Genomics of the honey bee microbiome

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Abstract

The guts of honey bee workers contain a distinctive community of bacterial species. They are microaerophilic or anaerobic, and were not clearly delineated by earlier studies relying on laboratory culture of isolates under atmospheric oxygen levels. Recently, a more complete picture of the potential metabolism and functions of these bacteria has been possible, using genomic approaches based on metagenomic samples, as well as cultured isolates. Of these, most are host-restricted and are generally absent outside adult guts. These species include both Gram negative groups, such as *Gilliamella apicola* and *Snodgrassella alvi*, and Gram positive groups such as certain *Lactobacillus* and *Bifidobacterium* species. These gut bacterial species appear to have undergone long term coevolution with honey bee and, in some cases, bumble bee hosts. Prediction of gene functions from genome sequences suggests roles in nutrition, digestion, and potentially in defense against pathogens. In particular, genes for sugar utilization and carbohydrate breakdown are enriched in *G. apicola* and the *Lactobacillus* species.

Introduction

The importance of gut-dwelling microbial communities in the health of animals, from humans to insects, has become widely appreciated only recently [1]. A key reason is that tools for studying microorganisms in non-laboratory environments have become much more powerful. Because most organisms in most environments, including guts, are not readily grown in laboratory culture, traditional culture-based methods gave an incomplete and often extremely misleading picture of microbial communities. Approaches based on DNA sequencing have enabled a more reliable picture, and the growth of nucleotide sequence databases has enabled routine classification of gut microorganisms characterized only by short fragments of sequenced DNA.

These new methods have revolutionized understanding of microbial ecology in general. They have now been applied to gut communities in honey bees (*Apis mellifera*), and have yielded insights into the dynamics of these distinctive communities and into potential effects of gut bacteria on bee hosts. Below, I summarize recent findings about the honey bee gut microbiota, mostly based on genomic methods.

Who are they?

Starting in 2003 with a study on honey bee workers from North America and South Africa ([2], several novel rRNA gene sequences were obtained, pointing to the occurrence of characteristic bacteria in the guts of this species. Later, more intensive sequencing repeatedly retrieved these same distinctive sequences and a few others from honey bee workers from samples in Europe, North America, and Australia ([3-6]. It became clear that honey bee guts harbor a specific set of bacterial species not retrieved from other environments. The guts of bumble bees (*Bombus* species) contain bacteria that are closely related to some of the honey bee associates [7] and that are classified as the same bacterial species [8]. These same distinctive groups are also found in other *Apis* species [9-11]. In contrast, bees outside of *Apis* and *Bombus*, whether solitary or social, usually lack these bacterial species [5,7,12,13]. The exceptions are certain eusocial stingless bees (*Meliponini*) in Australia which share some of the *Lactobacillus* clusters with honey bees [14].

Approximately eight bacterial species clusters dominate in the guts of *A. mellifera* workers (Table 1), making up over 95% of the bacteria in most individuals ([10,15]. Each such cluster corresponds to a set of closely related bacterial strains. Because species designations are somewhat arbitrary within bacteria, some of these clusters are described as a single species, while others are described as multiple species. Three are newly described as species within the Gram negative bacterial phylum Proteobacteria; these are *Snodgrassella alvi*, *Gilliamella apicola*, and *Frischella perrara* [8,16], all restricted to the guts of *Apis* species and *Bombus* species, with *F. perrara* confined to honey bees. Strains within each of these three species show <3% sequence variation within the 16S rRNA gene but much more extensive divergence in their overall genomes, implying differences in metabolic capabilities [17,18]. Three similarly closely related clusters of Gram positive bacteria are also largely restricted to bee guts; these

include two clusters within the Firmicutes phylum and specifically within the genus *Lactobacillus*, and one *Bifidobacterium* cluster within the phylum Actinobacteria. These have been called F-4, F-5 (or Firm4 and Firm5) and “Bifido” [3-5]. Within the two “Firm” clusters, strains have been given multiple species names within *Lactobacillus* [19]; likewise “Bifido” corresponds to a species cluster that includes *Bifidobacterium asteroides* from honey bee [20] and several species described from bumble bees [21-23] (Table 1). Two other species clusters are from distantly related clusters of Alphaproteobacteria, initially called Alpha1 and Alpha2 [4]. Alpha1 is a close relative of *Bartonella* species, a group of specialized animal pathogens within the Rhizobiales, and is present and often abundant in about half of sampled workers [15]. Alpha2 consists of numerous strains of Acetobacteraceae, including Alpha2.1, which is a gut specialist, and Alpha 2.2 (*Parasaccharibacter apium*) [24]. Among members of the typical honey bee gut community, only the Alpha 2.2 group, within Acetobacteraceae, appears to grow commonly in environments outside of bee guts. Closely related strains to Alpha2.2 are found in floral nectar, bee bread, and honey as well as both adult and larval guts [25].

