Yonsei Medical Journal
Vol. 40, No. 5, pp. 420−424, 1999

The Presumptive Identification of *Candida albicans* with Germ Tube Induced by High Temperature

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Abstract

For direct identification of *Candida albicans* from other *Candida* species, the chlamydospore formation and the mycelial transition induced by high temperature and by sera were examined in 198 *Candida* isolates. The germ tubes of *C. albicans* developed early at 30 min in high temperature-induction, but at 60 min in serum-induction. *C. albicans* generated germ tubes well at concentrations lower than 2×10^7 cells/ml, but the germ tube formation was markedly restrained at concentrations higher than 4×10^7 cells/ml. In a serum-free, yeast extract-peptone-dextrose (YEPD) medium, *C. albicans* grew as a yeast form at 30°C and as a mycelial form at 35−42°C. Mycelial development was maximal at 37°C in serum and at 39°C in YEPD. Germ tubes were formed within 30 min in YEPD at 39°C, but after 60 min in serum at 37°C. Our examination showed that the 39°C-induced germ tube formation tests were very reliable (sensitivity 100%, specificity 100%) at discerning *C. albicans* from other *Candida* species. These results suggest that the high temperature-induced germ tube formation testing could be a useful identification method of *C. albicans* in clinical laboratories.

Key Words: *Candida albicans*, high temperature, germ tube

INTRODUCTION

Candida albicans is the most frequently isolated fungal pathogen in humans. As the primary agent of mucocutaneous and disseminated candidiasis, the opportunistic pathogen *C. albicans* causes significant morbidity and mortality, especially in immunocompromised, postoperative patients.[1-4]

C. albicans exhibits the ability to grow in either a yeast or a mycelial form in response to different environmental factors. In vivo, the mycelial forms found in infected tissues may be important as a virulence factor in the adherence of organisms to host epithelium.[5,6] In vitro, the morphological transition can be induced by environmental shifts in growing conditions or a variety of exogeneous factors including ambient pH,[7] nutritional status[8-11] and temperature.[12] However, the mechanism whereby these factors induce germ tube formation in *C. albicans* is virtually unknown.

In the identification of unknown yeast isolates, conventional methods including germ tube and chlamydospore formation, as well as sugar assimilation tests have been used.[13] To perform the germ tube formation test, yeast isolates are incubated in serum at 37°C for a few hours. In this study, for direct identification of *C. albicans* from other *Candida* species, we adopted germ tube formation induced by high temperature in serum-free medium substituting for the conventional method. The results were more reliable than the serum-induced germ tube test and chlamydospore test.

Received March 12, 1999
Accepted July 19, 1999
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This study was supported by a grant (# HMP-96-M-2-1060) of the '96 Good Health R&D Project, Ministry of Health and Welfare, Republic of Korea.
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MATERIALS AND METHODS

Organisms and culture

Standard strains of *C. albicans* ATCC 10231, *C. albicans* ATCC 36081 (serotype A), *C. albicans* ATCC 36082 (serotype B), *C. tropicalis* ATCC 14056, *C. parapsilosis* ATCC 7330, *C. glabrata* ATCC 38326,

C. krusei ATCC 2159, and *C. guilliermondii* ATCC 56802 were obtained from the National Institute of Health, Seoul, Korea. Sixty blood isolates and 138 commensal isolates were identified using standard methods including morphological characteristics and biochemical tests. All yeasts were precultured in YEPD broth composed of 1% (w/v) yeast extract, 2% (w/v) peptone and 2% (w/v) dextrose at 37°C for 18 h, then transferred into fresh YEPD medium, and incubated at 37°C for 4 h. Precultured yeast cells were washed twice by centrifugation with sterile PBS [50 mM-sodium phosphate buffer with 0.9% (w/v) sodium chloride, pH 7.2]. The concentration of yeast suspension was adjusted by haemocytometer measuring.

Carbohydrate assimilation

Carbohydrate assimilation test was performed by the disk method. Yeast suspension in saline equivalent to McFarland standard No. 0.5 was spread on a yeast nitrogen base agar plate. Paper disks soaked in 1% (w/v) carbohydrate solution were placed on the plate. The results were acquired after incubating at 25°C for 2−3 days.

Chlamydospore formation

Suspended yeast was deeply streaked with a loop in cornmeal tween 80 agar plate and covered with glass slips. The plate was incubated in high humidity at 25°C for 7 days. Chlamydospore was observed with a microscope (×400).

Germ tube formation

Adjusting cell concentration of yeasts, the washed *C. albicans* was inoculated into a culture flask in rabbit serum or in YEPD, and incubated at 30−45°C for 0.5−3 h. The germ tube was observed with a microscope (×400).

