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## A Comparison of Germ Tube Production by *Candida albicans* in Three Media

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**Abstract:** The conversion of *C. albicans* from commensal to the pathogenic state is associated with its ability to make the dimorphic transition from the yeast to the hyphal (germ tube) form. Three liquid media were compared in ability to promote germ tube production by *C. albicans*. Medium 199 and Glucose-Glycine Medium at 37°C were highly satisfactory and gave 90 % conversion of yeast cells to hyphal-form cells. Sucrose-Gelatine medium gave only 15% conversion. Glucose-Glycine medium appeared to give more rapid germ tube production than 199 Medium.

**Key Words:** *Candida albicans*, germ tube, medium

### Üç Ortamda *Candida albicans* 'ın Germ Tüp Oluşumunun Kıyaslanması

**Özet:** *C. albicans*' ın komersal durumdan patojenik duruma dönüşümü, maya formundan hif (germ tüp) formuna dimorfik geçişi yapılabilişle ilgilidir. Üç sıvı ortamda *Candida albicans* 'ın germ tüp oluşumu uyarabilmesi için karşılaştırıldı. 199 Besiyeri ve Glukoz-Glisin Besiyeri 37°C de oldukça başarılı sonuçlar vermiş olup, % 90 oranında maya formundan hif formuna dönüşümünü sağladı. Sukroz-Jelatin Besiyeri sadece %15'lik dönüşüm sağladı. Glukoz-Glisin Besiyeri, 199 Besiyerin'den daha hızlı germ tüp oluşturdu.

**Anahtar Sözcükler:** *C.albicans*, germ tüp, besiyeri

### Introduction

*C. albicans* is a opportunistic pathogen and is dimorphic. The conversion of *C. albicans* from the commensal to the pathogenic state is associated with transition from the yeast cell to the hyphal form (1), although the evidence in support of this is equivocal (2, 3). This phenomenon of dimorphism must involve reorganization of the cell wall at the molecular level as revealed by detection of antigens and adhesins on hyphal-form cells (4-9). The morphological development of *C. albicans* can be described as follows: The yeast-form cell is the unicellular form of the fungus which propagates itself by budding. Under certain environmental conditions this form cell produces a cylindrical structure - the germ tube which is the initial morphological form of hyphae. The two parts are then described as the germ-tube portion and the yeast-cell portion. The whole structure is referred to as a hyphal-form cell (9).

The yeast-hypha transition in *C. albicans* is controlled by environmental conditions in which the important variables are the carbon source, pH, ions and temperature. The production of germ tubes in serum has remained the method of choice for identifying *C. albicans* (10).

A pH value in the range 6 - 8 is critical for germ tube formation (3). Germ tubes are induced when yeasts are incubated at temperatures between 33 and 42°C in a medium containing aminoacids such as proline, glutamine and arginine, and aminosugars such as *N*-acetylglucosamine and *N*-acetylmannosamine (11). Furthermore, *N*-acetylhexose derivatives, such as chitin, mucin, hyaluronic acid and immobilized *N*-acetylglucosamine are capable of inducing germ tubes (12). Serum, serum derivatives and tissue culture media are also promote germ-tube production (5, 6, 10). These derivatives are not metabolized or transported into the cells and it is believed that the inducer binds to a cell-surface receptor and produces an intracellular signal, which primes the cells for germ-tube formation. Divalent cations, in particular magnesium, are also necessary for germ tube formation (13). Glucose-starved cells inoculated into media with added calcium ions grew as hyphal forms, while removal of the calcium from the media caused the morphology to revert to the yeast form (14). It has been demonstrated that actively growing cultures need first to be starved before producing germ tubes (15). When yeast cells which have entered the stationary phase of growth in modified Lee medium without aminoacids are transferred into 199 medium at 37°C. They have been shown to produce germ tube within 2-2.5h (5, 6).

In this study, three liquid media, 199 Medium, Glucose-Glycine Medium and Sucrose-Gelatine Medium were compared for ability to promote germ tube production by *C. albicans*.

