Department of Bacteriology

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General Summary

In the current year, clinical bacteriological studies with collaborators of clinical departments have greatly advanced in addition to basic researches. Basic bacteriological studies include 1) physiological and molecular analyses of bacteria under viable but noncultuable status, 2) analysis of a novel gene module of prophage contributing to high virulence of enterohemorrhagic *Escherichia coli* O157, 3) elucidation of the quality control mechanism of type-VIII-secretion system, 4) biofilm dispersal and pathogenicity of *Staphylococcus aureus*, and 5) nitrogen fixation in the mammalian intestine. Clinical bacteriological studies include 1) development of a novel method for infections by multi-drug resistant bacteria, 2) elucidation of the cause of interstitial cystitis, 3) development of a rapid detection method of drug-resistant bacteria. Active international collaborative researches 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. We found iron-dependent periplasmic oxidative burst-mediated cell death induced in stressed Gram-negative bacteria such as *Escherichia coli*, including enterohemorrhagic *E. coli* O157, depending on activity of the RNA polymerase sigma factor σ^s. 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 expressing H_2O_2-degrading enzyme catalase in the periplasm, but not the cytosol. We demonstrated that the dormant bacteria evaded cell death by catalase from a commensal bacterium *Pseudomonas aeruginosa* or meat; when administered with meat, dormant *E. coli* O157 caused fatal infections in mice. In addition, based on the identified physiological attributes, we developed a method that isolates dormant pathogen cells from contaminated food sources via avoiding cell death. This study provides evidence of the novel stress response and cell death pathway in gram-negative bacteria, including food-borne pathogens, which may affect public health.

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

In microbial evolution, bacteriophage has a great contribution. The contribution of viru-

lence factors such as toxin transferred into bacteria from bacteriophage is direct and clear; however, 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 sigma factor σ^S and that was found in enterohemorrhagic *E. coli* O157, a deadly foodborne pathogen emerged from a less virulent *E. coli*. Specifically, via *rpoS* repression, *pmoAB* decreased expressions of bacterial stress-response genes, but simultaneously, increased expressions 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 *pmoAB* control, *E. coli* O157 caused a drastically fatal infection in mice, and whereas, underwent high cell death via 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 may 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.

Quality control of the type 8 secretion system in Escherichia coli

The *E. coli* type 8 secretion system is involved in secretion and formation of extracellular curli amyloid fibers. We discovered that curli biogenesis depends on molecular chaperone DnaK, a bacterial Hsp70 homolog, via a quantity and quality control of RpoS, a stationary phase-specific alternative sigma factor regulating bacterial transcription, and CsgD, the master transcriptional regulator of curli formation. DnaK also keeps CsgA and CsgB in a translocation-competent state by binding to their signal peptides prone to aggregation. We also found that certain periplasmic chaperones and proteases participate in the quality control and degradation of CsgA and CsgB in the periplasmic space. These results provide mechanistic insights into curli biogenesis and robust biofilm formation.

Virulence of staphylococcal biofilm-dispersed cells induced by nuclease

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 found that *Staphylococcus aureus* caused biofilm dispersal by nuclease. To investigate the virulence of dispersed bacteria, we examined PMN phagocytosis of these bacteria in comparison to planktonic bacteria. Dispersed bacteria decreased phagocytosis by PMN. The expression of extracellular polysaccharide PNAG (poly-N-acetylglucosamine) which

protects bacteria from PMN phagocytosis was increased in dispersed bacteria. In mouse infection model, dispersed bacteria caused a lethal infection within 24 h, however planktonic bacteria did not. These results indicated that dispersed bacteria from biofilm showed highly virulence than planktonic bacteria in vitro and in vivo.

Publications

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Reviews and Books

Kanematsu H[1], Barry DM[2], Ikegai H[3], Michiko Y[4], Mizunoe Y ([1]The National Institute of Technology Suzuka College, Suzuka, Japan, [2]Clarkson University, Potsdam, NY, USA, [3]University of Human Arts and Sciences, Saitama, Japan, [4]National Institute for Materials Science; NIMS, Tsukuba, Japan). Biofilm evaluation methods outside body to inside — Problem presentations for the future — . *Medical Research Archives.* 2017; **5:** 1-17.