RESULTS

Optimal conditions for germ tube formation

The rate of germ tube formation of *C. albicans* was investigated during incubation time of 3 h (Fig. 1 and 2). When culturing in serum for at least 2 h, *C. albicans* generated germ tubes with more than 60% yeasts. In YEPD, a transition rate of >60% was shown at 39°C after an incubation time of 1 h. The germ tube was formed within 30 min in YEPD at 39°C, but after 60 min in serum at 37−39°C, and in YEPD at 37°C. The effect of cell concentration on germ tube formation of *C. albicans* was also examined in serum at 37°C for 2 h and in YEPD at 39°C for 1 h (Fig. 3). By increasing cell concentrations of *C.*

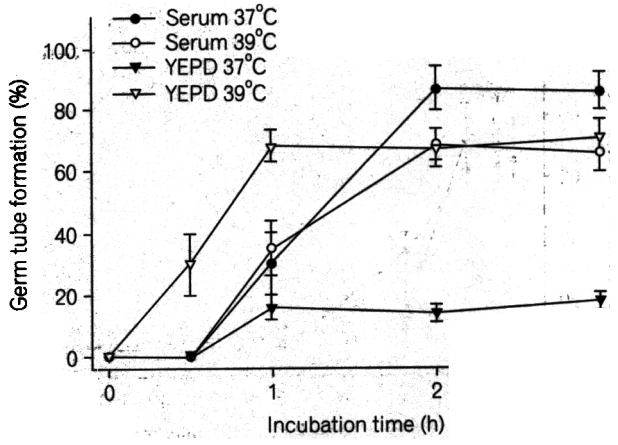

Fig. 1. Effect of incubation time on germ tube formation of C. albicans. At an initial cell concentration of 2×10^7 cells/ml, C. albicans was cultured in serum (37°C, ●; 39°C ○) and in YEPD (37°C, ▼; 39°C ▽). Data are expressed as mean±SD (n=5).

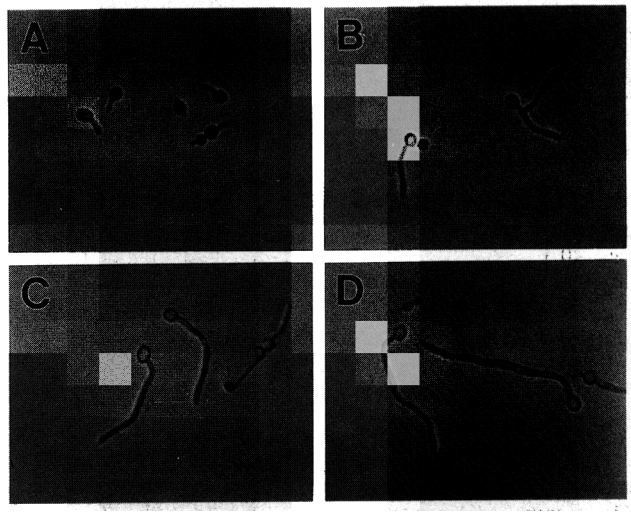

Fig. 2. Photographs of germ tube induced by high temperature. At an initial cell concentration of 2×10^7 cells/ml, C. albicans was cultured in YEPD at 39°C for various times. A, 30 min; B, 60 min; C, 90 min; D, 180 min.

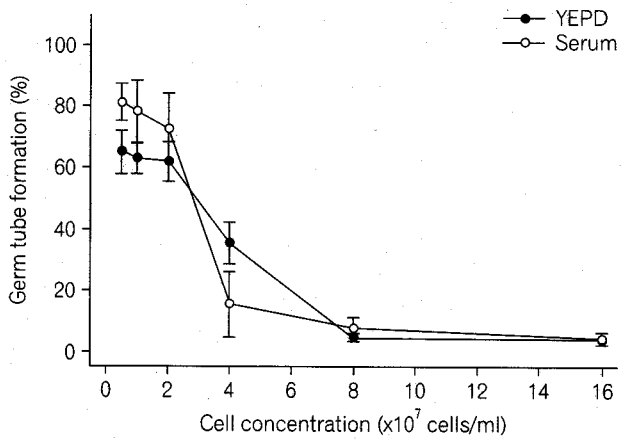

Fig. 3. Effects of cell concentration on germ tube formation of C. albicans. Various concentrations of C. albicans were cultured in serum (○) at 37°C for 2 h and in YEPD (●) at 39°C for 1 h. Data are expressed as mean±SD (n=5).

