学位論文内容の要旨

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学位論文題名

Elucidation of molecular bases underpinning insect-bacteria gut symbiosis

（昆虫と細菌の腸内共生を支える分子基盤の解明）

Insect is one of the most diverse animal group on the earth, many of which possess symbiotic microorganisms. Insect symbionts confer numerous physiological advantages to the hosts, including provision of essential nutrients. The bean bug *Riptortus pedestris* is a notorious pest of leguminous crops and harbors gut symbiotic bacteria of the genus *Burkholderia* in the posterior midgut region (M4) that consists of hundreds of sac-like tissues called crypts. The bean bug acquires *Burkholderia insecticola* horizontally from the environmental soil via oral ingestion every generation, where gut-colonizing *Burkholderia* provides numerous benefits to the host. However, the mechanism of how the highly specific association between the bean bug and *Burkholderia insecticola* is maintained is scarcely investigated. This study aimed to reveal molecular bases of how *Burkholderia insecticola* specifically colonizes, adapts, and lives in the midgut crypts.

1. Host-symbiont specificity determined by microbe-microbe competition in an insect gut

Despite the omnipresence of specific host-symbiont associations with acquisition of the microbial symbiont from the environment, little is known how the specificity of the interaction evolved and is maintained. The genus *Burkholderia* consists of over 100 species showing ecologically diverse lifestyles and including serious human pathogens, plant pathogens, and nodule-forming plant mutualists, as well as insect mutualists. Through infection tests of 34 *Burkholderia* species and 18 taxonomically diverse bacterial species demonstrate that non-symbiotic *Burkholderia* and even its outgroup *Pandoraea* could stably colonize the gut symbiotic organ. However, co-inoculation revealed that the natural symbiont always outcompeted the non-native bacteria inside

the gut symbiotic organ, explaining the predominance of the native *Burkholderia* symbiont in natural bean bug populations. Hence, the abilities for colonization and cooperation, usually thought as specific traits of mutualists, are not unique to the symbiont but competitiveness inside the gut is a derived trait of the symbiont lineage only and was thus critical in the evolution of the insect gut symbiont.

2. Dual oxidase enables insect gut symbiosis by mediating respiratory network formation

Dual oxidase (Duox) is a well-described enzyme, involved in gut mucosal immunity by the production of reactive oxygen species that antagonizes pathogenic bacteria and maintains gut homeostasis in insects. However, despite its non-specific harmful activity on microorganisms, little is known about the role of Duox in the maintenance of mutualistic gut symbionts. This study shows that in the bean bug, Duox-dependent ROS did not directly contribute to epithelial immunity in the midgut in response to its mutualistic symbiont, *B. insecticola*. Instead, the expression of Duox is tracheae-specific and its downregulation by RNAi results in the loss of dityrosine crosslinks in the tracheal protein matrix, a collapse of the respiratory system and a disruption of the gut symbiosis. Downregulation of the hypoxia-responsive transcription factor Sima or the regulators of tracheae formation Trachealess and Branchless produces similar phenotypes. Thus, in addition to known roles in immunity and the formation of diverse extracellular matrices, Duox is also a crucial enzyme for tracheae stability by establishing a dityrosine network of luminal matrix proteins, which maintains mutualistic symbionts.