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Microbiology of the insect gut: tales from mosquitoes and bees

The class Insecta consists of a large group of organisms with rich species diversity. There are an estimated 750,000 species of insects, but the actual number could be far more than this as some regions have been poorly studied and many ecosystems, especially in the tropics, have not been explored at all. Some estimates put the number as high as 10 million (Novotny *et al* 2002). All insect species are known to harbour a rich and complex community of microorganisms in their guts and other body regions. This microbiota participates in many types of interactions ranging from pathogenesis to obligate mutualism (Dillon and Dillon 2004). One reason for the microbial diversity is that different groups of insects have different feeding habits; this results in different gut structures and functions and promotes the establishment of different phylotypes. In recent years there has been renewed interest in the understanding of insect gut microorganisms for two reasons. First, this diverse microbiota is a potential source of novel bioactive compounds such as antimalarial, antiviral and antitumour peptides (Chernysh *et al* 2002), enzymes (Zhang and Brune 2004) and novel metabolites (Wilkinson 2001). Second, manipulating these microbial symbionts is thought to be an effective strategy for controlling the spread of pathogens that use insects as hosts (Mickes and Ferguson 1961; Lehane *et al* 1997; Beard *et al* 2002; Dillon *et al* 2005).

Approaches for studying microbial community structure have changed phenomenally over in the last decade. Methods like 16S rDNA cloning and sequencing, and genetic profiling using various methods – denaturant gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP) or terminal restriction fragment length polymorphism (T-RFLP) and fluorescent *in situ* hybridization (FISH) among them – are being used to explore the bacterial diversity of various ecosystems without resorting to actually culturing the microorganisms in the laboratory (Reeson *et al* 2003; Mohr and Tebbe 2006; Thimm and Tebbe 2003). Before a few years, the microbiota of insect gut systems were only rarely examined using these techniques, an example being work on the microbial community structure of termite guts (Brauman *et al* 2001). Two recent studies (Lindh *et al* 2005; Mohr and Tebbe *et al* 2006) extend this knowledge to other insect groups and provide new insights into the interplay between gut microbiota and insect physiology, development and social behavior. These studies also provide background information on para-transgenic approaches to the control of vector-borne diseases (Durvasula *et al* 1997. The 'para-transgenic' method involves removing a microbial symbiont from an insect, genetically modifying it and re-introducing it into another insect that has previously been cured of its normally resident symbionts. Thus the transgene is carried by the insect but not in its own genome.).

Pidiyar *et al* (2002, 2004) examined the mid-gut flora of *Culex quinquifasciatus*, responsible for the transmission of filaria and probably Japanese encephalitis. They used both the conventional, culture-based approach and culture-independent, 16S rRNA gene-based PCR–clone–sequencing. Many cosmopolitan bacteria were found in the mosquito mid-gut, as also a new species of the genus *Aeromonas*, namely *Aeromonas culicicola* (Pidiyar *et al* 2002). This bacterial species was 2000-fold more abundant in mosquitoes that were blood fed as compared to those which were not. Subsequently, the same species was also found to inhabit the mid-guts of other mosquito species – *Aedes aegyptii* and *Anopheles stephensi* – found the same locality.

In a later study, several species of bacteria were detected in the mid-guts of *Anopheles gambiae* and *A. funestus* by both methods (Lindh *et al* 2005). The isolates included at least two new species belonging to the genera *Thorsellia* and *Janibacter* (Kampfer *et al* 2006a,b). One isolate was identified as *Rhodococcus corynebacteroides* which is a relative of *R. rhodinii* that is found in *Rhodinus prolixus* and has been successfully used in the para-transgenic approach (Durvasula *et al* 1997). The 16S rRNA approach revealed the presence of various subgroups of proteobacteria and genera representing intracellular bacteria such as *Anaplasma* and *Spiroplasma*. (The proteobacteria are a major group of Gram-negative bacteria; among them, the gamma-proteobacteria include many medicinally important and pathogenic forms, for example *Enterobacteria*, *Vibrio*, *Salmonella*, *Yersinia* and *Pseudomonas*.) Interestingly, both studies showed the presence of members of genera *Bacillus*, *Stenotrophomonas* and *Pseudomonas* in the mosquito mid-gut.

