Influence of aeration of *Candida albicans* during culturing on their surface aggregation in the presence of adhering *Streptococcus gordonii*

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**Abstract**

*Candida albicans* surfaces are extremely sensitive to changes in growth conditions. In this study, adhesion to glass of aerated and non-aerated *C. albicans* ATCC 10261 in the presence and absence of adhering *Streptococcus gordonii* NCTC 7869 was determined in a parallel plate flow chamber. In addition, the influence of aeration on the yeast cell surface hydrophobicity, surface charge, and elemental cell surface composition was measured. *S. gordonii* adhering at the glass surface caused a reduction in the initial deposition rate of *C. albicans*, regardless of aeration. In a stationary end-point, only adhesion of non-aerated *C. albicans* was suppressed by the adhering *S. gordonii*. Non-aerated yeasts had a higher O/C elemental surface concentration ratio, indicative of cell surface polysaccharides, than aerated yeasts, at the expense of nitrogen-rich cell surface proteins. Both yeasts were essentially uncharged, but the nitrogen-rich cell surface of aerated yeasts had a slightly higher water contact angle than non-aerated yeasts. Summarizing, this study suggests that highly localized, hydrophobic cell surface proteins on *C. albicans* are a prerequisite for their interaction with adhering streptococci. © 1999 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

**Keywords:** Adhesion; *Candida albicans*; *Streptococcus gordonii*

1. **Introduction**

Mixed *Candida* and bacterial infections are becoming increasingly important in selected patient groups. Combined yeast and bacterial oropharyngeal infections are common in bone marrow transplant [1] and AIDS patients [2,3], while biomaterials-related biofilms consisting of yeasts and bacteria can lead to denture-associated stomatitis in edentulous patients, malfunctioning of silicone rubber voice prostheses in laryngectomized patients [4], and the disruption of function in nasogastric tubes [5].

Quantitative study of adhesive interactions between yeasts and bacteria is difficult and consequently a number of semi-quantitative methods have been developed (for a review see [6]), most of which are inadequate since they lack proper control of mass transport to the surface and use rinsing steps that introduce artifacts [7]. The use of a parallel plate flow chamber is effective for quantitative evaluation.
of adhesive interactions between yeasts and bacteria, although the five-fold difference in size between the microorganisms impedes direct in situ visualization of cell-cell contact. Using a parallel plate flow chamber, it was shown that the presence of adhering \textit{Streptococcus gordonii} NCTC 7869 on the glass surface caused a three-fold higher rate of \textit{Candida albicans} ATCC 10261 aggregate formation when the yeasts were grown at 37°C than when the yeasts were grown at 30°C [8]. Earlier work, based on microbial adhesion to hydrocarbons (MATH), demonstrated that the cell surface of \textit{C. albicans} was sensitive to growth conditions and an increase in growth temperature from 26°C to 37°C yielded a transition from a hydrophobic to a hydrophilic cell surface [9]. Yeast cell surface hydrophobicity has been implicated in the aggregation of \textit{Saccharomyces cerevisiae} [10] as well as the adhesion of \textit{Candida} to plastics [11], epithelial cells [12] and dental materials [13]. While yeasts have been shown to be sensitive to growth temperature, there have been very few studies investigating the influence of aeration on \textit{Candida} cell surface properties.

The aim of this study is to determine the influence of aeration of \textit{C. albicans} ATCC 10261 during culturing on the adhesion of yeasts to glass in the presence of adhering \textit{S. gordonii} NCTC 7869. In addition, the effects of aeration on the yeast cell surface hydrophobicity, charge, and elemental concentration are measured.

2. Materials and methods

2.1. Microorganisms and growth conditions

\textit{S. gordonii} NCTC 7869 (Channon) was cultured from brain heart infusion agar (BHI) in 10 ml tryptone yeast extract broth supplemented with glucose (TY-glucose, per liter: 5 g tryptone, 5 g yeast extract, 4 g K$_2$HPO$_4$, 5 g glucose) (24 h, 37°C). This preculture was used to inoculate 200 ml TY-glucose broth (16 h, 37°C). \textit{C. albicans} ATCC 10261 was cultured from BHI agar initially for 24 h at 37°C in 10 ml yeast medium (YM, per liter: 7.5 g glucose, 3.5 g (NH$_4$)$_2$SO$_4$, 1.5 g L-asparagine, 10 mg L-histidine, 20 mg DL-methionine, 20 mg DL-tryptophan, 1 g KH$_2$PO$_4$, 500 mg MgSO$_4$7H$_2$O, 500 mg NaCl, 500 mg CaCl$_2$2H$_2$O, 100 mg yeast extract, 500 mg H$_2$BO$_3$, 400 mg ZnSO$_4$7H$_2$O, 120 mg Fe(II)Cl$_3$, 200 mg Na$_2$MoO$_4$2H$_2$O, 100 mg KI, 40 mg CuSO$_4$5H$_2$O), which was subsequently used to inoculate 200 ml YM (16 h, 37°C). The yeasts were aerated by shaking in a water bath at 190 rpm, or incubated without shaking. Under these growth conditions \textit{C. albicans} ATCC 10261 remained in the yeast form. The bacteria and yeasts were harvested and washed two times in demineralized water by centrifugation (10,000×g, 5 min, 10°C), and resuspended in approximately 3 ml demineralized water.

