Macroscopic and microscopic approaches toward bacterial adhesion
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Dynamic cell surface hydrophobicity of lactobacillus strains with and without surface layer proteins

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Several studies have shown that bacterial strains, such as lactobacilli, can protect the host against infection by invading uropathogens. The mechanism by which lactobacilli exert this protection is not fully understood, but adhesion is a commonly accepted prerequisite (Sanders, 1993). Several lactobacillus species possess a surface layer protein (SLP) anchored to the cell envelope. This surface layer consists of a (glyco-)protein, the so-called S-protein, which assembles into characteristic two-dimensional crystalline layers at the cell surface (Sára & Sleytr, 2000). The function of the S-layer on these organisms is unknown, but S-layers of lactobacilli are important in their adhesion to surfaces, as SLP conveys

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hydrophobicity to the lactobacillus cell surface (Van der Mei et al., 2003). Yet, adhesion of lactobacilli to surfaces often does not proceed according to expectations based on their cell surface hydrophobicity and hydrophobic strains do not always adhere best to hydrophobic substrata (Millsap et al., 1996), as outlined by surface thermodynamics (Absolom et al., 1983). This suggests cell surfaces of lactobacilli may adapt their cell surface hydrophobicity in response to environmental changes, like in pH or ionic strength.

Macroscopic bacterial cell surface hydrophobicity is commonly inferred from water contact angle measurements on bacteria deposited on membrane filters (Busscher et al., 1984). If water molecules have a greater preference to surround each other than to contact a bacterial cell surface, the surface appears hydrophobic and water droplets do not spread. If water molecules favor a microbial cell surface rather than each other the surface appears hydrophilic. Hydrophobic lactobacillus isolates with water contact angles above 100 degrees (L. acidophilus RC14) have been described, but also extremely hydrophilic ones with water contact angles of 19 degrees (L. casei 36) (Van der Mei et al., 1998a). Although cell surface hydrophobicity arises from interactions at the molecular level, hydrophobicity has never been assessed at the level of molecular cell surface components.

Atomic force microscopy (AFM) has emerged as a valuable tool for probing interaction forces at the molecular level with a high spatial resolution (Dufrêne, 2002). A sharp tip located at the free end of a flexible cantilever is approached and retracted from the surface under study. Interaction forces between the tip and the sample surface cause the cantilever to deflect. The deflection signal during the approach and retraction process is acquired to provide so-called force-distance curves (see Figure 7.1 as an example).

![Figure 7.1](image.png)

Figure 7.1. Force-distance curve for L. acidophilus ATCC4356 interacting with an hydrophobic AFM tip at 10 mM KCl. The solid line represents the approach curve, while the dashed line indicates the retraction curve. The maximum adhesion force $F_{\text{adh}}$ probed upon retraction is indicated on the graph.
In this chapter, the surfaces of *L. acidophilus* ATCC4356 and *L. casei* ATCC393 with and without SLP, respectively, have been probed with regard to their interaction forces with chemically functionalized AFM tips, *i.e.* terminated with hydrophobic (CH$_3$) and hydrophilic (OH) groups. Experiments were done in a 10 mM and 100 mM KCl solution. The macroscopic cell surface hydrophobicity of the two strains has also been assessed by contact angle measurements with these low and high ionic strength solutions.

Bacterial strains were cultured in MRS (De Man, Rogosa Sharpe, Merck, Germany) at 37 °C in an atmosphere containing 5 % CO$_2$. This culture was used to inoculate a second culture that was grown for 16 h prior harvesting. Bacteria were harvested by centrifugation (5 min at 10,000 g), washed twice with demineralized water and suspended in demineralized water, 10 or 100 mM KCl solution. Contact angle measurements were performed on bacterial lawns prepared by depositing about 50 layers of bacteria suspended in demineralized water on a cellulose acetate membrane filter (pore diameter 0.45 µm) (Van der Mei *et al.*, 2003). For AFM experiments, bacteria were attached to a positively charged poly-L-lysine treated glass slide. “V”-shaped silicon nitride cantilevers with a spring constant of 0.06 N m$^{-1}$ were functionalized by coating them with a thin layer of titanium and gold followed by their immersion in HS(CH$_2$)$_{11}$OH or HS(CH$_2$)$_{17}$CH$_3$ solutions. Functionalized probes were always used immediately after preparation. AFM measurements were made at room temperature under 10 and 100 mM KCl solution using an optical level microscope (Nanoscope III Digital Instrument). An array of 32×32 force-distances curves were collected over the entire field of view, once a bacterium was imaged (see Figure 7.2a and 7.2a' as an example). Adhesion maps were produced by taking the most negative force detected during the retraction curve (see Figure 7.1) and by plotting that value against $x$-$y$ position of each force-distance curve (Figure 7.2b and 7.2b'). From the adhesion maps, a selected area of ~800×800 nm$^2$ over the top of each bacterium was used to generate an adhesion distribution histogram (Figure 7.2c and 7.2c') from which an average adhesion force $F_{adh}$ was calculated between functionalized AFM tips and the bacterial cell surfaces for each experimental condition studied. Three to five different organisms were studied in each particular case.

