The Effect of Rabbit Serum on the Expression of Cell Surface Hydrophobicity in *Candida albicans*

Woon Seob Shin, Donghwa Kim*, Kyoung Ho Lee*, Kyunghoon Kim, Yoon Sun Park and Choon Myung Koh***

Department of Microbiology, Kwandong University College of Medicine, Kangnung 210-701, Korea
**Department of Microbiology, Yonsei University Wonju College of Medicine, Wonju 220-701, Korea*
***Division of Life Sciences, College of Natural Sciences, Kangwon National University, Chunchon 200-701, Korea*

INTRODUCTION

Candida albicans, one of the best-known opportunistic pathogens, is an important infectious agent because the candidiasis is a common life-threating disease in immunocompromised patients. Despite numerous investigations on candidiasis, conclusive virulence factors are not well-defined because of several confusing and contradictory results. In general, adhesive factors, invasive factors, and toxins are regarded as major virulent factors for the pathogenesis of mocroorganis ms. The candidiasis, however, appears to be caused not by a single virulent factor, but by an array of various virulent factors such as protease, phospholipase adhesin, and germ tube formation[1]. Adhesive factors play an important role in the initial step of the *Candida* infection. Accordingly, the adherence properties of *C. albicans* to various surfaces, especially those of vaginal and buccal epithelial cells, have been the subject of extensive investigations[2]. It was reported that cell surface hydrophobicity (CSH) of *C. albicans* is involved in its adherence to host tissues and the level of the CSH expression varies with the isolates of *C. albicans*[3~5]. Hydrophobic cells of *Candida albicans* are more virulent than hydrophilic cells in mice[6]. The CSH expression of *C. albicans* is also dependent on environmental conditions[7,8]. *C. albicans* cells grown at room temperature are more virulent than those grown at 37℃ because they are less likely to be killed by phagocytes and are more likely to disseminate[6]. In general, *C. albicans* expresses CSH more actively at 25℃ than 37℃ and the germ tube form of *C. albicans* expresses CSH more than the yeast form. Moreover, hydrophobic proteins of *C. albicans* are produced while the yeasdt cells are invading the host tissues[9]. Hydrophobic cell wall proteins from *C. albicans*[10~13] grown in serum-free media have been extensively studied, but it is not clear whether the hydrophobic proteins produced in serum-free media are identical to those produced in serum culture. Because the culture condition of *C. albicans* in serum would be similar to that *in vivo*, we investigated the dynamic expression of CSH from *C. albicans* in serum and compared the cell wall protein patterns from YEPD-induced hydrophobic cells at 25℃ or 37℃, serum-induced hydrophobic cells, and hydrophilic cells.

MATERIALS AND METHODS

Organisms and culture conditions

C. albicans YWCM132 was chosen out of 198 clinical isolates by measuring the lethality of mice and the level of CSH. The YWCM132 strain was highly virulent to mice, and expressed CSH to a

†Corresponding author: Prof. Choon Myung Koh, Department of Microbiology, Yonsei University Wonju College of Medicine, 162 Ilsan-Dong, Wonju, Kangwon-Do 220-701, Korea. Tel. +82-371-741-0321; Fax. +82-371-748-2709; E-mail. kohcm@wonju.yonsei.ac.kr
This study was supported by a grant (# HMP96-M-2-1060) of the '96 Good Health R&D Project, Ministry of Health and Welfare, R.O.K

higher extent at 25°C but expressed CSH much less at 37°C. The strain was grown on a YEPD (1% yeast extract, 2% peptone, 2% dextrose, pH 6.8) plate at room temperature. Cells were harvested from the plate, suspended in 20% skim milk, and maintained at -70°C. For the seed culture, the *C. albicans* strain was grown in YEPD liquid at 37°C for 18 h, harvested by centrifuge, and washed twice with sterile PBS (0.05 M sodium phosphate buffer with 0.9% sodium chloride, pH 7.2). The cell number was monitored using a haemocytometer. For the CSH expression, the strain was cultivated for 2 h at 37°C in rabbit serum or in YEPD liquid, or grown overnight in YEPD at 25°C. The cells become hydrophilic when they are grown overnight in YEPD at 37°C. In order to investigate the effect of glucose concentration on the CSH expression from *C. albicans*, 0.1% or 1% glucose was added to rabbit serum.