## Materials and Methods

### Organism

The *C. albicans* strain GDH 2346 (NCYC 1467) used in this study had been isolated at Glasgow Dental Hospital from a patient with denture stomatitis (16). It was supplied as freeze-dried samples from which further freeze-dried ampoules were prepared. The organisms were maintained on slopes of Sabouraud dextrose agar (Difco) and subcultured monthly. Every two months, cultures were replaced by new ones freshly grown from freeze-dried stocks.

### Culture Conditions

#### Modified Lee Medium without amino acids for starved cells

Yeast -form cells were prepared by subculturing in modified Lee medium (17) without amino acids (MLMwAA) as modified by Tronchin, *et al.*, (5) containing (in grams per liter):  $(\text{NH}_4)_2\text{SO}_4$ , 5.0;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2;  $\text{K}_2\text{HPO}_4$ , 2.5;  $\text{NaCl}$ , 5.0; glucose, 10.0; and biotin, 0.04 at pH 6.8. After incubation for 36 h at 25°C with orbital shaking at 150 rpm, cells in stationary phase were

harvested by centrifugation and washed three times in distilled water. Stock cultures were inoculated into 250 ml flasks containing 50 ml MLMwAA and incubated at 25°C for 36 h with orbital shaking at 150 rpm. The cells were harvested by centrifugation (3500 rpm for 5 min) and washed twice with distilled water and resuspended in 2 ml distilled water.

#### **Medium 199 Method of Tronchin *et al.* (1988)**

Medium 199 (Modified) with Hank's salts and 20 mM HEPES buffer, but without glutamine and sodium bicarbonate, was purchased from Flow Laboratories, Irvine Scotland. Its pH was adjusted with HCl to 6.7 and it was filtered through a 0.45 µm membrane filter. The final pellet of starved yeast cells in Modified Lee Medium was resuspended in 2 ml distilled water and added dropwise to 10 ml 199 Medium in a 50 ml conical flask to give a count of  $10^7$  cells ml<sup>-1</sup> using an improved Neubauer haemocytometer. The culture was then incubated at 37 °C for up to 140 min with constant shaking at 150 rpm. Samples were removed at intervals and examined as wet preparations at 400x to monitor the appearance of germ tubes. The cells in 10 fields were counted, and the number of hyphal-form cells in each field recorded. The percentage germ-tube production was thus calculated and plotted in relation to incubation time.

#### **Glucose-Glycine Medium Method of Muerkoester *et al.* (1979)**

The medium was prepared by dissolving the glycine (10.0 g) and the yeast extract (1.0 g) in 500 ml DW and adjusted to pH 7.5 with solid NaHCO<sub>3</sub> and autoclaved (18). The glucose solution (10.0 g in 500 ml distilled water) was autoclaved separately at 10 p.s.i. for 10 min and added to the glycine-yeast extract. The pH of the final medium was adjusted to 7.2 with HCl, followed by sterile filtration. The procedure was basically the same as above except that the starved cells instead of being inoculating into 199 Medium were inoculated into Glucose-Glycine Medium.

#### **Sucrose-Gelatin Medium (SG), Method of Mayer *et al.* (1992)**

Unlike the two above procedures, a three-stage process was employed. Yeast nitrogen base (YNB) was supplemented with glucose (10.0 g) and L-asparagine (1.5 g). Sucrose-Gelatin Medium (SG) was prepared by dissolving sucrose (10.0 g) and gelatin (0.75 g) in 1 L distilled water. Yeast cells were inoculated into 50 ml of the YNB in a 250 ml flask and incubated at 27°C overnight on a shaker at 150 rpm. From the overnight culture, 5 ml was inoculated into fresh 45 ml of the same medium and grown at 27°C for 24 h, again with shaking at 150 rpm. In the third stage, 10 ml from stage two was harvested, the cells washed twice in saline and resuspended in 2 ml saline. This suspension was added dropwise to 10 ml SG Medium in a 50 ml conical flask to give a count of  $10^7$  cells ml<sup>-1</sup>. The culture was then incubated at 37 °C with constant shaking at 150 rpm for up to 140 min. Germ tube production of the yeast cells were counted as described above. The experiments in this study were repeated three times.

