

Short Report

## Comparison of Mycelial Production by *Candida Albicans* Isolated from Different Sources

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### ABSTRACT

The aim of this study was to compare the ability of 46 isolates of *Candida albicans* to produce mycelial form with oral source in three groups of individuals including, removable appliance wearers, non-wearers and oral medicine patients. Saliva samples were obtained from all subjects along with a foam imprint from the fitting surface of the upper removable appliance in the case of patients. Colonies that were green-blue were confirmed as *C. albicans* by the germ-tube test. The production of mycelia was measured *in vitro* using defined medium (Lee's medium) during 24 h. The results indicate that the production of mycelia in *C. albicans* isolated from removable appliance wearers and oral medicine patients are significantly different ( $p < 0.025$ ). *Iran. Biomed. J.* 7 (4): 187-189, 2003

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### INTRODUCTION

Many factors contribute to the virulence of *Candida albicans*, such as phospholipases, proteinases, acid phosphatase, etc. However, most investigators agree that the hyphal form of *C. albicans* adheres better to tissue and acrylic than yeast phase [1]. Furthermore, transformation of yeast to mycelium is necessary for the pathogenesis in humans [2-4]. Adherence of *C. albicans* to host cell surfaces is considered to be the first and essential step in colonisation and infection [5]. Thus, a close relationship has been established between the adhesive properties and their ability to colonize [6]. *C. albicans* when cause an infection is converted to its mycelial form or pseudohyphae under appropriate conditions [7]. Budtz-Jørgensen *et al.* [8] have reported that the hyphal form of *C. albicans* from the denture may be related to the pathogenesis of *Candida*-associated denture stomatitis. The aim of this study was to compare the ability of *C. albicans* to produce mycelial form from different sources.

### MATERIALS AND METHODS

Forty-six species of *C. albicans* were used in this study. These yeasts were oral isolates and obtained from the oral cavity of three groups of individuals including removable appliance wearers (18), non-wearers (6) and oral medicine patients (22) of the University of Manchester, Dental Hospital, Manchester, UK. In this study only healthy people were included and the medical history of each individual was checked for factors known to affect carriage of oral *Candida* i.e. diabetes, anaemia and immunosuppression. Similarly, individuals who smoked or who had received or were currently receiving treatment with antibiotics, antifungals or steroids in the past 3 months were excluded. Samples from subjects were inoculated on to CHROMagar *Candida* plates (CHROMagar *Candida* Company, Paris, France) and incubated at 37°C for 48 h aerobically, as recommended by the manufacturer. *C. albicans* were initially identified by colonial colour when compared with standard colour photographs supplied by the manufacturer. Colonies that were yellow-green to blue-green in colour were confirmed as *C. albicans* by germ tube-

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test, production of chlamydoconidia on Rice agar, growth at 42-45°C and ID32C Kits (After 48 h of incubation of ID 32C). *C. dubliniensis* does not assimilate D-L-lactate (LAT),  $\alpha$ -methyl-D-glucoside (MDG) or xylose (XYL) [9, 10].

In this study, for induction of mycelia, a chemically defined medium (Lee's medium) was prepared as describing by Lee *et al.* [11]. This liquid medium contained per 1000 ml of distilled water (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 5.0 g; MgSO<sub>4</sub>, 0.2 g; K<sub>2</sub>HPO<sub>4</sub> (Anhydrous), 2.5 g; NaCl, 5.0 g; glucose, 12.5 g; L-alanine, 0.5 g; L-leucine, 1.3 g; L-lysine, 1.0 g; L-methionine, 0.1 g; L-ornithine, 0.0714 g; L-phenylalanine, 0.5; L-proline, 0.5 g; L-threonine, 0.5 g and biotin 0.001 g. All ingredients (except biotin) were combined and autoclaved at 110°C for 20 min. Biotin was filtered by disc filter (0.2  $\mu$ m, Gelman Sciences, Wagner) and then added to the medium. Final pH of prepared Lee's medium was 6.8  $\pm$  0.05. To maximise and similar conditions yield, cultures were initiated with a standard inoculation (10<sup>6</sup>) CFU/ml of early stationary phase cells. The culture was incubated in a shaker water bath (Grant Instruments, Cambridge, England) at 150 rpm at 37°C. After 1, 3, 5, 7 and 24 h of incubation, yeasts germ-tubes and mycelia were counted in three fields of microscopic slide and the mean of percentages was calculated for each isolate (Fig. 1).