Determination of cell surface hydrophobicity

The CSH of *C. albicans* was measured by the methods of Kennedy *et al.*[14] and Minagi *et al.*[15]. After *C. albicans* was cultured under various conditions, it was harvested, and washed three times with sterile PBS. The concentration of washed cells was adjusted to an optical density of 0.5 at 580 nm. For the measurement of CSH, 1 ml of *n*-hexane was added to 4 ml of the adjusted cell suspension and vortexed for 1 min. The OD of the mixture was then measured at 580 nm with a spectrophotometer. The relative hydrophobicity was determined by measuring the difference in absorbencies of test and control cells, and by calculating the percentage of cells that entered the *n*-hexane phase.

Isolation of cell wall components

One hundred μl of wet cells was suspended in 440 μl of sterile PBS, and 60 μl of lyticase (ß-1,3 glucanase, 1250 units ml^{-1}), or proteinase K (5 mg ml^{-1}) was added to the suspension. The mixture treated with lyticase was incubated at 25°C for 2 h. The mixture with proteinase K was incubated at 37°C for 2 h. Each enzyme-treated mixture was then centrifuged at 5000 x g for 10 min. The cell pellet was washed three times with sterile PBS and used for the determination of the CSH. The supernatant was precipitated with 90% ethanol, centrifuged at 10000 x g for 10 min, and the harvested precipitate was used for the analysis of cell wall components by SDS-PAGE.

SDS-PAGE

The ethanol-precipitate containing the cell wall components was dissolved in sample buffer (62.5 mM Tris-HCl, 2% SDS, 10% glycerol, 5% 2 mercaptoethanol, 0.01% bromophenol blue, pH 6.8). After being boiled for 10 min, 20 μl of each sample was loaded on a 1.5 mm-thick slab gel of 15% polyacrylamide running gel with a 3% polyacrylamide stacking gel and electrophoresed at 15 V overnight[16]. After the electrophoresis, the gel was stained with Coomassie brilliant blue R-250 and apparent molecular weights of the proteins were estimated from a standard curve based on migrations of the molecular marker proteins

RESULTS

In order to investigate the dynamic expression of CSH in *C. albicans*, we measured the CSH of *C. albicans* which had been cultured in rabbit

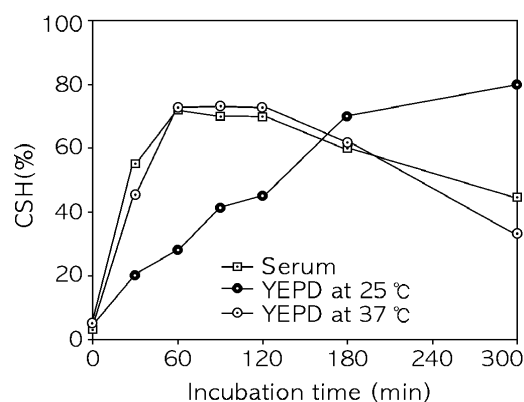


Fig. 1. Dynamic expressions of CSH during the growth of *C. albicans* YWCM132 in rabbit serum and YEPD medium. At intervals during the growth, the relative CSHs were determined with the *n*-hexane partition method. Initial cell concentration was 2 x 10^7 cells ml^{-1}.

