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Fungal hydrophobins form self-assembled monolayers (SAMs) and influence biofilm formation

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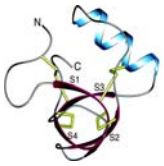
Problem and Objective

Bacteria establish biofilms in several environments on a great variety of natural and synthetic surfaces. Especially in industry and medicine biofilms can cause various problems such as pipe plugging, water and food contamination or infections.

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-area modification** of surface characteristics.

Introduction – What are hydrophobins?



Structure of HFBII with β-barrel and four symetrical disulfide bonds

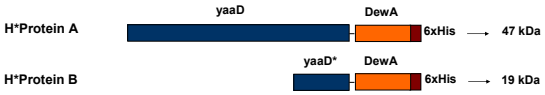
Hakanpää. et al. 2004, Journal of Biological Chemistry

Hydrophobins are fungal proteins which are highly surface active.

Hydrophobins

- are approximately **100 amino acids** long (molecular mass **10kDa**).
- show a **conserved pattern of 8 cysteine residues**, which form four **intramolecular disulfide bridges**.
- are **amphiphillic**.
- **self-assemble** on interfaces into **highly stable monolayers**.
- are very **efficient** (1mg coats 1m^2).
- are **non-toxic**.

Strategy – use of recombinant hydrophobins



Recombinant fusion-hydrophobins produced by BASF AG

DewA: hydrophobin of *Aspergillus nidulans;* **yaaD**: fusionprotein, synthase of *Bacillus subtilits;* **yaaD***: shortened fusionprotein

At first recombinant fusion-hydrophobins were used and coating protocols were developed for various materials.
Recombinant hydrophobins provide the opportunity that they can easily be modified and functionalized with e.g. enzymes or peptides.

Characterization of hydrophobin-coated surfaces

Surface coating

The materials were incubated in protein solutions whereas the hydrophobins self-assembled on the surfaces in α-helical conformation. To stabilize the coating a β-sheet shift was induced by heat (80°C) and SDS treatment.

Change of surface hydrophobicity

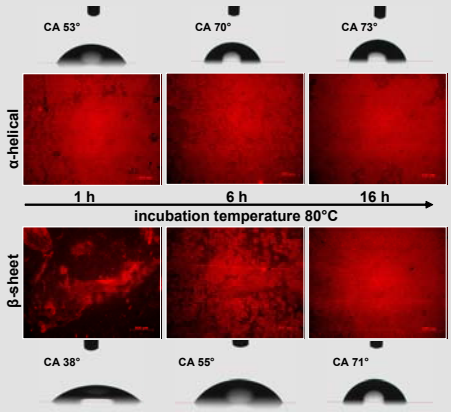
The surface hydrophobicity of coated surfaces was determined by contact angel measurements (CA). A **long incubation time** (16h) and **high incubation temperature** (80°C) **increased** the **surface hydrophobicity** of hydrophobin-coated surfaces significantly (original material: glass, CA 11°).

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** in **α-helical and β-sheet conformation**. SEM and AFM confirmed these results.

Adsorption characteristics of hydrophobins

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



	layer thickness [nm]
Hydrophobin **H*A**	17 ± 3
Hydrophobin **H*B**	14 ± 2

Biofilm formation

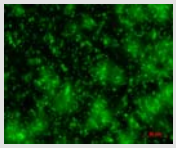
To analyse and characterize the influence of hydrophobin-coatings on biofilm formation, coated and uncoated glass slides were incubated in a biofilm reactor in natural waste water effluent for four weeks.

Bacterial adhesion

Adhesion of bacteria to hydrophobin-coated and uncoated glass slides was quantified by determination of colony forming units (CFU). In average **the same number of bacteria adhered to coated and uncoated surfaces (2 to 5x10^4 cm^{-2})**.

Spatial distribution

To analyze the biofilm distribution the bacteria were stained with the DNA intercalating dye Syto9. With fluorescence microscopy **no significant differences in spreading and appearance** were detected.



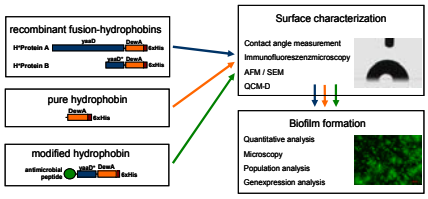
Biofilm on hydrophobin-coated surface

Community composition

For characterization of the community composition the total biofilm DNA was extracted. The ribosomal DNA of *Eubacteria* was specifically amplified by PCR and the amplicons were separated with denaturing gradient gel electrophoresis (DGGE). A **similar bacterial population can be found on hydrophobin-coated and uncoated glass-slides**.

Conclusion and Outlook

Recombinant fusion-hydrophobins are good targets for easy large-scale surface coatings. They adhere in a temperature and time dependent manner into stable monolayers but the change of the surface characteristics as the hydrophobicity is not sufficient to influence the formation of mature biofilms.



Since recombinant hydrophobins can easily be modified the pure hydrophobin and hydrophobins modified with antimicrobial peptides will be used to enhance the effect on biofilm formation. Furthermore, the potential impact of hydrophobin-coatings on the initial bacterial adhesion will be determined.

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