

Does surface wettability influence bacterial adhesion?

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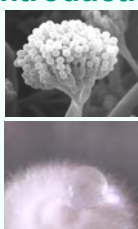
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Problem and Objective

Bacteria establish biofilms on a great variety of natural and synthetic surfaces. Especially in industry and medicine biofilms can cause various problems.

➔ In order to **influence bacterial adhesion** and **manipulate biofilm formation** the surface properties of a material need to be changed. Hydrophobins are potential candidates for the **large-scale surface modifications** and **change of surface hydrophobicity**.

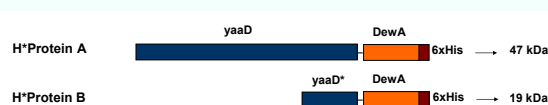
Introduction – What are hydrophobins?



- highly **surface active fungal proteins**
- approximately **100 amino acids** long (molecular mass **10kDa**)
- **amphiphilic**
- **self-assemble** on interfaces into **highly stable monolayers**
- very **efficient**
- **non-toxic** and **non-immunogenic**

Strategy – use of recombinant hydrophobins

Recombinant fusion-hydrophobins produced by BASF AG



DewA: hydrophobin of *Aspergillus nidulans*;
yaaD: synthase of *Bacillus subtilis*; yaaD*: shortened form of yaaD (40 amino acids)

Characterization of hydrophobin-coated surfaces

Surface coating

The materials were incubated in hydrophobin solution (10 μM) and subsequently treated with 2% SDS at 80°C for coating stabilization (β-sheet shift).

Change of surface hydrophobicity

The surface hydrophobicity was determined by contact angle measurements (CA).

A **long incubation time** (16h) and **high incubation temperature** (80°C) **increased the surface hydrophobicity** of hydrophobin-coated surfaces significantly (starting material: glass, CA 11°).

Homogeneity of hydrophobin coating

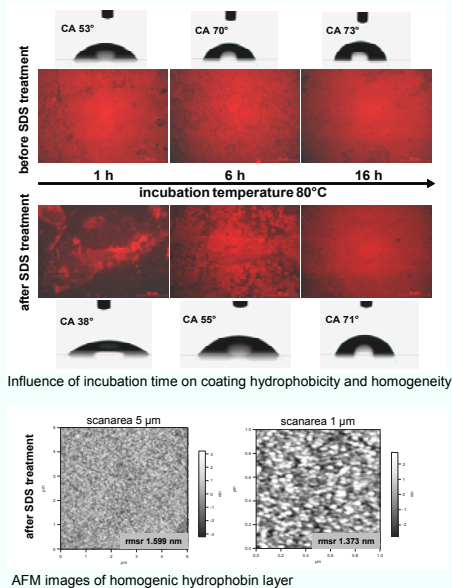
The hydrophobin-coating was specifically detected with His-tag directed fluorescent labeled antibodies and analyzed by epifluorescence microscopy.

It was essential to incubate the materials for **16 hours at 80°C** in the protein-solution to form a **homogenous hydrophobin-layer** after SDS treatment. Atomic force microscopy confirmed these results.

Adsorption characteristics of hydrophobins

The adsorption behavior of hydrophobins was analyzed with quartz crystal microbalance with dissipation monitoring (QCM-D). The layer thickness was estimated using Voigt equation.

	layer thickness [nm]
Hydrophobin H*A	17 ± 1
Hydrophobin H*B	15 ± 3



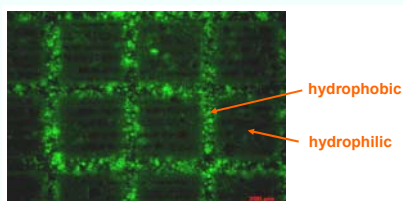
Biofilm formation

The influence of hydrophobin-coatings and changed surface hydrophobicity on different stages of biofilm development was analyzed.

Initial bacterial adhesion

The adhesion of a GFP-tagged *Escherichia coli* was monitored for **two days**.

A **preference of initial bacterial adhesion on hydrophobic surfaces** was detected.



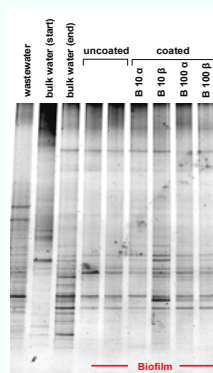
Bacterial adhesion on patterned surface

Mature Biofilm

Biofilms were grown on hydrophobin-coated and uncoated glass slides in a biofilm reactor in natural wastewater effluent for **four weeks**.

A **similar bacterial population** was found on **hydrophobin-coated and uncoated glass-slides**.

➔ The **effects of hydrophobic surfaces are time dependent**.

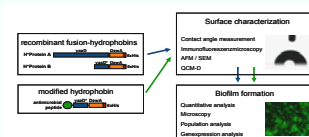


PCR-DGGE

Conclusion and Outlook

• Recombinant fusion-hydrophobins are well suited for easy **large-scale surface coatings**. They adhere in a temperature and time dependent manner into stable monolayers and **change surface hydrophobicity**.

• **Surface wettability has an impact on primary bacterial adhesion but not on biofilm formation.**



• Recombinant hydrophobins modified with **antimicrobial agents** will be used to influence biofilm formation.