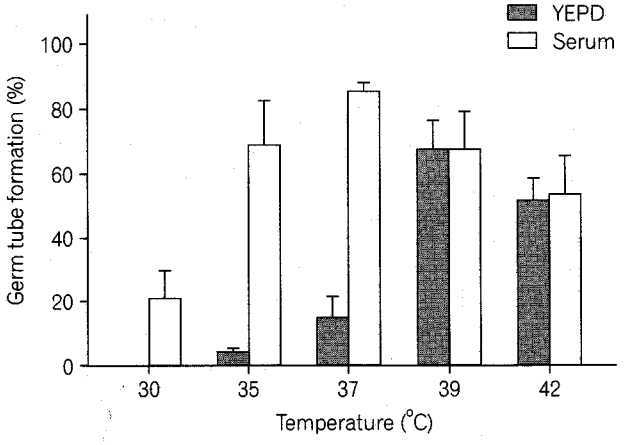

Fig. 4. Effects of temperature on germ tube formation of C. albicans. At an initial cell concentration of 2×10^7 cells/ml, C. albicans was cultured in serum (□) for 2 h and in YEPD (■) for 1 h at an range of 30−45°C. Data are expressed as mean±SD (n=5).

albicans, the rates of germ tube formation were decreased in both conditions. *C. albicans* generated germ tubes well at concentrations lower than 2×10^7 cells/ml, but the germ tube formation was markedly restrained at concentrations higher than 4×10^7 cells/ml. As in culturing at a concentration of 2×10^7 cells/ml in YEPD for 1 h, the rate of germ tube formation was more than 50% at 39−42°C (Fig. 4). In serum, *C. albicans* generated germ tubes well at a wide range of 30−42°C. *C. albicans* grew as a yeast form at 30°C and as a mycelial form at 35−42°C in serum-free YEPD medium. Mycelial development was

Table 1. Microscopic Morphology of *Candida* Species

Strains	Morphology*	
	Serum, 37°C	YEPD, 39°C
C. albicans ATCC 10231	M	M
C. albicans ATCC 36081 serotype A	M	M
C. albicans ATCC 36082 serotype B	M	M
C. tropicalis ATCC 14056	M	Y
C. parapsilosis ATCC 7330	M	Y
C. glabrata ATCC 38326	Y	Y
C. krusei ATCC 2159	Y	Y
C. guilliermondii ATCC 56802	Y	Y

With the initial cell concentration of 2×10^7 cells/ml, each yeast was incubated in serum (37°C, 2 h) and in YEPD (39°C, 1 h).
* M, mycelial form; Y, yeast form.

maximal at 37°C in serum and at 39°C in YEPD. In both conditions, germ tube was not formed at 45°C. Although the rate of mycelial transition in serum was higher than that in YEPD at less than 37°C, the rate was similar in both conditions at a higher temperature, 39−42°C.

Mycelial transition

Eight standard yeasts were cultured in serum at 37°C for 2 h and in YEPD at 39°C for 1 h (Table 1). All three *C. albicans* formed germ tubes in serum at 37°C and in YEPD at 39°C. However, *C. glabrata*, *C. krusei*, and *C. guilliermondii* did not form germ tubes and grew only as a yeast form in serum and in YEPD. In cases of *C. tropicalis* and *C. parapsilosis*, they grew as a single yeast form in YEPD, but as a pseudohyphae or a chain form in serum.

Identification with high temperature-induced germ tube formation

Among the 198 strains of clinical isolates, 131 strains formed germ tubes or pseudohyphae in serum, 105 strains produced germ tubes in YEPD at 39°C, and 102 strains produced chlamydospores in cornmeal tween 80 agar. However, of the 198 *Candida* isolates tested, *C. albicans* comprised only 105 strains (Table 2). The sensitivities/specificities of the high temperature-induced germ tube test, the serum-induced germ

Table 2. Sensitivity and Specificity of Identifying Methods for *C. albicans*

	Hyphal transition induction		Chlamydospore
	Serum, 37°C	YEPD, 39°C	
Sensitivity	98.1	100.0	96.2
Specificity	78.6	100.0	99.0

For hyphal transition, with initial cell concentration of 2×10^7 cells/ml, each yeast was incubated in serum (37°C, 2 h) and in YEPD (39°C, 1 h). Chlamydospore test is described in Materials and Methods.

tube test, and the chlamydospore test were 100/100%, 98.1/78.6%, and 96.2/99.0%, respectively.

DISCUSSION

C. albicans can reproduce by budding, giving rise to the formation of yeast cells. The production of germ tubes results in conversion to a filamentous growth phase or mycelial form. The formation of pseudohyphae occurs by polarized cell division when yeast cells grown by budding have elongated without detaching from adjacent cells. Under certain nonoptimal growing conditions, *C. albicans* can undergo the formation of chlamydospores, which are round, refractile spores with a thick cell wall. These morphological transitions often represent a response of the fungus to changing environmental conditions and may permit the fungus to adapt to different biological nicks.[14]

It has been known that serum, heme, albumin, N-acethylglucosamine or glucose starvation have an influence on germ tube formation of *C. albicans*. For germ tube formation, the concentration of yeast cells and culture temperature also play a role as limiting factors. With heavy inoculum of more than 8×10^7 cells/ml, germ tubes were formed from less than 10% of yeast cells in serum and serum-free medium. For germ tube development, the ranges of temperature were different between serum and serum-free medium. Germ tube induction of *C. albicans* may involve complicated pathways triggered by distinct environmental factors which regulate different or overlapped subsets of controlling systems for dimorphism.