Table 1. Effects of enzyme treatments on the CSH expression from *C. albicans* YWCM132

	Initial	Lyticase	Proteinase K
37°C hydrophilic cell	5.3	5.5	17.2
25°C hydrophobic cell	90.3	36.9	75.4
37°C hydrophobic cell	51.6	16.3	28.6
Serum hydrophobic cell	80.1	40.2	66.7

For hydrophobic induction, 1 x 10^9 cells were inoculated in 50 ml of rabbit serum or YEPD liquid, and cultured for 2 h at 37°C or overnight at 25°C

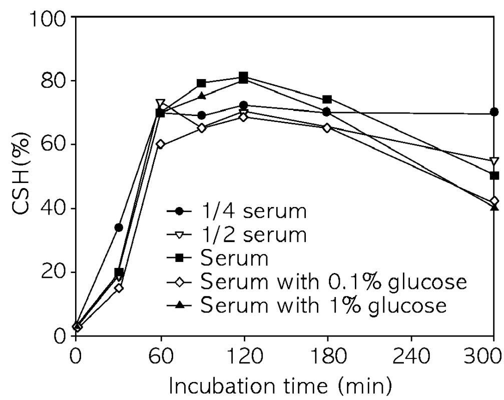


Fig. 2. Effects of serum and glucose concentrations on the CSH expression from *C. albicans* YWCM132. Initial cell concentration was 2 x 10^7 cells ml^{-1}.

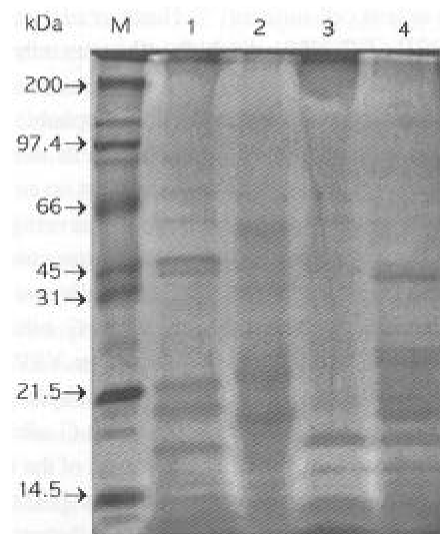


Fig. 3. SDS-PAGE of cell wall extracts of hydrophobic and hydrophilic *C. albicans* YWCM132. Each lane was loaded with 20 μl of the cell wall extract prepared as described in Materials and Methods. lane 1, Hydrophilic; lane 2, Serum-induced hydrophobic; lane 3, 37°C YEPD-induced hydrophobic; lane 4, 25°C YEPD-induced hydrophobic.

serum at 37°C, and in YEPD at 25°C or 37°C for 5 h (Fig. 1). The CSH increased during the initial 60 min growth upon release into serum at 37°C and fresh YEPD at 37°C and thereafter slowly decreased. However, at 25°C, the CSH increased gradually throughout the entire culture period of 5 h.

We also investigated the effects of glucose and serum concentrations on the expression of CSH in *C. albicans* (Fig. 2). The serum and glucose concentrations did not influence the initial expression of the CSH. Interestingly, the initially increased CSH in 1 to 4-diluted serum was maintained with no decrease for the rest of culture period.

Effects of lyticase and proteinase K treatments on the CSH expression from *C. albcans* were examined (Table 1). The CSH was not affected by proteinase K and lyticase treatments in case of the hydrophilic yeast grown overnight in YEPD at 37°C. However, levels of the CSH of the hydrophobic yeasts were lowered by the hydrolytic enzyme treatments.

Since the enzyme treatments resulted in a decrease in the CSH of the hydrophobic cells, we compared the cell wall protein patterns from the 25°C-, 37°C-induced hydrophobic cells, serum-induced hydrophobic cells, and hydrophilic cells using a 15% SDS-polyacrylamide gel (Fig. 3). The protein profiles of the hydrophobic cells and the hydrophilic cells were not identical to each other. The major proteins of the hydrophilic yeast were 47, 44, 24, 20, and 17 kDa (Fig. 3, lane 1). While pro-

teins of 52.4, 25, and 19 kDa were extracted from the hydrophobic cells induced in serum (Fig. 3, lane 2), extraction of the hydrophobic cells induced in YEPD at 25℃ yielded specific proteins of 42.5, 29, 27, and 19 kDa (Fig. 3, lane 4).