## Results and Discussion

In this investigation, experiments were made to establish satisfactory conditions for obtaining rapid and consistent germ tube production by *C. albicans*. Germ tube production by *C. albicans* can occur rapidly in serum, serum substitutes and other natural and synthetic media. In order to find a suitable medium, the three defined media were tested. The figure shows that for the first 40 min in 199 Medium, there was no germ tube production, but thereafter the germ tubes started to appear and reached a plateau of about 90% at 140 min. GG Medium appeared to give more rapid germ tube production than 199 Medium, whereas SG Medium gave much slower and less complete germ tube production. With both, 199 Medium and GG Medium, an essential part of the overall procedure was 36 h starvation beforehand in Modified Lee Medium without aminoacids (MLMwAA), as reported by Tronchin *et al.* (5).

Complex synthetic media such as 199 Medium which were originally designed for tissue culture work have been widely used for production of hyphal-form *C. albicans* (5, 7-9, 20, 21) Tronchin *et al.* described 199 Medium as giving a high percentage of hyphal-form cells within 140 min at 37°C (5). The principal nitrogen source is various aminoacids and the main carbon source is glucose. The mixture of aminoacids in 199 Medium may therefore stimulate hypha formation by processes similar to those of serum.

Muerkoester *et al.* (18) compared GG medium with five other media, namely neopeptone-starch broth, Lee medium, non-sterile pooled human serum, sterilized pooled serum and Sabouraud's dextrose broth. They showed that GG Medium gave 70% germ tube production within 3.5 h at 37°C, which was a higher percentage and more rapid production of these structures than with the other media tested. Mr A.I. Altabet from our laboratory (personal communication) also used these six media and observed that 90% hyphal-form cells were produced by *C. albicans* in GG Medium at 37°C for 3.5 h. Among the less suitable media for germ-tube production was SG Medium which, in this study, gave only about 15 % germ-tube production after previous growth of the organisms in yeast nitrogen base containing glucose and L-asparagine, as described by Mayer *et al.* (19). These authors prepared yeast cells from three consecutive passages in yeast nitrogen base containing glucose and L-asparagine, with final resuspension in SG. After the 1 h incubation at 37°C used by these authors, the percentage of germ tubes was not reported. But it should be remembered that a low percentage of germ-tube production by *C. albicans* could be due to inappropriate preincubating conditions.

Although the production of germ tubes by yeast cells of *C. albicans* in serum is a useful rapid test for identification since it gives results within 2-3 h, the chemical complexity of serum is probably a disadvantage for basic studies of components on the surface of the germ-tube and yeast-cell portions of the cell.

In addition to promotion of germ tube production at similar percentage by GG and 199 Medium, as shown in this study, expression of adhesin molecules of hyphal-form *C. albicans* GDH 2346 specific for fucose were found to be similar in both media (7, 9). GG Medium can be preferred to use in similar studies since it has been easy to prepare in our laboratory conditions and is cheaper than 199 Medium.

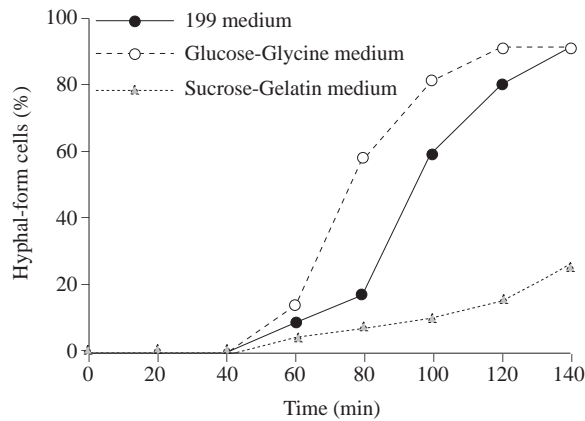


Figure 1. Production of germ tubes of *C. albicans* in three different media. Each point is based on the count of 60 cells per field over 10 fields.

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