Department of Bacteriology

Yoshimitsu Mizunoe, *Professor*
Tadayuki Iwase, *Assistant Professor*
Kenichi Okuda, *Assistant Professor*
Akiko Tajima, *Assistant Professor*
Shinya Sugimoto, *Assistant Professor*

General Summary

Research projects of our department have focused on: (1) a novel strategy for biofilm inhibition with small molecules targeting molecular chaperone, (2) thioflavin T as a fluorescence probe for monitoring RNA metabolism, (3) analysis of staphylococcal biofilm structuring and dispersal, (4) biofilm formation with *Propionibacterium acnes* isolated from pacemakers, (5) high-throughput screening of antibiofilm compounds, (6) the role of gut microbes on the host nitrogen cycle, and (7) analysis of the viable but nonculturable state.

Research Activities

Novel strategy for biofilm inhibition with small molecules targeting molecular chaperone
Biofilms are complex communities of microorganisms that attach to surfaces and are embedded in a self-produced extracellular matrix. Because these cells acquire increased tolerance against antimicrobial agents and host immune systems, biofilm-associated infectious diseases tend to become chronic. We show here that the molecular chaperone DnaK is important for biofilm formation and that chemical inhibition of DnaK cellular functions is effective in preventing biofilm development. Genetic, microbial, and microscopic analyses revealed that deletion of the *dnaK* gene (chaperone Hsp70, with co-chaperone DnaJ) markedly reduced the production of the extracellular functional amyloid curli, which contributes to the robustness of *Escherichia coli* biofilms. We tested the ability of the DnaK inhibitors myricetin, telmisartan, pancuronium bromide, and zafirlukast to prevent biofilm formation of *E. coli*. Only myricetin, a flavonol widely distributed in plants, inhibited biofilm formation in a concentration-dependent manner; however, it did not affect growth. Transmission electron microscopy demonstrated that myricetin inhibited the production of curli. These findings provide insights into the significance of DnaK in curli-dependent biofilm formation and indicate that DnaK is an ideal target for antibiofilm drugs.

Thioflavin T as a fluorescence probe for monitoring RNA metabolism
The intrinsically stochastic dynamics of messenger RNA (mRNA) metabolism have important consequences on gene regulation and nongenetic cell-to-cell variability; however, no generally applicable methods exist for studying such stochastic processes quantitatively. Here, we describe the use of the amyloid-binding probe thioflavin T for monitoring RNA metabolism in vitro and in vivo. Thioflavin T fluoresced more strongly in complex with bacterial total RNA than with genomic DNA. Thioflavin T bound purine

oligoribonucleotides preferentially over pyrimidine oligoribonucleotides and oligodeoxyribonucleotides. Cellular analyses, in combination with genetic approaches and the transcription-inhibitor rifampicin treatment, demonstrated that thioflavin T stained mainly mRNA in actively dividing *E. coli* cells. Thioflavin T also facilitated mRNA metabolism profiling at the single-cell level in diverse bacteria. Furthermore, thioflavin T can also be used to visualize transitions between nonpersister and persister cell states, a phenomenon of isogenic subpopulations of antibiotic-sensitive bacteria that acquire tolerance to multiple antibiotics due to stochastically induced dormant states. Collectively, these results suggest that probing mRNA dynamics with thioflavin T is a broadly applicable approach ranging from the molecular level to the single-cell level.

Analysis of staphylococcal biofilm structuring and dispersal

Staphylococcus aureus colonizes prosthetic implants as biofilm, which is multiple layers of bacteria. Inside the biofilm, bacteria are embedded in self-produced matrixes, such as extracellular DNA (eDNA), proteins, and polysaccharide, which elevate levels of resistance to antibiotics and host defenses. Dispersal of biofilm can result in spread to secondary sites and worsening of the infection.

In the staphylococcal biofilm development, bacteria formed biofilm within 8 hours in the growth medium brain-heart infusion with or without glucose; however, biofilm was dispersed in the brain-heart infusion without glucose after 24 hours. Analysis of the extracellular matrix of biofilm showed that this dispersal correlated with degradation of eDNA or extracellular RNA (eRNA) in the matrix. The addition of DNase I had no effect on biofilm dispersal, but RNase A caused biofilm disassembly and inhibited biofilm formation. This finding suggests that eRNA is important for biofilm structure. The culture supernatant from dispersed biofilm caused biofilm disassembly, which suggested biofilm was dispersed by a self-produced secretion factor. It has been reported that *S. aureus* produces thermonuclease (nuclease A, 16.8 kDa; nuclease B, 18.8 kDa). A fraction of 10 to 50 kDa was applied to a cation exchange column, and the active fraction showed DNase activity in DNA zymography. These results suggest the importance of eRNA in biofilm formation and dispersal. Analysis of biofilm disassembly mechanism is in progress.

Biofilm formation by P. acnes isolated from pacemaker

The bacterium *P. acnes* is a facultative anaerobic Gram-positive commensal of the human skin, mouth, conjunctiva, and large intestine. It is usually responsible for late chronic infections and rarely causes acute infections related to medical devices. In this study, the colonization of bacteria on the surfaces of explanted cardiac devices (pacemaker generators) that show no signs of infection was consecutively analyzed. As a result of culture tests with agar plates followed by 16S ribosomal RNA gene sequencings, *P. acnes* was isolated from 7 of 31 devices. To examine whether certain lineages associate with characteristics of the *P. acnes* isolates, sequence typing of *P. acnes* isolates was performed with the multilocus sequence typing scheme. Isolates of *P. acnes* were categorized into 4 different sequence types (STs), including ST2 (2 isolates), ST4 (1 isolate), ST53 (1 isolate), ST6 (2 isolates), and novel ST (1 isolate). As a result of a biofilm formation assay using polystyrene plate, biofilm formations of all *P. acnes* isolates were enhanced by addition

of glucose in growth media. Sensitivity testing of biofilms against proteinase K, DNase I, and dipersin B, which target potential biofilm matrix components, suggested that eDNA is important for the formation and stability of biofilm. This work was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT)-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

High-throughput screening of antibiofilm compounds

One of the potential strategies for preventing and treating biofilm-associated infections is to use small molecules that inhibit biofilm development. We are now pushing ahead high-throughput screening to identify the compounds effective against bacterial biofilm development in collaboration with the University of Tokyo, which has a chemically diverse small-molecule library. Before now, screening has been run with 59,600 compounds, and 2 compounds — ABC-JK1 and ABC-JK2 — that show strong antibiofilm activity against staphylococci, including methicillin-resistant *S. aureus*, were obtained. Both ABC-JK1 and ABC-JK2 affected the extracellular polysaccharide synthesis and cell wall synthesis of staphylococci. Molecular mechanisms of action for ABC-JK1 and ABC-JK1 are now under investigation with a multi-omics approach that uses transcriptomics, proteomics, and metabolomics. This work was supported by the MEXT-supported Program for the Strategic Research Foundation at Private Universities, 2012-2016.

Role of gut microbe on host nitrogen cycle

Nitrogen, like oxygen, hydrogen, and carbon, is an important element for the growth, maintenance, and survival of organisms. Nitrogen itself is abundantly present on Earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be utilized by organisms. Compared with the amount of the bioavailable forms of other elements, that of nitrogen can often be insufficient, and this insufficiency can restrict the increase in the biomass of organisms. We investigated the roles of gut microbes on the nitrogen cycle in hosts.

Publications

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