# Department of Bacteriology

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## General Summary

Research projects of our department have focused on: 1) the analysis of *Staphylococcus aureus* biofilm formation, 2) the analysis of biofilm detachment factor secreted by *S. aureus*, 3) the molecular mechanisms of *S. aureus* biofilm disassembly triggered by *Staphylococcus epidermidis* Esp, 4) the effects of bacteriocins against methicillin-resistant *S. aureus* (MRSA) biofilm, 5) the mechanism of *Escherichia coli* O157 entering a viable but nonculturable (VNC) state, and 6) the mechanism of bacterial ATP secretion.

## Research Activities

*Intranasal application of S.epidermidis prevents MRSA in mice*

Recently, MRSA has emerged as a leading cause of infection worldwide. Colonization with MRSA predisposes to infection and facilitates transmission of the pathogen; however, available treatments do not prevent MRSA colonization. Studies of human nasal flora suggest that resident bacteria play a critical role in limiting *S. aureus* colonization and prompted us to ask whether application of commensal resident bacteria can prevent nasal colonization by MRSA. We established a murine model to answer this question and showed that mice with nasal precolonization by *S. epidermidis* became more resistant to colonization by MRSA. Our study suggests that application of commensal bacteria and antibiotics is a more effective strategy for preventing MRSA colonization.

*Analysis of biofilm detachment factor*

The bacteria within a biofilm matrix are protected from the host immune system and from antibiotic attack. Therefore, a substance that disassembles biofilms might have wide medical and industrial applications for preventing or eradicating biofilms. We found that *S. aureus* secretes a factor that causes its own biofilm to detach. The culture supernatant of *S. aureus* also detached the biofilms of *S. epidermidis*, MRSA, *Pseudomonas aeruginosa,* and *E. coli.* The factor responsible for the detachment effect has a molecular weight less than 500 Da and is heat-stable. The culture supernatant was fractionated with gel filtration chromatography and subjected to reverse-phase column chromatography. The flow-through was subjected to hydrophilic interaction chromatography and eluted with decreasing concentrations of acetonitrile (90% to 0%). The fraction that had detachment activity was analyzed with mass spectrometry. We are now attempting to identify the factor.

*Molecular mechanisms of S. aureus biofilm disassembly triggered by S. epidermidis Esp*

*S. aureus* is frequently found in the nasal cavities of healthy persons, but the colonization

often causes pathogenic infection. *S. aureus* exhibits a strong capacity to attach to biotic or abiotic surfaces and to form biofilms, which lead to chronic infections. We have recently shown that Esp, a serine protease secreted by commensal *S. epidermidis*, inhibits *S. aureus* biofilm formation and nasal colonization. However, the substrate specificity and target proteins of Esp remain unclear. Therefore, the aim of this study was to elucidate these factors and thereby determine the mechanism by which Esp inhibits the formation of *S. aureus* biofilms. We used a mutant Esp protein ($Esp^{S235A}$) with defective proteolytic activity; this protein did not disassemble the biofilm formed by a clinically isolated MRSA strain, thereby indicating that the proteolytic activity of Esp is essential for biofilm disassembly. Proteomic and immunological analyses showed that Esp degrades at least 73 proteins, including 11 proteins, such as extracellular adherence protein, fibronectin-binding protein A, protein A, and a putative lytic transglycosylase, associated with biofilm formation and colonization. Esp selectively degraded several human *S. aureus* receptor proteins (e.g., fibronectin, fibrinogen, and vitronectin) that are involved in its colonization or infection. These results suggest that Esp inhibits *S. aureus* colonization and biofilm formation by degrading specific proteins that are crucial for biofilm construction and host-pathogen interaction.