## RESULTS AND DISCUSSION

During the first 1-h, 72.2% of removable appliance wearers, 66.7% of non-wearers and 36.4% of oral medicine patient isolates had hyphal growth. Statistical analysis was shown that there is a significant differences between the production of mycelia in removable appliance wearers and oral medicine patients ( $p < 0.025$ ). However, the differences between removable appliance wearers and non-wearers, and non-wearers and oral medicine patients was not significant ( $p < 0.5$ ). In this study, 54.3% of the isolates were germinated after 1 h and 84.8% after 3 h. All isolates were germinated after 5, 7 and 24h. There were differences between the percentages of mycelial production in each isolate from different patient groups during 24 h (Fig. 2).

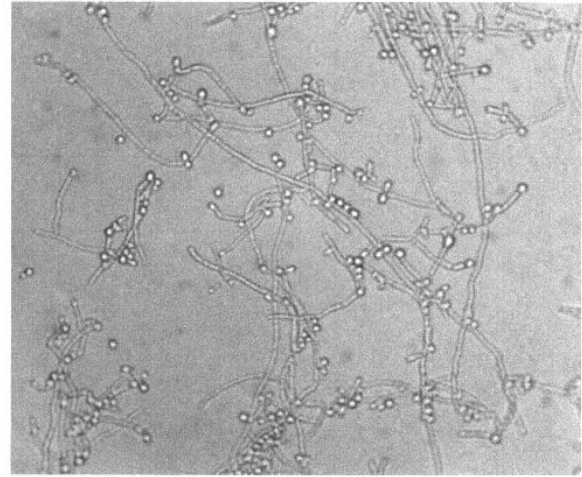


Fig. 1. Mycelia in *C. albicans* after 24 h growing on Lee's medium ( $\times 40$ ) (arrow shows a long branched mycelium).

For example, after 24 h, 10.7% of *C. albicans* isolates from removable appliances wearers and 6.5% from oral medicine patients (with denture) were produced mycelia. In this study, it was found that there is a difference between production of mycelia in Lee's medium and serum. The composition of Lee' medium and incubation period probably affect mycelia production.

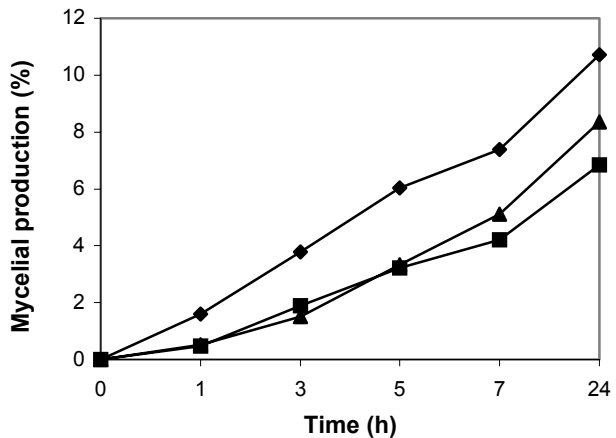


Fig. 2. Mycelial production during 24h in *C. albicans* in removable appliance wearers ( $\blacklozenge$ ), non-wearers ( $\blacksquare$ ) and oral medicine patients ( $\blacktriangle$ ).

The hyphal form of *C. albicans* is thought to be more invasive and facilitates adherence, colonisation and subsequent tissue invasion. Therefore, it has been recognised as one of the most important virulence factor [6]. Martin *et al.* [2] concluded that the pathogenic potential of *C. albicans* in the mouth depends on the formation of germ-tube and Budtz-

Jørgensen *et al.* [8] have found a large number of hyphae in patients with denture wearers. Moreover, it was claimed that proliferation of yeasts hyphal was occurred on the denture rather than palate [12].

