrophs (white colonies) and prototrophs (dark brown colonies) counted.

RESULTS AND DISCUSSION

The mutant MM2002 was isolated by a physical separation technique. A single mutation in the dimorphic process was required so that direct comparisons between mutant and parental strains could be made. Thus the isolation of a spontaneous mutant was preferable to subjecting the cells to non-specific mutagens.

Physical separation methods rely upon the incomplete transition of a culture from the yeast to the mycelial morphology. Of those yeast cells not undergoing the transition, some will be dead, some will be at the wrong stage of the cell growth cycle and some may be spontaneous mycelial mutants. Cells failing to undergo the mycelial transition were isolated by differential filtration. The mycelia in a 3.5 h 'mycelial' culture (SSV medium at 37 °C) were removed by filtration through a sintered glass funnel of porosity 3. The yeast cells were removed from the filtrate by further filtration through a sterile glass fibre disc (Whatman GF/C). These cells were used as an inoculum for 5 ml YNBG in which cells grew as yeast at 37 °C. After 18 h incubation, cells were harvested, washed and inoculated into fresh SSV medium to a cell density of 3 x 10^8 cells ml^-1. These cells were incubated for 3-5 h at 37 °C before being filtered as before. This cycle was repeated, amplifying each time the proportion of cells not undergoing the transition, until after 10 cycles none of the cells in the SSV incubation developed germ tubes.

The organism isolated was in fact derived from strain MEN and not a contaminating yeast which was not dimorphic. The cells appeared similar under microscopic examination and showed the same growth kinetics when growth curves were studied. API diagnostic tests showed that strains MEN and MM2002 were of the species C. albicans. Sugar fermentation tests for both strains gave the results expected for C. albicans according to the classification of Barnett et al. (1983). Strain MEN formed germ tubes in horse serum at 37 °C whereas strain MM2002 did not. This is expected, assuming that the mycelium forming process is the same in horse serum as in SSV medium. Both strain MEN and strain MM2002 produced chlamydospores on corn meal agar containing 1% (v/v) Tween 80, but the rate of formation was slow and the hyphal network was scarce for both strains compared to C. albicans 6406. Further definitive evidence that strain MM2002 was derived from strain MEN was the discovery that, like MEN, MM2002 was heterozygous for 5-fluorocytosine resistance. Both MEN and MM2002 produced heterogenous colony sizes on MIN agar containing 5-fluorocytosine (50 μg ml^-1). These findings are similar to those of Whelan et al. (1981, Fig. 2) where the heterozygosity of C. albicans MEN was indicated by the segregation of cells into those growing into large resistant colonies, and those giving small sensitive micro-colonies on drug-impregnated plates.

BiGGY agar contains bismuth sulphite indicator and prototrophic yeast, which possess active sulphite reductase, can reduce the sulphite to sulphide, which gives the colonies a dark brown colouration (Nickerson, 1953). Methionine auxotrophs lacking sulphite reductase, however, do not produce the pigmentation and grow as white colonies. Table 1 shows the frequency of methionine auxotrophs after UV-induced mitotic recombination of C. albicans...
strains MEN, MM2002 and 6406. Strain 6406 did not produce methionine auxotrophs at a measurable frequency, indicating that it is homozygous for this gene involved in methionine biosynthesis. Strains MEN and MM2002 gave rise to methionine auxotrophs at an increased, and at approximately the same, frequency (0.92% and 0.95% of survivors respectively). No auxotrophs were found among 2232 colonies grown from unirradiated MEN cells. One explanation of these results is that both strains are heterozygous for methionine requirement. The frequency of auxotrophic survivors was higher than that found by Whelan et al. (1980) and this may be due to the fact that different strains and media were used, and that their method relied on replica plating, which is not always totally accurate.

Thus, using a physical separation and enhancement technique, a spontaneous mycelial mutant has been isolated. A comparison of various biochemical parameters between the mutant and parental strains might help elucidate the nature and possibly the cause of the dimorphic transition in *C. albicans*.

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**REFERENCES**


