Role of cell surface hydrophobicity in *Candida albicans* biofilm

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**Abstract:** Overall cell surface hydrophobicity (CSH) is predicted to play an important role during biofilm formation in *Candida albicans* but is the result of many expressed proteins. This study compares the CSH status and *CSH1* gene expression in *C. albicans* planktonic cells, sessile biofilm, and dispersal cells. Greater percentages of hydrophobic cells were found in non-adhered (1.5 h) and dispersal forms (24 or 48 h) (41.34±4.17% and 39.52±7.45%, respectively), compared with overnight planktonic cultures (21.69±3.60%). Results from quantitative real-time PCR confirmed greater up-regulation of the *CSH1* gene in sessile biofilm compared with both planktonic culture and dispersal cells. Up-regulation was also greater in dispersal cells compared with planktonic culture. The markedly increased CSH found both in *C. albicans* biofilm, and in cells released during biofilm formation could provide an advantage to dispersing cells building new biofilm.

**Keywords:** *Candida albicans* • Cell surface hydrophobicity • Biofilm • Planktonic cells • Dispersal cells

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1. Introduction

The cell surface hydrophobicity (CSH) of the yeast *Candida albicans* is believed to be a putative virulence factor [1]. It has been postulated that the CSH1 protein significantly affects the overall CSH status of *C. albicans*. *CSH1* has been detected on the *C. albicans* cell surface, although its expression is temperature- and strain-dependent [2-4]. Contradictory results evaluating direct associations between CSH and adherence or an ability to form biofilm in *C. albicans* suggest that the hydrophobic phenotype is not exclusive but a contributing factor in irreversible adhesion [4,5]. In respect to certain conditions, adhered cells can continue to build biofilm – a community of self-controlling microorganisms. The formation of biofilm is a process composed of several phases [6]. Hydrophobic phenotype can promote formation during the first phase (i.e. adhesion). CSH status is also predicted to play an important role in the dispersal of cells from mature biofilm, the main function of which is probably to build new biofilm.

Previous studies have found changes in gene expression of sessile biofilm and dispersal cells [7,8]. Based on these previous results, different gene transcription of the *CSH1* gene was expected in dispersal cells (non-sessile cells spread from biofilm) and sessile biofilm in comparison with planktonic culture (cells cultivated overnight in liquid medium). To determine whether this is the case, this work compared CSH status and *CSH1* gene expression in *C. albicans* planktonic cells with biofilm and dispersal cells.

2. Experimental Procedures

2.1 Preparation of yeast suspension for CSH and biofilm

For this study, *C. albicans* SC5314 was used [9]. The strain was cultivated on YPD plates (1% yeast extract, 1% mycological peptone and 1% *D*-glucose, supplemented with 2% agar, Applichem) at 28°C for
24 h before use. A large loop of cells was transferred to 20 mL of YNB (Yeast Nitrogen Base broth, SIGMA-Aldrich) containing 0.9% D-glucose. After incubation at 37°C for 16 h with shaking (planktonic culture), the cells were centrifuged and washed twice with 1 mL of phosphate-buffered saline (PBS), then vortexed and centrifuged at 5000xg for 5 min. The cells were then re-suspended in 1.0 mL of YNB broth. Afterwards, the cell suspension was calculated in a haemocytometer and prepared to a final inoculum 10⁷ cells/mL.

2.2 CSH and biofilm formation

Overall CSH was measured for planktonic cells and dispersals using the biphasic separation method, as previously described by Klotz et al. [10]. Planktonic cells were collected from overnight cultures and dispersals were collected from various time points. Both samples were adjusted to 10⁷ cells/mL then 1.2 mL of appropriate suspension was placed into a clean glass tube and overlaid with 0.3 mL of n-octane (SIGMA-Aldrich). The 5 parallel samples from every group of collected cells were vortexed for 3 min, and then the phases were allowed to separate. The OD₂₅₀ was measured for the aqueous phase. The OD values of strains in the YNB broth without n-octane overlay were used as negative controls. The relative CSH was calculated according to the formula: [(ODₓ₅₀ of the control - ODₓ₅₀ after n-octane overlay)/ODₓ₅₀ of the control] x 100. The results were expressed as an average ± standard deviation (SD).