DISCUSSION

It was generally accepted that cell surface structures play important roles in adhesion of *C. albicans* to host cell surfaces[17,18]. Hazen *et al.*[11] studied the CSH of *C. albicans* which influences adhesion of the yeast to epithelial cells and virulence of the organism[3,4]. He also reported that hydrophobic cells were more virulent than hydrophilic cells in mice, and the CSH expression was dependent on environmental conditions[6]. In this study, we investigated the effect of rabbit serum on the expression of CSH in *C. albicans*. The CSH of *C. albicans* was expressed early within 60 min when *C. albicans* YWCM132 was cultured in serum or YEPD at 37℃. According to Glee *et al.*[9], several hydrophobic proteins are actually expressed from *C. albicans in vivo*. Therefore, the early expression of the CSH from cells grown in serum or YEPD at 37℃ may play a role in initial growth of *C. albicans* and pathogenesis of *C. albicans*. The CSH was maintained highly at 25℃ even after overnight culture. Consistently with this result, Antley *et al.*[6] reported that the cells grown at room temperature are hydrophobic and less likely to be killed by phagocytes, and more likely to disseminate[6]. However, the CSH decreased after 120 min in serum or YEPD culture at 37℃. We do not know exactly why the CSH decreases. When the cells are grown for more than 2 h in YEPD at 37℃, no germ tube is formed and cell aggregation does not occur. On the contrary, when the cells are cultured for more than 2 h in serum at 37℃, germ tube formation and cell aggregation become active. Thus, the observed decrease in hydrophobicity of serum-cultured cells may be not due to the real decrease in CSH but due to the germ tube formation and cell aggregation. In fact, the germ tube form of *C. albicans* expresses CSH to a higher extent but the CSH can not be measured by the *n*-hexane partition method used in this study because the aggregated cells are precipitated so easily.

We investigated the effects of serum and glucose concentrations on the expression of hydrophobicity in *C. albicans* YWCM132 (Fig. 2). Glucose addition or serum dilution did not affect the CSH expression in the initial 60 min growth of *C. albicans*. This result is similar to the reports of Hazen *et al.*[11] There are many reports about the CSH of *C. albicans* in various environments[5,7,8]. However, there is little information about hydrophobic proteins of *C. albicans* expressed in serum. It is not clearly known whether the hydrophobic proteins of *C. albicans* expressed in serum are identical to those expressed in serum-free media.

In order to investigate the cell wall components of the hydrophobic yeasts induced by various conditions, we studied the effects of hydrolytic enzymes on the CSH, and analyzed the cell wall proteins by SDS-PAGE (Table 1, Fig. 3). Hydrolytic enzymes decreased the CSH of hydrophobic yeasts induced by serum or YEPD at 37℃. Extraction of cell wall proteins by hydrolytic enzymes from the hydrophilic and the hydrophobic yeasts revealed different protein profiles, indicating that different kinds of proteins are involved in the CSH expression from C. albicans under various conditions.

