

PK-19**Functions and evolution of a dual bacterial symbiosis in a serious citrus pest, *Diaphorina citri***

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カンキツの重要害虫ミカンキジラミにおける複合細菌共生系の機能的役割と進化

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Key word : insect, symbiosis, intracellular, genome, toxin

The Asian citrus psyllid, *Diaphorina citri*, is an important pest, as it transmits a serious citrus disease, Huanglongbing or greening disease. It is widely distributed in Asia including Japan, and is spreading into other citrus growing regions worldwide. *D. citri* has a symbiotic organ called the bacteriome, which harbors two distinct intracellular symbionts, *Candidatus Carsonella ruddii* (*Carsonella_DC*, *Gammaproteobacteria*), and an undescribed 'symbiont' (SSDC, *Betaproteobacteria*). This dual symbiotic system is potentially a promising target for developing effective and highly selective pest control methods, as it seems to play crucial roles for the survival of the host insect. To understand basics of this symbiotic system, we determined their genome sequences, followed by biochemical and physiological analyses related to the gene inventory. The extremely small genome of *Carsonella_DC* (174,014 bp) appears to be specialized in synthesizing essential amino acids that are scarce in the phloem sap, the sole food of the host psyllid. On the other hand, the genome of SSDC (459,399 bp) encodes enzymes for synthesizing apparent novel toxins, which is further verified by subsequent analyses. Our results strongly suggest that *Carsonella_DC* and SSDC are nutritional and defensive symbionts, respectively.

PK-21**Microdiversity analysis provides insights into the evolution of termite gut protists**

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シロアリ腸内原生動物の種内多様性解析

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Key word : insect, diversity, symbiosis, endosymbiont

Termites harbor diverse gut-symbiotic microorganisms, at least some of which are strictly specific to their host species. In such microbial communities, microevolution and the resulting microdiversification may play an important role in their macroevolutionary processes. Here, we analyzed the microdiversity of the cellulolytic protist *Pseudotriconympha grassii* and its endosymbiotic, nitrogen-fixing bacterium, *Candidatus "Azobacterium pseudotriconymphae"*, in the gut of the termite *Coptotermes formosanus*. Single cells of *P. grassii* were isolated by means of micromanipulation, and each cell was subjected to whole genome amplification. From these samples, we PCR-amplified 18S rRNA gene and an rRNA-ITS region for *P. grassii* and an rRNA-ITS and nif-operon intergenic region for the endosymbiont "*A. pseudotriconymphae*" by using a proofreading DNA polymerase, and determined the sequences. Interestingly, these sequences were completely identical among the protist cells in a single gut. Thus, the microdiversity of the protist and its intracellular symbiont was unexpectedly low. We hypothesize that a population bottleneck of the gut microbiota at each colony foundation of the termites maintains such an extremely low microdiversity.

PK-20**Methanogens associated with cellulolytic protists in termite guts**

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シロアリ腸内原生動物共生メタン菌の進化生態学的解析

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Key word : unculture, symbiosis, termite, TEM, phylogeny

Termites harbor diverse gut microbes for host survival. Among them, methanogens are consistently found in various termites and occasionally ecto- and/or endosymbionts of certain cellulolytic gut protists. Because of the difficulty in cultivation of these protist-associated methanogens, the details of their phylogeny, morphology, and functions remain unclear. Here, we performed phylogenetic and TEM analyses of the methanogens associated with the termite gut protists. Methanogens were detected with their specific autofluorescence, and the protist cells possessing methanogens were collected using a micromanipulator. These were subjected to whole genome amplification, and 16S rRNA genes were amplified by PCR, cloned, and sequenced. Almost all were related to *Methanobrevibacter filiformis*, an isolate from termite gut epithelia. The sole exception was that recovered from the cells of a novel *Mixotricha* species, a large parabasalid protist. This phylotype shared 98 - 99% sequence identity with *Methanobrevibacter arboriphilus*, which is common in various anaerobic environments. In the TEM analyses, we found that the *M. filiformis*-related symbionts were enclosed in the host membrane, while the *M. arboriphilus*-like symbionts were directly in contact with the host cytoplasm. We will discuss the symbiotic associations between these methanogens and the protist hosts.

PK-22**An absence of symbiotic methanogens in termite guts can cause a disadvantage to the nutrition economics of the host termite colony**

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シロアリは腸内共生メタン菌の脱落によって不利益を被り得る

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Key word : acetogen, hydrogen emission, colony parameter, metabolome analysis

Methanogens as well as acetogens are a major hydrogen sink in termite guts, whereas an apparent absence of methanogens has been reported for some termite colonies. Here we compared hydrogen emissions, colony parameters, and metabolic profiles of the termite *Neotermes koshunensis* between presence and absence of methanogens in their colonies. First, a total of 138 colonies were examined by specific PCR amplification of the 16S rRNA gene, epifluorescence microscopy, and gas chromatography. Methanogens/methanogenesis were detected from 111 colonies (MET colonies) and not from 27 colonies (non-MET colonies). Hydrogen emission measurements showed there were neither expected increases in non-MET colonies nor significant differences between MET and non-MET colonies. Given that a previous study has demonstrated higher acetate concentrations in the guts of non-MET colonies, hydrogen could have been utilized by acetogens, which should provide more energy source for the host termites. However, in reality, a mean weight of soldier termites was significantly lower in non-MET colonies, while metabolome analysis indicated a great difference between MET and non-MET colonies. These results suggest that an absence of methanogens not only represents an absence of methanogenesis, but also causes a poor nutritional status of the colonies possibly due to the different metabolic processes in the guts.