SHORT COMMUNICATION

Inducibility of Germ-tube Formation in *Candida albicans* at Different Phases of Yeast Growth

By ELENA MATTIA AND ANTONIO CASSONE

*Institute of Microbiology, Medical Faculty,
University of Rome, 00100 Rome, Italy*

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Yeast cells of *Candida albicans* growing at 25 °C in glucose/yeast extract medium cannot germinate by dilution into fresh medium at 37 °C before reaching the stationary phase of growth. However, cells at any stage of growth are able to form germ-tubes giving true hyphae when incubated at 37 °C in the presence of inducers such as serum or *N*-acetyl-D-glucosamine. Thus, stationary phase cells are not endowed with a specific 'competence' to germinate.

INTRODUCTION

In recent studies, Chaffin & Sogin (1976) and Soll & Bedell (1978) have proposed that the dimorphic fungus *Candida albicans*, growing as a budding yeast at 25 °C, is able to form germ-tubes by a temperature shift to 37 °C in fresh nutrient medium only during the stationary phase of growth. The dimorphism of *C. albicans* is classically known to depend on both temperature and nutritional factors (Romano, 1966). The above studies did not establish whether the dependence of germ-tube formation on a 'competence' of stationary phase cells is limited to the temperature-controlled dimorphism or is a general property, particularly in those systems where the dimorphic transition is governed by complex [e.g. serum (Taschdjian *et al.*, 1960)] or simple [e.g. *N*-acetyl-D-glucosamine (Simonetti *et al.*, 1974)] nutritional inducers. We have therefore investigated further the relationship between the phases of growth of *C. albicans* at 25 °C and the ability to form germ-tubes, either after simple dilution into fresh medium or by dilution in different germination-inducing media, at 37 °C.

METHODS

Organism and growth conditions. Two strains of *Candida albicans* were used throughout these studies: *C. albicans* A₄₄ has been described previously (Cassone *et al.*, 1973); *C. albicans* BP was kindly given by Dr P. Marconi of the University of Perugia, Italy. Cultures were routinely maintained at 37 °C on slants of Winge solid medium containing (g l⁻¹): glucose, 20; yeast extract, 3; agar (BBL), 1.5. For experimental purposes, the organisms were inoculated into Winge liquid medium and incubated in a water bath with vigorous agitation at 25 °C to a final density of 5×10^7 to 1×10^8 cells ml⁻¹. This preculture was then diluted into fresh prewarmed medium at 25 °C to give an initial density of 2×10^6 cells ml⁻¹. The growth of this culture was recorded together with the ability of the cells to form germ-tubes during the different phases of growth.

Growth and germ-tube formation. At intervals during growth, the cell concentration was determined by direct counting in a Burke haemocytometer; here we defined 'cell' as meaning any presumptive colony-forming unit, i.e. single cells (singlets), an unseparated pair (doublet) and so on, up to large clusters of four or more attached cells. The ability of blastospores to form germ-tubes was tested by diluting the

Fig. 1. Relationship between the phases of growth of *Candida albicans* strain BP in Wingé medium at 25 °C and the inducibility of germ-tube formation at 37 °C: (a) exponentially growing cells; (b) stationary phase cells; (c) cells from stationary phase diluted in prewarmed Wingé medium and incubated for 1 h at 37 °C; (d) cells treated as in (c), but incubated for 20 h; (e) exponentially growing cells (7 h in Wingé medium), diluted in prewarmed serum plus GlcNAc and incubated at 37 °C for 30 min; (f) cells treated as in (e), but incubated for 18 h. Bar markers represent 5 µm.

yeast growing at 25 °C into either prewarmed medium of the same composition or into other inducing media, including diluted horse serum [S.I.A.L., Rome, Italy; 10 % (v/v) in water], *N*-acetyl-D-glucosamine (GlcNAc; 1 mg ml⁻¹ in water or Wingé medium), as well as a mixture of serum and GlcNAc at the above concentrations. Germ-tube formation was measured by counting the number of individual cells showing a definite outgrowing tube and expressing this as a percentage of the total cell population.

RESULTS

Qualitatively similar results were obtained with both strains of *C. albicans* used, so only details of the experiments performed with strain BP are reported here.

Budding, cell division and growth of C. albicans at 25 °C

After inoculating fresh Wingé medium with a preculture stopped in the stationary phase, the kinetics of growth at 25 °C were essentially similar to those described by Soll & Bedell (1978) in Lee's medium (Lee *et al.*, 1975); the slight quantitative differences were probably due to the different strain of *C. albicans* and medium used. There was a lag of about 3 h, with the exponential phase of growth characterized by cycles of budding not followed by cell separation. Clusters of cells were therefore prominent and singlets were few (Fig. 1a). The number of clusters began to decrease after the mid-exponential phase of growth and the stationary phase showed a marked increase of singlets in the population (Fig. 1b). The final cell concentration was about 2.5×10^8 cells ml⁻¹ after 30 h growth, more than 80 % of the cells being singlets. Less than 20 % of the stationary phase population persisted as doublets after 48 h growth.