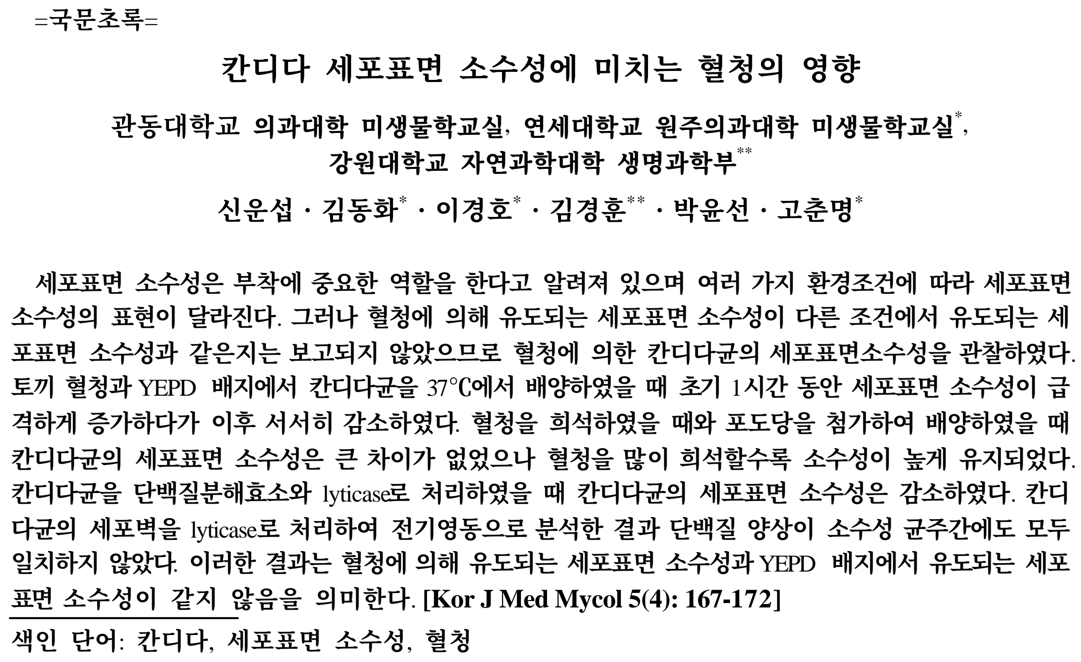
SUMMARY AND CONCLUSION

The CSH of *Candida albicans* YWCM132 increased rapidly during the initial 60 min growth in YEPD or serum at 37℃. and then decreased slowly. However, in YEPD at 25℃, the CSH increased in progression for 300 min. Glucose addition or serum dilution did not affect the initial expression of the CSH. The CSH of the hydrophobic yeasts was decreased by the treatment of hydrolytic enzymes such as lyticase and proteinase K. Upon SDS-PAGE analysis of cell wall proteins with lyticase treatment, the protein profiles of the hydrophilic and the hydrophobic cells were revealed to be different. The major cell wall proteins from the hydrophilic yeast were 47, 44, 24, 20, and 17 kDa.

Patterns of the cell wall proteins of the serum-induced and the YEPD-induced hydrophobic cells were different from each other. While proteins of 52.4, 25, and 19 kDa were extracted from the hydrophobic cells induced in serum, extraction of the hydrophobic cells induced in YEPD at 25℃ yielded specific proteins of 42.5, 29, 27, and 19 kDa. These results indicate that various hydrophobic cells induced by serum or YEPD at 37℃, or YEPD at 25℃ express different hydrophobic proteins.