The streptococci were sonicated for approximately 40 s, and the yeasts were sonicated for 10 s to disrupt aggregates. This treatment did not cause lysis of either organism. Both the bacteria and yeasts were enumerated in a Bürker-Turk counting chamber. Concentrations of $3 \times 10^8$ bacteria ml$^{-1}$, and $3 \times 10^6$ yeasts ml$^{-1}$ were subsequently suspended in TNMC buffer (1 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 1 mM MgCl$_2$, 1 mM CaCl$_2$).

2.2. Parallel plate flow chamber system and substratum surface

The flow chamber is constructed of nickel-coated brass and has been previously described [14,15]. Two glass plates with dimensions 5.5×3.8 cm, separated 0.06 cm using Teflon spacers, constituted the top and bottom plates of the chamber. The glass plates were cleaned by sonication for 5 min in a commercially available surfactant solution (2% RBS in water, Société des Traitements Chimiques de Surface, Lamber- sat, France), rinsed thoroughly with water, then methanol, and finally with demineralized water again prior to fixing in the flow chamber.

Laminar fluid flow was achieved in the middle of the flow chamber by the gradual slope of the inlet and outlet channels, and the suspending fluid flow (shear rate 15 s$^{-1}$) corresponded to a Reynolds number of around 1, well within the laminar flow range. The flow chamber was mounted on the stage of a phase-contrast microscope (Olympus BH-2) with an ultralong working distance 40× objective (Olympus ULWD-CD Plan 40 PL) for observation of bacteria, and a 10× objective for observation of the yeasts. A CCD camera (CCD-MX High Technology, Eind-
hoven, The Netherlands) mounted on the microscope was linked to and controlled by an image analyzing computer program (TEA image-manager; Difa, Bre- 

daa, The Netherlands). With this system it was possible to directly monitor, in real time, the initial adhe- 

sion of yeasts to the bottom glass plate in a field of view of $2.58 \times 10^{-3}$ cm$^2$.

2.3. Adhesion protocol

First, streptococci were seeded on the bottom glass plate to saturation (approximately 30% surface coverage) from a pulse-free flow, created by hydrostatic pressure at a shear rate of 15 s$^{-1}$, while the bacterial suspension was recirculated by a roller pump (LKB, Bromma, Sweden). After saturation was reached, flow was switched to buffer for approximately 30 min in order to remove non-adhering bacteria from the chamber and system tubing. Subsequently, a yeast suspension was perfused through the chamber for 2 h at the same shear rate, and images were 'grabbed' at defined time points. After 2 h, flow was switched to buffer to remove non-adhering yeasts and the chamber was drained, therewith passing an air-liquid interface over the substratum surface and adhering microorganisms. Pre- and post-draining im- 

ages were compared to determine the number of $C.\ albicans$ that were detached by the surface tension force resulting from the passage of an air-liquid in- 

terface [16,17].

Control experiments were conducted with yeasts adhering to bare glass in the absence of adhering streptococci. All adhesion experiments were done in triplicate with freshly cultured yeasts and bacteria.

2.4. Data analysis

The initial deposition rate, $j_{0,\text{total}}$, is defined as the number of microorganisms adhering per unit time and area, and can be expressed as:

$$j_{0,\text{total}} = \frac{dn(t)}{dt}|_{t=0}$$

(1)

where $n(t)$ represents the number of yeasts adhering at time $t$. The initial deposition rate, $j_{0,\text{total}}$, is sub- 

sequently divided into a deposition rate, $j_{0,\text{agg}}$, for yeasts adhering in aggregates, and a deposition rate, $j_{0,\text{singles}}$, for singly adhering yeasts.

In a stationary end-point of the adhesion process, yeast adhesion was quantified by evaluation of the total number of adhering yeasts and the average ag- 

gregate size in yeast numbers.

2.5. Contact angle measurement, particulate microelectrophoresis, and X-ray photoelectron spectroscopy (XPS)

Water contact angles were determined on lawns of yeasts using the sessile drop technique [18]. Briefly, the yeasts were layered onto 3 μm pore size filters (Millipore) from a suspension in TNMC buffer using negative pressure. The filters were left to air-dry until so-called plateau water contact angles could be measured.

Zeta potentials of the microorganisms were deter- 

mined by particulate microelectrophoresis [19] in TNMC buffer. The electrophoretic mobilities were measured at 150 V in a Lazer Zee Meter 501 (Pen- Kem, USA) and converted to zeta potentials according to the Helmholtz-Smoluchowski equation [20].