Again, whether these genera have a specific role in the biology of the mid-gut or the finding is an offshoot their presence in breeding waters is an open question. The presence of several gamma-proteobacteria in both studies is promising from the point of raising para-transgenic *Anopheles* mosquitoes; well established methods for genetic modification are available for proteobacteria.

Mohr and Tebbe (2006) compared the gut communities of three species of bees using a culture-independent approach. Here, the SSCP technique was used for community dynamics analysis and the relevant bands were sequenced to determine the phylogenetic affiliations of uncultured gut bacteria. The bee species used in the study can co-exist in the same habitat but had different social behaviour and feeding habits. In *Apis mellifera* (the European honey-bee), workers have mouth-to-mouth contact with larvae and with each other. Adult forager bees use nectar as a food source; the larvae are first fed on secretions from the hypopharyngeal gland and later on the secretion is mixed with pollen and nectar. In *Bombus terrestris* (the bumble-bee), adult workers have only indirect contact with larvae and each other by feeding on the same food, which has been collected and mixed with nectar by forager bees. In the third species, *Osmia bicornis* (red mason bee) is a typical solitary bee. The female lays eggs on a stored mass of pollen, separates them with mud and dies before the eggs hatch. The emerged larvae feed on the stored pollen and thus there is no direct feeding contact between larvae and adults at all.

These studies were carried out over three years and included different developmental stages. There were substantial qualitative as well quantitative differences in the microbial types depending on the species, developmental stage and the diet. *Apis mellifera* adults predominantly contained *Lactobacilus* wheras larval SSCP patterns had a predominance of bands corresponding to *Salmonella enterica* var *typhi*, uncultured *Simonesiella* and uncultured *Serratia*. This is presumably because the food source for forager bees (honey and nectar) has a low pH of approximately 3.9 and lactobacilli can tolerate this pH. The pH of larval gut is around 7 and is less favourable for Lactobacilli. On the other hand, the gut from the larvae of solitary bee *O. bicornis* showed SSCP patterns quite different from the other two species, which could be due to different social habit and also difference in development. The gut of this species opens during the early development of the larvae whereas for the other two species it opens much later, just before pupation. This would result in differences in physicochemical conditions and thus differences in the microbiota.

Despite these differences, the bacteria from the three different bee species reflected clusters of highly similar sequences even from specimens collected from different continents. Both larvae and adults of *A. mellifera* contained sequences related to uncultured species of *Simonsiella, Serratia, L. crispatus* and *Gluconacetobacter*. The bacteria could have either survived pupation or were inoculated through food and/or mouth-to-mouth contact. Interestingly, these sequences, found in all three bee species, were also reported in other two *A. mellifera* subspecies. The earlier study on *A. mellifera* sub-species in South Africa showed that out of 10 unique 16S rRNA sequences, bacteria from six genera were shared in both subspecies (Jeyaprakash *et al* 2003). Studies by Mohr and Tebbe (2006) retrieved 179 16S rRNA sequences, which represented 68 phylotypes. Among these, the overlap was very high for five genera and these may represent bacterial species that are highly abundant and cosmopolitan, adapted to survival in the gut.

In summary, it appears that insect guts are reservoirs for a large variety of microbes. Many are poorly characterized and considering the diversity of insects, there must be novel microbes awaiting discovery. Our understanding of the biology of insects will be incomplete without a comprehensive understanding of their gut microbes, as these have a significant impact on various life processes of the hosts. While the roles of endosymbionts like *Wolbachia* and *Buchnera* are better understood, not much is known about the normal microbial community flora. Characterization of midgut microbes using molecular tools is the first step in understanding their role in insect biology. Application of genomics and proteomics would further our understanding of their interaction. Genome sequencing projects of such bacteria are underway and they will eventually help in defining the minimal essential genes required for the bacteria to multiply and survive in insect gut. They will help in distinguishing transient from resident populations and in understanding interactions between bacteria and their host insects at molecular level.

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