REFERENCES

1. Cutler JE. Putative virulence factors of *Candida albicans*. Annu Rev Microbiol 1991; 45: 187-218
2. Martinez JP, Gil MU, Lopez Ribot JL, Chaffin WL. Serologic response to cell wall mannoproteins and proteins of *Candida albicans*. Clin Microbiol Rev 1998; 11: 121-141
3. Kennedy MJ, Rogers AL, Yancey RJ Jr. Environmental alteration and phenotypic regulation of *Candida albicans* adhesion to plastic. Infect Immun 1989; 57: 3876-3881
4. Hazen KC. Participation of yeast cell surface hydrophobicity in adherence of *Candida albicans* to human epithelial cells. Infect Immun 1989; 57: 1894-1900
5. Silva TM, Glee PM, Hazen KC. Influence of cell surface hydrophobicity on attachment of *Candida albicans* to extracellular matrix proteins. J Med Vet Mycol 1995; 33: 117-122
6. Antley PP, Hazen, KC. Role of yeast cell growth temperature on *Candida albicans* virulence in mice. Infect Immun 1988; 56: 2884-2890
7. Kennedy MJ, Sandin RL. Influence of growth conditions on *Candida albicans* adhesion, hydrophobicity and cell wall ultrastructure. J Med Vet Mycol 1988; 26: 79-92
8. Hazen BW, Hazen KC. Dynamic expression of cell surface hydrophobicity during initial yeast cell growth and before germ tube formation of *Candida albicans*. Infect Immun 1988; 56: 2521-2525
9. Glee PM, Sundstrom P, Hazen KC. Expression of surface hydrophobic proteins by *Candida albicans in vivo*. Infect Immun 1995; 63: 1373-1379
10. Lopez-Ribot JL, Navarro D, Sepulveda P, Nogueira JM, Casanova M, Martinez JP. A comparative study on cell wall antigens and cell surface hydrophobicity in clinical isolates of *Candida albicans*. Mycopathologica 1994; 127: 1-13
11. Hazen KC, Glee PM. Hydrophobic cell wall protein glycosylation by the pathogenic fungus *Candida albicans*. Can J Microbiol 1994; 40: 266-272
12. Lopez-Ribot JL, Casanova M, Martinez JP, Sentandreu R. Characterization of cell wall proteins of yeast and hydrophobic mycelial cells of *Candida albicans*. Infect Immun 1991; 59: 2324-2332
13. Masuoka J, Hazen KC. Cell wall protein mannosylation determines *Candida albicans* cell surface hydrophobicity. Microbiology 1997; 143: 3015-3021
14. Kennedy MJ, Volz PA, Edwards CA, Yancey R. Mechanism of association of *Candida albicans* with intestinal mucosa. J Med Microbiol 1987; 24: 333-341
15. Minagi S, Miyake Y, Fujioka Y, Tsuru H, Suginaka H. Cell surface hydrophobicity of *Candida* species as determined by the contact angle and hydrocarbon-adherence methods. J Gen Microbiol 1986; 132: 1111-1115
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680-685
17. Fukazawa Y, Kagaya K. Molecular bases of adhesion of *Candida albicans*. J Med Vet Mycol 1997; 35: 87-99
18. Chaffin WL, Lopez-Ribot JL, Casanova M, Gozalbo D, Marinez JP. Cell wall and secreted proteins of *Candida albicans*: identification, function, and expression. Microbiol Mol Biol Rev 1998; 62: 130-180



=국문초록=

칸디다 세포표면 소수성에 미치는 혈청의 영향

관동대학교 의과대학 미생물학교실, 연세대학교 원주의과대학 미생물학교실*,
강원대학교 자연과학대학 생명과학부**

신운섭 · 김동화* · 이경호* · 김경훈** · 박윤선 · 고춘명*

세포표면 소수성은 부착에 중요한 역할을 한다고 알려져 있으며 여러 가지 환경조건에 따라 세포표면 소수성의 표현이 달라진다. 그러나 혈청에 의해 유도되는 세포표면 소수성이 다른 조건에서 유도되는 세포표면 소수성과 같은지는 보고되지 않았으므로 혈청에 의한 칸디다균의 세포표면소수성을 관찰하였다. 토끼 혈청과 YEPD 배지에서 칸디다균을 37℃에서 배양하였을 때 초기 1시간 동안 세포표면 소수성이 급격하게 증가하다가 이후 서서히 감소하였다. 혈청을 희석하였을 때와 포도당을 첨가하여 배양하였을 때 칸디다균의 세포표면 소수성은 큰 차이가 없었으나 혈청을 많이 희석할수록 소수성이 높게 유지되었다. 칸디다균을 단백질분해효소와 lyticase로 처리하였을 때 칸디다균의 세포표면 소수성은 감소하였다. 칸디다균의 세포벽을 lyticase로 처리하여 전기영동으로 분석한 결과 단백질 양상이 소수성 균주간에도 모두 일치하지 않았다. 이러한 결과는 혈청에 의해 유도되는 세포표면 소수성과 YEPD 배지에서 유도되는 세포표면 소수성이 같지 않음을 의미한다. **[Kor J Med Mycol 5(4): 167-172]**

색인 단어: 칸디다, 세포표면 소수성, 혈청