Thus, a close relationship has been established between the adhesive properties and their ability to colonisation pathology [6]. Germination, cell wall mannoproteins and the presence of saliva are important factors in adhesion of *Candida* to denture [13, 14]. Long-term wearing of dental removable appliances is a major factor for the colonisation of *Candida* on mucous surfaces; this colonisation may lead to levels sufficiently high to give rise to oral candidosis, particularly affecting the mucosa beneath the appliance aid. The data show that *C. albicans* isolated from different individuals has varying potential for the production of mycelia *in vitro*. Orthodontic isolates produced faster mycelia than other isolates, suggesting that this feature may be an important attribute contributing to the mycelial invasion of host tissues.

## REFERENCES

1. McCourtie, J. and Douglas, L.J. (1981) Relationship between cell surface composition of *Candida albicans* and adherence to acrylic after growth on different carbon sources. *Infect. Immun.* 32: 1234-1241.
2. Martin, M.V., Craig, G.T. and Lamb, D.J. (1984) An investigation of the role of true production in the pathogenesis of experimental oral candidiasis. *Sabouraudia* 22: 471-476.
3. Sobel, J.D., Muller, G. and Buckley, H.R. (1984) Critical role of germ-tube formation in the pathogenesis of candidal vaginitis. *Infect. Immun.* 44: 576-580.
4. Vartivarian, S. and Smith, C.B. (1993) Pathogenesis, host resistance, and predisposing factors. In: *Candidiasis: Pathogenesis, Diagnosis and Treatment.* (Bodey, G.P. ed.), 2nd Edition, Raven Press, New York, pp. 59-84.
5. Douglas, L.J. (1987) Adhesion of *Candida* species to epithelial surfaces. *Crit. Rev. Microbiol.* 15: 27-42-59.
6. Odds, F.C. (1988) *Candida* and candidosis, a Review and Bibliography. 2nd Edition. Bailliere Tindall, London, pp. 468.
7. Murphy, J.W. (1993) Introduction. In: *Fungal Infections and Immune Response.* (Murphy, J.W., Friedman, H. and Bendinelli, M. eds.), Plenum Press, New York, pp. 1-5.
8. Budtz-Jørgensen, E., Stenderup, A. and Grabowski, M. (1975) An epidemiology study of yeasts in elderly denture wearers. *Commun. Dent. Oral Epidemiol.* 3: 115-119.
9. Giammanco, G.M., Pignato, S., Salvo, S. and Giammanco, G. (2000) Carbohydrate assimilation profiles of the first Italian *Candida dubliniensis* clinical isolates recovered from an HIV-infected individual. *Res. Microbiol.* 151:889-891.
10. Tintelnot, K., Haase, G., Seibold, M., Bergmann, F., Staemmler, M., Franz, T. and Naumann, D. (2000) Evaluation of phenotypic markers for selection and identification of *Candida dubliniensis*. *J. Clin. Microbiol.* 38: 1599-1608.
11. Lee, K.L., Buckley, H.R. and Campbell, C.C. (1975) An amino acid liquid synthetic medium for the development of mycelial and yeast forms of *Candida albicans*. *Sabouraudia* 13: 148-153.
12. Olsen, I. and Bondevik, O. (1978). Experimental *Candida*-induced denture stomatitis in the Wistar rat. *Scand. J. Dent. Res.* 86: 392-398.
13. Maza, J.L., Elguezabal, N., Prado, C., Ellacuria, J., Soler, I. and Ponton, J. (2002) *Candida albicans* adherence to resin-composite restorative dental material: Influence of whole human saliva. *Oral. Surg. Oral. Med. Oral. Pathol. Oral. Radiol. Endod.* 94:589-592.
14. Herrero, A.B., Uccelletti, D., Hirschberg, C.B., Dominguez, A. and Abeijon, C. (2002) The Golgi GDPase of the fungal pathogen *Candida albicans* affects morphogenesis, glycosylation, and cell wall properties. *Eukaryot Cell* 1:420-431.