The biofilm was prepared according to the protocol of Li et al. [11], with minor modifications. Polystyrene Petri dishes (10 cm diameter, Sarstedt) were inoculated with 10⁷ cells/mL in 20 mL of YNB supplemented with 0.9% D-glucose at 37°C for 90 min (adhesion phase). Non-adherent (detached) cells were removed and collected for RNA isolation, and adherent cells were washed three times with 1x PBS, then scraped and collected for RNA isolation as well. In parallel Petri dishes, adherent cells were overlaid with 20 mL of YNB medium. After 24 h of cultivation the procedure of collecting dispersal cells and sessile biofilm was repeated. Parallel Petri dishes with mature biofilm were cultivated for a final 48 h. Mature biofilm was collected as described for 24 h biofilm.

2.3 Quantitative real time PCR (qRT-PCR)

Quantitative real time PCR used to measure the expression of the CSH1 gene in planktonic and non-attached cells as well as cells from different biofilm stages of C. albicans. Total RNA was isolated from previously collected planktonic cells, sessile biofilm, non-attached cells, and dispersal cells using RNeasy Mini kits (Qiagen). Subsamples (3 µg) of RNA were treated with DNase I and used for cDNA synthesis using the Taqman Reverse Transcription kit (Applied Biosystems). Quantitative RT-PCR was performed with SYBR (Protocol) Maxima™ (Fermentas), according to the manufacturer’s instructions for ABI Prism 7900HT (Applied Biosystems). Data for the CSH1 gene were calculated and expressed as a fold regulation in comparison to the reference gene ACT1 for every condition tested, using the Standard curve quantification method [12]. The expression data from planktonic culture or 24 h dispersal cells were used as reference samples to determine ΔΔCt values. The primers for ACT1 and were previously described by Řičicová et al. [13]. The primers for CSH1 were designed by Dr. I. Hikkel; forward primer: 5’-TGG TTG GCA CCA ATT CAT CTC-3’ and reverse primer: 5’-AAA CAC CAC CGT TTG GA-3’. All primers were synthesized by Metabion International AG. The qRT-PCR assay was performed in three independent experiments.

3. Results and Discussion

Some genes encoding for proteins with multiple functions, including those affecting the hydrophobic properties of C. albicans, have already been described [3,14]. The protein Csh1, coded by the already cloned CSH1 gene, has been hypothesized to affect total CSH status, and to contribute to virulence of C. albicans in mice [3]. To date, only limited information about the role of the CSH1 gene during biofilm formation has been published [8].

Hydrophobicity is most frequently associated with adherence and biofilm formation. In previous work, 50 C. albicans and C. dubliniensis isolates did not prove a relevant correlation that strains with high CSH were also high biofilm producer [4]. In that study, CSH was determined only in planktonic culture. In contrast, this study evaluated and compared CSH status and changes in the expression of the CSH1 gene of the C. albicans SC 5314, not only in respect to planktonic cells, but also to different phases of biofilm. In this work, the hydrophobic population of C. albicans determined in the planktonic overnight culture was 21.69±3.60%. However, CSH increased in dispersal cells collected from 24 and 48 h biofilm to 41.34±4.17% and 39.52±7.45%, respectively. It is of interest that the percentage of hydrophobic cells markedly increased (to 35.23±3.31%) in the adhesion phase during biofilm formation (1.5 h). However, this short adhesion period was satisfactory for changing CSH status. These results were in agreement with those obtained from qPCR, which confirmed the up-regulation of the CSH1 gene in biofilm and dispersal cells.