Centers of Advanced Medicine
Center for Biofilm Science and Technology

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General Summary

The Jikei Center for Biofilm Science & Technology (JCBST) was established in April 2015 as a member of the Centers of Advanced Medicine of The Jikei University with the support of the Ministry of Education, Culture, Sports, Science and Technology–Supported Program for the Strategic Research Foundation at Private Universities. The JCBST will promote research for the prevention and control of biofilm-associated infections. Research projects of the JCBST have focused on: 1) the importance of extracellular RNA in bacterial biofilms, 2) redundancy and complexity in biofilms, 3) identification of anti-biofilm compound (ABC)-JK2, a small molecular inhibitor of staphylococcal biofilm formation, 4) visualization of biofilms in solution by atmospheric scanning electron microscopy (SEM), 5) biofilm formation mechanisms triggered by excreted molecular chaperones, and 6) genotypic and biofilm profiles of *Propionibacterium acnes* isolated from pacemakers without clinical signs of infection.

Research Activities

Importance of extracellular RNA in bacterial biofilms

We recently explored the presence of extracellular RNA in *Staphylococcus aureus* biofilms. In this study, we analyzed the roles of extracellular RNA in biofilm development. The molecular size of the extracellular RNA was estimated with urea–polyacrylamide gel electrophoresis to be 20 to 200 nucleotides. We observed localization of extracellular RNA in the 3-dimensional structure of biofilms with confocal laser scanning microscopy. In addition, RNase A inhibited biofilm formation and dispersed preformed biofilms, representing the importance of extracellular RNA in the structural integrity of biofilms. The extracellular RNA was also identified in a biofilm formed by *Pseudomonas aeruginosa*, indicating that this phenomenon is not limited to staphylococcal biofilms. Our findings provide evidence of a novel function for RNA that has important implications for understanding biofilm physiology.

Redundancy and complexity in biofilms

We detected the clinically isolated strain of *S. aureus* that has capacity to form robust proteinaceous biofilms. This strain has a large amount of extracellular adherence protein (Eap), a secreted protein specific to *S. aureus*, in the biofilm matrix. Although we predicted that Eap contributed to biofilm formation, single knock-out of the Eap gene (*eap*) had little effect on the biofilm biomass. We then deleted other genes involved in biofilm formation in the Δ*eap* strain. We found that deletion of a few genes significantly reduced biofilm formation. One of the genes was the sortase A gene (*srtA*), which encodes sortase A, a membrane protein responsible for the binding of some proteins harboring the leucine-proline-any-threonine-glycine motif to the cell wall. Hence, we predict that one or more of these cell wall–anchored proteins has function similar to that of Eap. Elucidation of the role of these proteins will lead to the development of a specific treatment for infection with *S. aureus*.

Identification of ABC-JK2, a small molecular inhibitor of staphylococcal biofilm formation

In this study, we aimed to identify compounds that inhibit biofilm formation by *S. aureus*. One of the screening hit compounds, named ABC-JK2, inhibited biofilm formation of several strains of *S. aureus*, including methicillin-resistant *S. aureus*, and *S. epidermidis* at a half-maximal inhibitory concentration range of 12.0 to 22.5 μM. Metabolomic analysis showed that ABC-JK2 decreased intracellular levels of glycolytic metabolites. The microarray/quantitative real-time polymerase chain reaction revealed up-regulation of genes related to peptidoglycan biosynthesis and hydrolases. In addition, transmission electron microscopy showed that the thickness of bacterial cell walls and the number of abnormal septa are increased in the presence of ABC-JK2. These results suggest that ABC-JK2 inhibits staphylococcal biofilm formation by affecting glycolysis and cell-wall synthesis.

Visualization of biofilms in solution by atmospheric SEM

We visualized biofilms immersed in aqueous solution, including biofilms formed by the Gram-positive coccus *S. aureus* and the Gram-negative bacillus *Escherichia coli* by recently developed atmospheric SEM. Because atmospheric SEM allows a biofilm cultured on electron-transparent film windows to be observed from below with an inverted SEM, biofilm formation was able to be studied near the substrate and the extracellular matrix at high resolution. Membrane vesicles, delicate spiral flagella, straight curli fibrils, and filamentous extracellular DNA networks were visualized with atmospheric SEM and effective labelling methods, such as labelling with positively charged Nanogold, heavy metals, and immuno-gold. Collectively, our results suggest that atmospheric SEM is a broadly applicable approach for microbial research and diagnostic purposes.

Biofilm formation mechanisms triggered by excreted molecular chaperones

Previously, we identified excreted cytoplasmic molecular chaperones DnaK and ClpB that exist in the extracellular matrix of *S. aureus* biofilms and promote biofilm formation. Mutational analysis for DnaK demonstrated that the N-terminal nucleotide binding

domain alone stimulated biofilm formation of *S. aureus*. In addition, neither mutations into amino acid residues involved in ATPase activity nor substrate-binding affected biofilm-promotion ability. These results suggest that DnaK acts independently as a biofilm-promoter on functions as a classical molecular chaperone involved in the control of intracellular protein homeostasis.

Genotypic and biofilm profiles of P. acnes *isolated from pacemakers without clinical signs of infection*

The colonization of bacteria on the surfaces of cardiac pacemakers explanted in patients without clinical evidence of infection was consecutively analyzed. The *P. acnes* was isolated from pacemakers without clinical signs of infection at high frequency (23%). Biofilm-forming capacities of the *P. acnes* isolates and biochemical properties of the biofilms differed among strains regardless of the sequence types; however, extracellular DNA was suggested to be a factor commonly involved in the biofilm formation of diverse *P. acnes* strains. High-resolution observation of nanostructures in the biofilms with transmission electron microscopy and atmospheric SEM visualized the leakage of cytoplasmic components along with cell lysis and fiber structures connecting cells in biofilm.

Publications

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