Several other bacteria have been found at low abundance in some honey bee guts. These include a specific cluster from Bacteroidetes [4,15] that has been retrieved from both European and American honey bee workers and from some bumble bees, but that is usually absent or at low abundance (<1%). In addition, several species of Enterobacteraceae that are related to common insect pathogens are commonly present in low numbers (<0.1%), occasionally reaching higher frequencies in individual bees [15,26].

Where are they?

The honey bee adult worker harbors a large bacterial community in the gut, with roughly 1 billion bacterial cells in a mature worker [27,28]. Of these bacteria, ~95% are in the hindgut. The crop (honey stomach or foregut) contains bacteria [29], but studies that quantify cell numbers have shown that crop populations are very small [25, 28]. The crop community is dominated by *Lactobacillus kunkeei*, other environmental *Lactobacillus*, and Acetobacteraceae (Alpha 2.2), taxa also present in the hive and in food, including nectar, dilute honey and bee bread [30]. Thus, the crop does not seem to harbor a specifically crop-adapted microbiota [25]. The midgut also contains relatively few bacteria, with most concentrated at the distal proventriculus region, adjoining the hindgut [28].

The hindgut is divided into two compartments, the anterior ileum, a narrow tube with six longitudinal invaginations, and the rectum, a larger, sac-like compartment; each region contains a characteristic community (Fig. 1). The three main proteobacterial species, *G. apicola*, *F. perrara*, and *S. alvi* dominate in the ileum, forming a dense biofilm beginning at the junction with the Malpighian tubules and continuing along the length of the ileum wall [27,28]. *S. alvi* forms a layer directly on the cuticle lining the gut, and *G. apicola* is towards the lumen. Firm-5 is also present as small clusters in the lumen. *F. perrara* is typically less abundant and occasionally entirely absent, but often dominates ileum communities around day 8 of adult life, corresponding to the nurse stage [27]. The

three dominant Gram-positives (Firm4, Firm5 and Bifido) dominate in the rectum, which contains a large bacterial community [27,28].

For adult worker guts, RNA and DNA samples give similar pictures of community composition, based on 16S rRNA amplicons, indicating that these bacteria are alive and replicating within bee guts [15]. Likewise, metagenomic studies based on random sequencing of non-amplified DNA or RNA from guts indicate a similar taxonomic composition [31,32]. All of these studies point to a characteristic bacterial community that has evolved to specialize upon the honey bee adult gut as its sole ecological niche.

Although these specialized bacteria were first detected by non-culture based sequencing approaches, each of the species has since been successfully cultured in the laboratory [8,16,19,24, 33]. In general, they grow on rich media, consistent with their host-associated lifestyle, and require anaerobic or microaerophilic conditions, that is, consistent with the likely lowered oxygen availability within the gut lumen, although some strains appear to grow at atmospheric oxygen levels [25]. Interestingly the *Bifidobacterium asteroides* present in honey bee guts retains capacity for aerobic respiration, unlike *Bifidobacterium* from mammalian guts, which are entirely anaerobic [20], reflecting the likelihood of higher oxygen concentrations in bee guts compared to mammalian guts. Culturing the bacteria enables full characterization of individual strains and facilitates progress in understanding the roles of the bacteria in bee health.

How are they acquired?

The characteristic gut bacteria in adult guts colonize during the first few days following emergence from the pupal stage [27]. When quantified, numbers of bacteria in larvae are small [28] and consist primarily of environmental *Acetobacteraceae* (Alpha2.2) and *Lactobacillus* [34]. Since these groups are also present in nectar and pollen, their presence in larvae may represent ingested bacteria present in the food, as suggested by phylogenetic analyses of strains from different sources [25]. Immediately following eclosure, adults lack gut bacteria. If pupae are removed from the hive and allowed to eclose within a clean dish in the laboratory, workers will remain nearly germ-free throughout adult life and will lack the typical gut bacterial species [27]. Colonization occurs through contact of newly eclosed adults with nurse bees and with the hive environment. Oral trophallaxis alone is insufficient for normal community establishment, whereas exposure to live nurses or hindgut contents results in typical communities [27]. Thus, at least some gut species, including those dominating in the ileum (*G. apicola*, *S. alvi*, *F. perrara*), are transmitted via a fecal-oral route. Adults that emerge in isolation in the lab can be experimentally colonized with known bacterial strains to determine effects on hosts [27].