Germ-tube formation by temperature shift in Wingé medium

At different phases of growth, cells were diluted into prewarmed medium either at 25 °C or at 37 °C, at a concentration in the range 2×10^6 to 5×10^6 cells ml⁻¹. In no case were germ-tubes produced from the organism at any phase of growth after incubation at 25 °C for 4 h. (Rare germinated elements were seen after 20 h incubation at 25 °C only in

serum plus GlcNAc, irrespective of the growth stage.) Cells from early- to mid-exponential phases of growth were also unable to develop germ-tubes when diluted into prewarmed Winge medium and incubated for up to 10 h at 37 °C. In contrast, more than 50 % of stationary phase cells formed germ-tubes after 1 h incubation (Fig. 1c). These germ-tubes appeared to be similar to those formed in serum (see below) and elongated normally during the subsequent 3 to 4 h. On further incubation, however, secondary budding took place and the population showed a mixed morphology at 20 h (Fig. 1d).

Some germination (about 10 % after 2 h) was detected when stationary phase cells were diluted in water and incubated at 37 °C.

Germ-tube formation induced by temperature and nutrients

The relationship between the phase of growth and the inducibility of germ-tube formation following exposure to suitable nutrients at 37 °C was different from that reported above. Exponentially growing cells, after 5, 7 and 9 h growth, were able to form germ-tubes when incubated in serum plus GlcNAc and, to a lesser extent, in serum or GlcNAc alone. Germ-tubes produced by early-exponential phase cells after 30 to 40 min in serum (Fig. 1e) elongated normally during the following 2 to 4 h and gave rise to a submerged hyphal growth after 12 to 18 h (Fig. 1f). Germination after 2 h incubation was 75 to 80 % for cells from early-exponential phase and more than 80 % for cells from late-exponential phase.

Apparently, any element of triplets or larger clusters could form germ-tubes while doublets often showed germ-tubes emerging from the smaller cell or bud. Germination was not preceded or accompanied, at least during the first hours of incubation, by any pronounced cell separation into singlets. Percentages of germination, the elongation rate and final growth morphology of cells from the stationary phase of growth induced to germinate in serum and GlcNAc were similar to those described for exponentially growing cells.

DISCUSSION

Work by Chaffin & Sogin (1976) and, more extensively, by Soll & Bedell (1978) suggested the existence of a close relationship between the stationary phase of growth of *Candida albicans* as a budding yeast and the inducibility of yeast cells to form germ-tubes. We have confirmed this relationship for two strains of *C. albicans* grown in Winge medium, where they can germinate by simple dilution into fresh medium at 37 °C. However, the results reported here also show that the intrinsic capacity of this organism to form germ-tubes is not an exclusive property of stationary phase cells. Given suitable chemical inducers, exponentially growing cells will also germinate efficiently. It is important to note that these germ-tubes produced true hyphae and never reverted to blastospores. Since any member of cell triplets or larger clusters, as well as both the younger and older elements of a doublet, could germinate, the existence of a close relationship between a precise stage of the cell cycle and the ability of a blastospore to germinate seems unlikely.

There is a classical body of evidence on the nutritional control of dimorphism in *Candida* (Romano, 1966). Recently, Shepherd & Sullivan (1976), working with organisms growing in continuous cultures, showed that a mycelial morphology was induced by maltose and a yeast morphology by lactate, at 30 °C. Overall, the results obtained by Chaffin & Sogin (1976), Soll & Bedell (1978) and the data reported here consistently show that, when temperature is the only critical factor for dimorphic transition, only stationary phase cells may undergo mycelial development. As suggested by Soll & Bedell (1978), these cells may reach a particular 'commitment' stage, possibly related to variations in the intracellular pools of metabolic intermediates and/or macromolecular components or other unknown factors. The use of nutritional inducers of germination may somehow by-pass or eliminate this requirement and force the exponentially growing cells to enter the hyphal cycle.

This conclusion stresses the importance of further research on the mechanism(s) by which certain natural products, such as serum, or defined inducers, such as GlcNAc, stimulate germ-tube formation and hyphal development in *C. albicans*.

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REFERENCES

- CASSONE, A., SIMONETTI, N. & STRIPPOLI, V. (1973). Ultrastructural changes in the wall during germ-tube formation from blastospores of *Candida albicans*. *Journal of General Microbiology* **77**, 417–426.
- CHAFFIN, W. L. & SOGIN, S. J. (1976). Germ-tube formation from zonal rotor fractions of *Candida albicans*. *Journal of Bacteriology* **126**, 771–776.
- LEE, K. L., BUCKLEY, H. R. & CAMPBELL, C. I. (1975). An aminoacid liquid synthetic medium for development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* **13**, 148–153.
- ROMANO, A. H. (1966). Dimorphism. In *The Fungi*, vol. 2, pp. 181–209. Edited by G. C. Ainsworth & A. S. Sussman. New York & London: Academic Press.
- SHEPHERD, M. G. & SULLIVAN, P. A. (1976). The production and growth characteristics of yeast and mycelial forms of *Candida albicans* in continuous culture. *Journal of General Microbiology* **93**, 361–370.
- SIMONETTI, N., STRIPPOLI, V. & CASSONE, A. (1974). Yeast–mycelial conversion induced by *N*-acetyl-D-glucosamine in *Candida albicans*. *Nature, London* **250**, 344–346.
- SOLL, D. R. & BEDELL, G. W. (1978). Bud formation and the inducibility of pseudo-mycelium outgrowth during release from stationary phase in *Candida albicans*. *Journal of General Microbiology* **108**, 173–180.
- TASCHDJIAN, C. L., BURCHALL, J. J. & KOZINN, P. J. (1960). Rapid identification of *Candida albicans* by filamentation in serum and serum substitutes. *American Journal of Diseases of Children* **99**, 212–215.