For more convenient identification of *Candida* species, commercial methods have been developed.[15-17] Recently, molecular methods including PCR have been also studied.[18,19] However, the observation of germ tube production as a method for the presumptive identification of *C. albicans* has been widely used for many years without limitations of skill and cost, and has provided quite reliable results. Besides *C. albicans*, other *Candida* species which can grow mycelial form in serum have been known. Since some other *Candida* species can generate germ tubes or pseudohyphae in serum and their assimilation patterns are similar to that of *C. albicans*, it is often difficult to discern *C. albicans* from other *Candida* species. We investigated *C. albicans* generated germ tubes only by the high temperature-induction in serum-free medium. In the application of this method for identifying *C. albicans*, our results obtained from the high temperature-induced method were not only more reliable than those of the serum-induced germ tube formation test, but they were also more sensitive than those of the specific chlamydospore formation test. Germ tube formation induced by high temperature could apply to a presumptive identification method in clinical laboratories.

REFERENCES

1. Hurley R, de Louvois J, Mulhall A. Yeasts as human and animal pathogens. In: Rose AH, Harrison JS, editors. The yeasts. 2nd ed. London: Academic Press INC; 1987. p.212-39.
2. Greenfield RA. Host defence system in interactions with *Candida*. J Med Vet Mycol 1992;30:89-104.
3. Jarvis WR. Epidemiology of nosocomial fungal infections with emphasis on *Candida* species. Clin Infect Dis 1995; 20:1526-30.
4. Pfaller MA. Epidemiology of fungal infections. J Hosp Infect 1995;30 Suppl:329-38.
5. Odds FC. *Candida* and candidosis. A review and a bibliography. 2nd ed. London: WB Saunders; 1988.
6. Cutler JE. Putative virulence factors of *Candida albicans*. Annu Rev Microbiol 1991;45:187-218.
7. Pollack JH, Hashimoto T. The role of glucose in the pH regulation of germ-tube formation in *Candida albicans*. J Gen Microbiol 1987;133:415-24.
8. Barlow AJ, Aldersley T, Chattaway FW. Factors present in serum and seminal plasma which promote germ-tube formation and mycelial growth of *Candida albicans*. J Gen Microbiol 1974;82:261-72.
9. Mattia E, Carruba G, Angiolella L, Cassone A. Induction of germ tube formation by N-acetyl-D-glucosamine in

Candida albicans: uptake of inducer and germinative response. J Bacteriol 1982;152:555-62.
10. Bruatto M, Gremmi M, Nardacchione A, Amerio M. Effect of glucose starvation on germ-tube production by *Candida albicans*. Mycopathologia 1993;123:105-10.
11. Casanova M, Cervera AM, Gozalbo D, Martinez JP. Hemin induces germ tube formation in *Candida albicans*. Infect Immun 1997;65:4360-4.
12. Odds FC. Morphogenesis in *Candida albicans*. Crit Rev Microbiol 1985;12:45-93.
13. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn Jr WC. Color atlas and textbook of diagnostic microbiology. 4th ed. Philadelphia: J.B. Lippincott Company; 1992. p.813-50.
14. Chaffin WJ, Lopez-Ribot JL, Casanova M, Gozalbo D, Martinez JP. Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. Microbiol Mol Biol Rev 1998;62:130-80.
15. Odds FC, Bernaerts R. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J Clin Microbiol 1994; 32:1923-9.
16. Rousselle P, Freydiere AM, Couillerot PJ, de Montclos H, Gille Y. Rapid identification of *Candida albicans* by using albicans ID and Fluoroplat agar plates. J Clin Microbiol 1994;32:3034-6.
17. Quindos G, San Millan R, Robert R, Bernard C, Ponton J. Evaluation of bichro-latex *albicans*, a new method for rapid identification of *Candida albicans*. J Clin Microbiol 1997;35:1263-5.
18. Weissman Z, Berdicevsky I, Cavari B. Molecular identification of *Candida albicans*. J Med Vet Mycol 1995;33: 205-7.
19. Shin JH, Nolte FS, Morrison CJ. Rapid identification of *Candida* species in blood cultures by a clinically useful PCR method. J Clin Microbiol 1997;35:1454-9.