XPS was applied to obtain the overall chemical composition of the microbial cell surfaces [21]. After culturing and washing, the microbial pellets were transferred to stainless steel troughs, and immediately frozen in liquid nitrogen. The samples were subsequently freeze-dried in a Leybold Heraeus Combibron CM30 freeze drier. The freeze-dried sam- 

ples were pressed into small stainless steel cups and inserted into the XPS chamber (Surface Science In- 

struments, S-Probe, Mountain View, CA, USA). X-rays (10 kV, 22 mA) at a spot size of 250 × 1000 μm were produced using an aluminum anode. Scans of the overall spectrum in the binding energy range of 1–1200 eV at low resolution (pass energy 150 eV), then peaks over a 20-eV binding energy range were recorded at high resolution (pass energy 50 eV) for $C_{1s}$, $O_{1s}$, $N_{1s}$, and $P_{2p}$. The area under each peak, after linear background subtraction, was used to calculate peak intensities, yielding elemental surface concentration ratios for nitrogen, oxygen, and phosphorus to carbon, after correction with sensitivity factors provided by the manufacturer.
3. Results and discussion

Table 1 presents the initial deposition rates of the yeasts and the numbers of yeasts adhering in a stationary end-point, for both aerated and non-aerated yeasts. Initial deposition rates of the yeasts were greatest in the absence of adhering streptococci, with non-aerated yeasts showing an approximate 20-fold increase in the deposition of single yeasts as compared to in the presence of adhering streptococci. In a stationary end-point, aerated yeasts adhered in greater numbers in the presence of adhering streptococci than non-aerated yeasts. Furthermore, the presence of adhering streptococci caused a two- to three-fold increase in yeast aggregate size, irrespective of aeration. Fig. 1 illustrates the surface aggregation of non-aerated *C. albicans* ATCC 10261 after 2 h on glass in the presence and absence of adhering bacteria. Clearly, large aggregates of adhering yeasts do not form in the absence of adhering streptococci.

Table 2 summarizes the water contact angles, zeta potentials by microelectrophoresis, and elemental cell surface compositions of *C. albicans* ATCC 10261. Aerated yeasts showed a slightly higher water contact angle, and a less positive zeta potential than the non-aerated yeasts. Non-aerated yeasts had increased O/C elemental surface concentration ratios relative to aerated yeasts, indicative of the presence of polysaccharide. Aerated yeasts, however, had increased N/C and P/C elemental cell surface concentration ratios, suggesting exposure of (lipo-)proteins at the expense of polysaccharides.

Aeration of *C. albicans* is therefore likely to be accompanied by the exposure of a cell surface protein that is important in a stationary end-point to

![Image](https://example.com/image.png)

**Table 1**

Initial deposition rates on glass for the total number of yeasts adhering ($J_{\text{total}}$), and single yeasts ($J_{\text{singles}}$), together with the stationary end-point adhesion ($n_{2h}$), and average aggregate size in yeast numbers (size) for aerated and non-aerated *C. albicans* ATCC 10261 grown at 37°C in the absence and presence of adhering *S. gordonii* NCTC 7869.

<table>
<thead>
<tr>
<th>Bacterial surface coverage (%)</th>
<th>Condition</th>
<th>$J_{\text{total}}$ (cm$^2$ s$^{-1}$)</th>
<th>$J_{\text{singles}}$ (cm$^2$ s$^{-1}$)</th>
<th>$n_{2h}$ (10$^5$ cm$^{-2}$)</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>aerated</td>
<td>181</td>
<td>131</td>
<td>9.5</td>
<td>7</td>
</tr>
<tr>
<td>30</td>
<td>aerated</td>
<td>90</td>
<td>55</td>
<td>9.7</td>
<td>19</td>
</tr>
<tr>
<td>0</td>
<td>non-aerated</td>
<td>227</td>
<td>143</td>
<td>11.3</td>
<td>10</td>
</tr>
<tr>
<td>30</td>
<td>non-aerated</td>
<td>37</td>
<td>8</td>
<td>2.6</td>
<td>20</td>
</tr>
</tbody>
</table>

All data are the results of three experiments with separately grown bacterial and yeast strains, yielding standard deviations of around 25% for all parameters.
overcome suppression of yeast adhesion by adhering streptococci. A similar suppression of yeast adhesion has been seen for *C. albicans* adhering on PMMA [8], that could be overcome by salivary adhesion mediators, most notably proteins.

*S. cerevisiae* cultured under reduced nutrient conditions also had less protein and more polysaccharide on its cell surface [22], similar to the non-aerated yeasts in this study. However, the relatively higher N/C elemental surface concentration ratio observed for aerated yeasts is still significantly lower than theoretically expected for a fully proteinaceous surface (estimated N/C ratio is 0.279 [23]). Possibly, aerated yeast cell surfaces only possess proteins at highly localized areas which explains why the water contact angle, as an overall surface property, is only slightly higher for aerated yeasts. However, these localized yeast cell surface proteins are responsible for overcoming the suppression in their adhesion due to the presence of streptococci on a surface.

In summary, this study shows that adhesion of non-aerated *C. albicans* ATCC 10261, with a relatively hydrophilic, uncharged polysaccharide-rich cell surface, is suppressed by the presence of adhering *S. gordonii* NCTC 7896. Upon aeration of the yeast during growth, an increased exposure of highly localized, hydrophobic cell surface proteins appeared able to overcome this suppression. Therewith, these cell surface proteins perform a similar role in mediating yeast adhesion in the presence of adhering bacteria as previously attributed to salivary proteins.

### References


