学位論文審査の要旨

博士の専攻分野の名称　博士（農学）　氏　名　Jang Seonghan

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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)

The thesis consists of 110 pages, 32 figures, seven tables, and seven chapters, and seven published/accepted papers are attached for reference.

The Insecta is one of the most diverse animal groups on earth. Many species of them possess symbiotic microorganisms. Insect symbionts confer numerous physiological advantages to the hosts, including provision of essential nutrients. Despite the accumulation of a large body of knowledge concerning the physiological bases of the insect-microbe symbiotic associations, the genetic and molecular bases of these intimate interactions are poorly investigated. 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. These gut-colonizing *Burkholderia* provide numerous benefits to the host. The bean bug acquires *B. insecticola* horizontally from the environmental soil via oral ingestion every generation. However, the mechanisms of how the highly specific association between the bean bug and *B. insecticola* is established and maintained are still unclear. The thesis work by Jang Seonghan aimed to reveal molecular bases of how *B. insecticola* specifically colonizes, adapts, and lives in the midgut crypts. The results section of the thesis consist of two complementary chapters, one chapter demonstrating bacterial competition as a primordial mechanism in the specific colonization of the midgut crypts with symbionts and the second one revealing the role of the Duox enzyme in tracheal development in insects as well as the importance of the insect tracheal network for the establishment of the symbiosis.

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

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, it was demonstrated that non-symbiotic *Burkholderia* and even its outgroup *Pandoraea* could stably colonize the gut symbiotic organ of the bean bug. 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. The second result chapter of the thesis shows that in the bean bug *R. pedestris*, 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 gut symbionts. Furthermore, reactive oxygen species generated by Duox can be scavenged with antioxidants such as N-acetylcysteine and feeding insects with this compound prevents tracheal formation and symbiosis, suggesting that antioxidants can be used as novel insecticides to control pests.

In conclusion, by diverse experimental approaches from both host and symbiont sides, this study demonstrates genetic, molecular, and evolutionary mechanisms that underpin the specific insect-microbe symbiosis. Moreover, this study not only increases our fundamental knowledge about symbiosis but also give a practical clue to control pest insects.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.