*Quality control of bacterial biofilm by extracellular molecular chaperones*

Proteomic analysis has been used to identify dozens of cytoplasmic proteins, including molecular chaperones DnaK and ClpB in the biofilm matrix fraction of MRSA. However, the biological significance of these chaperones in the extracellular environment is largely unknown. Here, we show the importance of these excreted chaperones in the quality control of biofilms. Both DnaK and ClpB were detected with Western blotting in the biofilm matrix fractions of various clinically isolated and laboratory *S. aureus* strains (n=49), and the abundance of these proteins varied among the strains. Indirect immunofluorescence microscopy revealed that DnaK and ClpB are attached to the cell surface of *S. aureus* embedded in biofilms. Interestingly, supplementation of media with DnaK and ClpB stimulated *S. aureus* biofilm formation in a dose-dependent manner. A cell-based pull-down assay demonstrated that the added DnaK and ClpB proteins were associated with *S. aureus* cells. In addition, DnaK and ClpB supported the development of biofilms produced by the clinically important pathogens *E. coli* and *P. aeruginosa*. These findings provide a novel insight into the extracellular functions of molecular chaperones DnaK and ClpB and also suggest the importance of these chaperones in infectious diseases.

*Amyloidosis mediated by bacterial biofilm infection*

Some bacteria secret extracellular amyloid fibrils, leading to the production of robust biofilms on biotic or abiotic surfaces. In this study, we investigated a novel hypothesis that bacterial biofilm infection triggers amyloidosis in host organisms using an *E. coli* and *Caenorhabditis elegans* amyloidosis model. We found that *E. coli* curli amyloid fibrils promote aggregation of amyloid beta peptides *in vitro* and that feeding of curli-producing *E. coli* accelerates paralysis of *C. elegans* expressing amyloid beta peptide. These findings support our hypothesis.

*Effects of bacteriocins against MRSA biofilm*

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by Gram-positive bacteria. Nisin, a bacteriocin produced by the lactic acid bacterium *Lactococcus lactis*, is used as a food preservative in many countries because it is nontoxic for humans and highly stable against heat and acids. In this study, we investigated the antibacterial activities of several kinds of bacteriocin (nisin A, lacticin Q, and nukacin ISK-1) against clinical isolates of biofilm-forming MRSA. After purified bacteriocins were added to MRSA biofilms, the antibacterial effects of the bacteriocins were analyzed with a colony-counting method and the live/dead staining of bacterial cells in biofilms. Vancomycin, which showed bacteriocidal activity against planktonic cells of MRSA, did not show activity against MRSA biofilm. On the other hand, nisin A showed high bactericidal activity against both planktonic cells and biofilm cells. Lacticin Q also showed bacteriocidal activity against both planktonic cells and biofilm cells, but its activity against biofilm was 1/10th that of nisin A. Nukacin ISK-1, which showed bacteriostatic activity against planktonic cells of MRSA, did not show activity against MRSA. Nisin A and lacticin Q are known to kill bacterial cells by forming pores on cytoplasmic membranes of target bacteria. Our results suggest that pore-forming bacteriocins are highly potent for the treatment of MRSA biofilm infections.

*High-throughput screening of antibiofilm compounds*

Potential strategies for preventing and treating *S. aureus* biofilm infections are to use small molecules to inhibit biofilm development or to promote biofilm dispersal without the use of lethal selection pressure. High-throughput screening (HTS) could be used to identify other compounds effective against *S. aureus* biofilm development. We will perform HTS in collaboration with the University of Tokyo, which has a chemically diverse small-molecule library (200,000 compounds). First, we have established a crystal violet staining assay of biofilm that is suitable for the HTS method. Additionally, we have designed a screening robot system that automates the dispensing of compounds to assay plates, cell culture handling, and activity measurement. Hereafter, HTS of antibiofilm compounds that are active against MRSA biofilm will be performed.

*Mechanism of E. coli O157 entering a VNC state*

Some *E. coli* O157 strains become VNC under environmental stress conditions and escape detection by conventional culture methods. We showed that the addition of catalase to the culture media resuscitated O157 from a VNC state to a culturable state and that the decrease of sigma factor S activity (encoded by *rpoS* gene) caused bacteria to enter a VNC state. An *rpoS* gene knockout strain was generated from the O157 Sakai strain. The *rpoS* mutant strain entered a VNC state, but the wild-type Sakai strain did not.

*Characterization of ATP-secreting bacteria from mice and humans*

We have reported that ATP-secreting bacteria are present in the intestines of mice and humans. However, the mechanisms of ATP secretion in bacteria are not completely understood. We are investigating the mechanisms of bacterial ATP secretion.

## Publications

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