Department of Bacteriology

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General Summary

Research projects of our department have focused on: (1) a novel single point mutation in domain 2 of the stress-inducible sigma factor ʃS (RpoS) attenuates RpoS activity, (2) a straightforward assay for measuring glycogen levels and RpoS activity, (3) the role of gut microbes on the host nitrogen cycle, (4) the molecular mechanisms of type 8 secretion system, (5) an exploration of novel physiological functions of polyphenols, and (6) the analysis of staphylococcal biofilm dispersal.

Research Activities

A novel single-point mutation in domain 2 of the stress-inducible RpoS attenuates its activity

RpoS regulates stress resistance genes in *Escherichia coli*, such as the *katE* encoding catalase hydroperoxidase (HPII) and the *glg* encoding glycogen synthesis protein. Monitoring RpoS activity can provide information on the stress sensitivity of *E. coli* isolates in clinical settings because its RpoS is often mutated. In the present study, we found a novel, missense point mutation at RpoS domain 2 in a clinical *E. coli* isolate. The mutant RpoS protein was non-functional according to the HPII activity and glycogen levels, which are positively regulated by RpoS. A reporter assay with β-galactosidase indicated that the dysfunction occurred at the transcriptional level. Substitution analysis indicated that the hydrophobicity of the amino acid at domain 2 was critical for RpoS activity. However, no RpoS activity was observed when RpoS domain 2 was substituted with the hydrophobic amino acid proline, which can destroy the alpha-helix structure at domain 2, suggesting that the structure near this residue may also play an important role in RpoS activity. These results contribute to a deeper understanding of RpoS regulatory mechanisms and bacterial stress responses.

A straightforward assay for measuring glycogen levels and RpoS activity

Bacterial cellular glycogen levels reflect the activity of RpoS. In this study, a straightforward assay for measuring glycogen levels and RpoS activity was developed to combine the ease and simplicity of qualitative approaches. The assay reagent comprised 2% iodine solution (2% iodine/1 M NaOH), and the basic principle of this assay is the iodine-glycogen reaction, which produces a reddish brown color that can be measured using a spectrophotometer. A calibration plot using the known amount of glycogen yielded the best linear fit over a range of 10-300 μg/assay (R^2 = 0.994). The applicability of the assay was assessed; glycogen was detected and quantified in clinical isolates with functional RpoS but not in isolates with dysfunctional RpoS. This assay constitutes a simple method for

measuring RpoS activity and was successfully applied for measuring glycogen levels in human cells.

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.

Molecular mechanisms of type 8 secretion system
Previously, genome-wide screening of genes involved in the type 8 secretion system (T8SS), which secretes and assembles extracellular amyloid fibrils, termed curli, on the surface of bacteria identified the highly conserved molecular chaperone 70 kilodalton heat shock protein (HSP 70 or DnaK). The protein DnaK is known to play important roles in quality control of diverse cytoplasmic proteins, membrane proteins, and secretion proteins. The aim of this study was to clarify molecular mechanisms by which DnaK regulates the expression and quality of T8SS. Mutational analysis combined with in-cell protein folding and cell-free protein folding analyses demonstrated that DnaK engages in the folding of some transcriptional regulators involved in the expression of T8SS-related genes. Comprehensive approaches will provide mechanistic insights into the quality control of T8SS by DnaK and may lead to an understanding of the regulation of severe neurodegenerative diseases caused by protein misfolding, such as Alzheimer's disease and prion disease.

Exploration of novel physiological functions of polyphenols
Exploration of the potential functions of food constituents provides an additional value for health and offers applications for preventing diseases. In this study, we sought to identify small natural compounds that inhibit bacterial biofilm formation without attenuating growth. We found that myricetin, a type of polyphenol, effectively prevented biofilm formation by *E. coli* and *Staphylococcus aureus,* including methicillin-resistant strains, in a dose-dependent manner. Myricetin inhibited production of curli, presumably via inactivation of DnaK. In addition, a more effective myricetin-derivative with approximately 10-fold higher activity than myricetin was identified. Its mode of action is now being elucidated.

Analysis of staphylococcal biofilm dispersal
In the staphylococcal development of biofilm, the bacteria formed the biofilm within 8 hours; however, the biofilm was dispersed after 24 hours. Analysis of the extracellular matrix of the biofilm showed that this dispersal correlated with nucleic acids being degraded in the matrix. The culture supernatant from dispersed biofilm caused the biofilm to disassemble. The fraction of 50 to 10 KDa was applied to the cationic exchange col-

umn, and the active fraction showed nuclease activity in DNA zymography. The dispersion of the biofilm was not detected in a *nuc* mutant, which suggested that nuclease is a key factor for the biofilm disassembly mechanism.

Publications

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