Adhesion maps indicated a heterogeneous surface distribution of interaction forces between the cell surfaces and functionalized tips for both strains, regardless of ionic strength (see the examples in Figure 7.2b and 7.2b'). Histograms showing the distribution of these interaction forces over the top of each bacterium are presented in Figures 7.2c and 7.2c’. The interaction forces detected by hydrophobic and hydrophilic AFM tips were averaged for each strain into an adhesion force $F_{adh}$ and compared with contact angles measured with aqueous, low and high ionic strength solutions (see Table 7.1). In general, high interaction forces with a hydrophilic tip were found to coincide with low contact angles, whereas a cell surface with high contact angle showed the strongest interaction with a hydrophobic tip. In addition, both strains reversed their hydrophobic nature upon increasing the ionic strength from 10 to 100 mM. The lactobacillus strain with SLP
Figure 7.2. Array of 32×32 force distance curves over the AFM field of view for *L. acidophilus* ATCC4356 (SLP) (a) and *L. casei* ATCC393 (no-SLP) (a’) together with their corresponding adhesion maps (b and b’) obtained using an hydrophobic AFM tip at 10 mM KCl. Histograms (c and c’) show the distribution of adhesion forces over a selected area of about 800×800 nm² on the bacterial cell surface.
Table 7.1. Summary of contact angles with aqueous, low and high ionic strength solutions for two lactobacillus strains with and without SLP together with the average adhesion force $F_{\text{adh}}$ as probed by hydrophobic and hydrophilic AFM tips.

Contact angles represent a mean value of two independent sets of measurements. AFM data are representative of results obtained on three to five cells, using different probes and independent preparations. ± denote the standard deviation associated to the values calculated.

<table>
<thead>
<tr>
<th></th>
<th>SLP strain</th>
<th>no-SLP strain</th>
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<tbody>
<tr>
<td>Ionic strength</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 mM</td>
<td>10 mM</td>
<td>100 mM</td>
</tr>
<tr>
<td>Contact angle</td>
<td>76 ± 4</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>$F_{\text{adh, phobic}}$ (nN)</td>
<td>-1.34 ± 0.19</td>
<td>-0.88 ± 0.13</td>
</tr>
<tr>
<td>$F_{\text{adh, philic}}$ (nN)</td>
<td>-0.11 ± 0.02</td>
<td>-2.14 ± 0.17</td>
</tr>
</tbody>
</table>

was found hydrophobic in 10 mM and became more hydrophilic in 100 mM, while the strain without SLP was hydrophilic in 10 mM and became hydrophobic in 100 mM.

The structure of the S-layer on *L. acidophilus* ATCC4356 is known to be composed of two sub-domains: an external N-terminal region showing predominantly hydrophobic amino acid residues and a C-terminal region, serving to attach the S-layer to the cell wall, which is mainly composed of positively charged hydrophilic residues (Smit *et al.*, 2002). The dynamic cell surface hydrophobicity observed may be explained by a shrinkage of the S-layer due to reduced intra-molecular electrostatic repulsion at high ionic strength. Then, the inner hydrophilic region may become (partly) exposed at the aqueous periphery of the bacterial surface, rendering it more hydrophilic.

*L. casei* ATCC393 on the other hand, does not possess an S-layer. Yet, its cell surface shows dynamic hydrophobicity as well. X-ray photoelectron spectroscopy indicated that the surface of *L. casei* ATCC393 is rich in polysaccharides (Van der Mei *et al.*, 2000b). At low ionic strength, this layer presents itself as a hydrophilic polyelectrolyte coating. At high ionic strength the polysaccharide layer is known to collapse and this evidently results in exposing a more hydrophobic surface.

It is interesting, that the dynamic behavior of the cell surface hydrophobicity of the lactobacilli was not only measurable macroscopically by contact angles on bacterial lawns, but also by AFM at a more microscopic level. Stronger interaction forces between the cell surfaces and hydrophobically or hydrophilically modified tips coincide with higher or lower contact angles with aqueous solutions. This is fully in line with surface thermodynamics, stating that hydrophobic surfaces favor interaction with hydrophobic surfaces. Analogously, hydrophilic surfaces show greater affinity for hydrophilic surfaces.

In conclusion, this study is the first to report the dynamic behavior of cell surfaces of lactobacilli with regard to their hydrophobicity in response to changes in environmental ionic strength. Dynamic cell surface hydrophobicity was
demonstrated both at a macroscopic and a more microscopic level by contact angle measurements and AFM, respectively. This dynamic behavior of bacterial cell surfaces upon changes in ionic strength offers to *L. acidophilus* ATCC4356 and *L. casei* ATCC393 a versatile mechanism to adhere to hydrophobic and hydrophilic surfaces in low and high ionic strength solutions, respectively.