

論文 / 著書情報
Article / Book Information

題目(和文)	イエバエとキンバエの腸内細菌叢と抗生物質耐性菌の解析
Title(English)	Analysis of the bacterial community and antibiotic-resistant bacteria in the digestive tract of houseflies (<i>Musca domestica</i>) and green bottle flies (<i>Calliphoridae</i>)
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Outline of Doctoral Thesis

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Houseflies and green bottle flies are two groups of most commonly occurring insects in Japan. They are classic examples of synanthropic flies, for they live in close association with humans and domesticated animals. Like other filth flies, they feed and oviposit on decaying organic matter such as animal manure, human excrement, garbage, and animal bedding, which is heavily colonized by microorganisms. These flies are important hosts for a variety of bacteria, including pathogenic and antibiotic-resistant strains. However, the structure of the bacterial communities associated with houseflies and green bottle flies have not been comprehensively studied. This study revealed the structure of the fly-associated bacterial community as well as the prevalence of antibiotic-resistant bacteria, and compared the persistence of antibiotic-resistant and -sensitive bacterial strains.

In Chapter 2, housefly eggs were collected from a residential area of Tokyo and cultured under laboratory condition. Individuals from three developmental stages (maggot, pupa, and adult fly) were collected, and the bacterial community associated with flies of each stage was analyzed by culture-independent methods, PCR–DGGE and 454 pyrosequencing. DGGE results showed clear individual divergence in the maggot and adult fly stages, but not in pupa stage. Pyrosequencing results revealed that the bacterial communities associated with maggots and pupae were predominated by an unclassified *Neisseriaceae*, while the community in adult flies was predominated by *Enterobacter* and *Acinetobacter*. Maggots and pupae shared more common bacterial genera than with adult flies. Antibiotic-resistant bacteria were screened on LB agar supplemented with six antibiotics: ampicillin, cefpodoxime, tetracycline, kanamycin, nalidixic acid, and ciproflaxacin. The isolated resistant strains were identified to species and their antibiotic susceptibility was tested with a standard broth microdilution method. The results showed that across all stages, the numbers of bacteria resistant to at least one antibiotic in each housefly individual ranged from 10^4 to 10^6 CFU, which were only one to three orders of magnitude lower than the number of the total culturable bacteria. Most strains subjected to susceptibility tests were resistant to two or more antibiotics.

In Chapter 3, green bottle flies were captured from five densely populated metropolitan districts of Tokyo and one suburban district in Yokohama in spring, summer, and autumn. The fly-associated bacterial community was revealed by 454 pyrosequencing and compared among seasons and collection sites. In spring, the major bacterial community consisted of *Staphylococcus* and *Pseudomonas*, and the composition was highly similar although the two samples were collected from two sites far apart. In summer, the five samples collected from different sites shared a common predominant genus, *Ignatzschineria*. Other abundant genera included *Dysgonomonas*, *Vagococcus*, and *Lactobacillus*. In autumn, no core genus common among all five samples collected from different sites was observed. Predominant genera in different samples included *Vagococcus*, an unclassified *Acetobacteraceae*, an unclassified *Entomoplasmatales*, and *Dysgonomonas*. Overall, there was an upward trend in the bacterial

community diversity from spring to autumn. Antibiotic-resistant bacteria were studied by culturing method as mentioned in Chapter 2. The concentrations of antibiotic-resistant ranged from 10^3 to 10^8 CFU/g fly gut, which were one to four orders of magnitude lower than that of the total culturable bacteria. Across all samples, 129 strains spanning 32 species of resistant bacteria were isolated and identified. The most frequently recovered species were *Pseudomonas putida* and *Pseudomonas moteilli* in spring, *Enterococcus hirae* in summer, and *Enterococcus faecalis* in autumn. In total, 20 out of 64, and 25 out 35 bacterial strains were resistant to two or more antibiotics.

In summary, this research revealed shifts in the composition of fly-associated bacterial community during the fly development and among different seasons, and highlighted the potential risks of fly-mediated transmission of antibiotic-resistant bacteria, especially multidrug-resistant pathogenic bacteria. Further efforts were made to investigate the persistence of antibiotic-resistant bacteria in the fly digestive tract without antibiotic selective pressure.