Department of Bacteriology

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General Summary

We are studying bacterial pathogenesis and host immune responses to bacteria and fungi. In particular, we focus on elucidating the molecular mechanisms of biofilm formation by *Staphylococcus aureus* and the pathogenesis of enterohemorrhagic *Escherichia coli*. We also conduct research for finding molecular targets to prevent biofilm-associated infections and for developing new pneumococcal vaccines. Active collaborative research with several basic and clinical laboratories have been conducted.

Research Activities

Iron-dependent periplasmic oxidative burst-mediated cell death identified in Gram-negative bacteria

Bacterial cell death and dormancy are keys against microbial infections, but the details, including underlying mechanisms, are poorly understood. Here, we identified iron-dependent periplasmic oxidative burst-mediated cell death induced in stressed Gram-negative bacteria, such as *E. coli*, including enterohemorrhagic *E. coli* O157, depending on the activity of the RNA polymerase sigma factor σ^s. Remarkably, this type of cell death, which was induced in dormant persister cells of stressed bacteria exhibiting outer-membrane disintegrity and periplasmic redox imbalance, was blocked by inhibitors of ferroptosis, a newly identified type of cell death in mammalian cells, or by the expression of a H_2O_2-degrading enzyme catalase in the periplasm but not in the cytosol. Furthermore, we demonstrated that the dormant bacteria evaded cell death by catalase from a commensal bacterium *Pseudomonas aeruginosa* or meat; when administered with meat, dormant food-borne pathogen *E. coli* O157 caused fatal infections in mice. In addition, on the basis of identified physiological attributes, we successfully developed a method that isolates dormant pathogen cells from contaminated food sources by avoiding cell death. In conclusion, this study provides evidence of the novel stress response and cell death pathway in Gram-negative bacteria, including food-borne pathogens, which might affect public health.

Modification of virulence and survival in the E. coli evolution by prophage module pmoAB that regulates bacterial gene expression

In microbial evolution, bacteriophages make a great contribution. The contribution of virulence factors, such as toxins, transferred into bacteria from bacteriophages is direct and clear; however, the evolutionary change of bacterial characteristics via prophage-derived genes acting such as a trans-species regulator is poorly understood. Here, we show that the virulence and survival of *E. coli* were regulated by a prophage module (designated

pmoAB) that represses expression of *rpoS* encoding the bacterial RNA polymerase sigma factor σ^S and that was found in enterohemorrhagic *E. coli* O157, a deadly food-borne pathogen emerged from a less-virulent *E. coli*. Specifically, via *rpoS* repression, *pmoAB* decreased the expression of bacterial stress-response genes but simultaneously increased the expression of virulence genes, such as type V-secreted serine protease and type-III secretion systems encoded within the virulence plasmid pO157 and exogenous elements in the bacterial genome, respectively. Under the control of *pmoAB*, *E. coli* O157 caused a drastically fatal infection in mice, and whereas, underwent high cell death via a periplasmic oxidative burst in dormant persister cells induced by stress-like *rpoS* mutants, suggestive of collateral damage. In conclusion, this study provides novel insights into microbial ecology and evolution, including bacteria-prophage interactions, and pathogenicity and survival, which might have implications for public health and food safety.

Role of gut microbe on host nitrogen cycle

Like oxygen, hydrogen, and carbon, nitrogen is an important element for the growth, maintenance, and survival of organisms. Nitrogen is abundantly present on Earth; however, it predominantly exists in the air as molecular nitrogen, which is inactive and cannot be used by organisms. Compared with the amount of the bioavailable forms of other elements, the amount of bioavailable 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.

Roles of lytic transglycosylases in biofilm formation and β-lactam resistance in methicillin-resistant S. aureus

While the functions of lytic transglycosylases in relation to cell division, biofilm formation, and antibiotic-resistance have been determined for several bacteria, their roles in *S. aureus* remain largely unknown. The only lytic transglycosylases known to occur in *S. aureus* are immunodominant staphylococcal antigen A (IsaA) and *Staphylococcus epidermidis* D protein. Our study demonstrated that, in a strain of methicillin-resistant *S. aureus*, IsaA and *S. epidermidis* D protein contribute differently to biofilm formation and β-lactam resistance. Our results suggest that IsaA is a potential therapeutic target for methicillin-resistant *S. aureus* infections.

Virulence of staphylococcal biofilm-dispersed cells

The biofilm dispersal process is the final stage of biofilm development and a necessary step for bacteria to leave the biofilm and spread in new locations. We have found that *S. aureus* causes biofilm dispersal by nuclease. We investigated the virulence of dispersed bacteria in comparison to planktonic bacteria in vitro and in vivo. Dispersed bacteria decreased phagocytosis by polymorphonuclear neutrophils. In a mouse model of infection, dispersed bacteria caused a lethal infection within 24 hours, but planktonic bacteria did not. These results indicate that dispersed bacteria from biofilm show higher virulence than do planktonic bacteria.

Basic research against biofilm formation of S. aureus

We found new insights of *S. aureus* biofilms. First, we demonstrated that RNA is a new component in biofilms. The RNA localized in the biofilm by binding to polysaccharides. Using several synthetic RNAs, we found several conditions of sequences to promote biofilm formation. Second, we demonstrated biofilm biomass is complemented by the cell wall anchoring protein *S. aureus* surface protein G and secreted extracellular adherence protein. Alternatively, these proteins might play different roles in the formation of the steric structure. Third, *Bacillus subtilis natto* inhibits the growth of *S. aureus*. Transcriptome analysis suggests that sporulation is important for this effect.

Publications

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