The worker gut community reaches its typical composition 3-5 days after eclosure [27] and does not change appreciably when workers shift from within-hive activities to foraging [30]. Thus the adult gut seems to have a stable and distinct community that is not found elsewhere. Other bacteria are present in hive components, and these include a diversity of taxa such as *L. kunkeei* and *Acetobacteraceae* strains, [35,36]. These organisms occur in stored nectar and beebread, where they appear to have a role in preserving but not in fermenting or digesting these stored products [35].

What are they doing?

A metagenomic study of the worker gut community [31] contained an excess of carbohydrate-processing genes, particularly in strains of *G. apicola*. Pectate lyase, which can digest pectin present in cell walls of pollen grains, was present and functional in some strains of *G. apicola*, while absent in others. This indicates strain diversity in ability to use different dietary components, suggesting that the particular set of strains present in individual workers or in colonies might affect nutritional ecology of the bees or might act to neutralize dietary toxins. A study based on sequencing RNA from gut communities verified an excess of carbohydrate-processing genes actively expressed in the bee gut [32]. Sequencing DNA from single bacterial cells from worker guts also indicated that *G. apicola* shows a striking enrichment for carbohydrate-processing genes and that gene sets in this functional category were highly variable among strains [18].

Complete genome sequencing of cultured isolates of *G. apicola* and *S. alvi* shows that these two species have highly complementary metabolic capabilities [17]. Whereas *G. apicola* strains contain a large number of sugar transporters and sugar utilization pathways, *S. alvi* cannot use sugar as a carbon source and instead must use carboxylates produced as downstream products of sugar metabolism. This metabolic complementarity was found in all strains of *G. apicola* and *S. alvi*, suggesting a long coevolution as metabolic partners within the bee gut. In both *G. apicola* and *S. alvi*, individual isolates can vary in the presence of hundreds of genes despite near-identity of 16S rRNA sequences [17]. Interestingly, the *G. apicola* strains from honey bee contain far more genes for use of carbohydrates including diverse sugars than did the bumble bee-associated strains [17,18], consistent with the expanded role of nectar-processing and honey in the ecology and nutrition of honey bees. The *Lactobacillus* and *Bifidobacterium* species also likely play a central function in carbohydrate catabolism and thus in nutrition of their hosts [32].

Another potential role for the gut community is protection against parasites and pathogens. Genomic analyses have revealed many genes that produce toxins, potentially affecting bee parasites [17,37]. The related gut bacteria in bumble bees have been shown to protect against infection by the trypanosomatid parasite *Crithidia bombi* [38], and variation in the gut microbiota underlies most observed variation in resistance to particular strains of *C. bombi* [39]. Community analyses of gut microbiota in three species of wild bumble bees in eastern North America revealed higher *Crithidia* incidence in individuals with low colonization by the core gut bacterial species *S. alvi* and *G. apicola*, consistent with a protective role by one or both of these bacterial species [40]. Honey bees also can be parasitized by *Crithidia* species as well as by other parasites and pathogens. Some other bacterial symbionts of insects are known to lower infection rates by RNA viral pathogens of the hosts [41]. Thus, honey bee gut bacteria plausibly can affect ability to limit proliferation of important bee diseases such as Deformed Wing Virus. Whether the characteristic gut bacteria play a role in resistance to these remains to be tested.

Some strains of bacteria associated with honey bees or bumble bees have antimicrobial properties, suggesting the possibility of inhibition of potential pathogens [21,42-46].

Probably gut communities are largely beneficial to their hosts, but this is rarely shown directly, even for well-studied mammalian systems. For bees, gut bacteria cannot be assumed to be entirely beneficial; possibly they confer a mixture of benefits and costs. For example, some strains of *Escherichia coli* in human guts contain a large locus (~50 kilobases) encoding a hybrid nonribosomal peptide-polyketide synthase pathway for production of a molecule called colibactin, which is implicated as a cause of colorectal cancer and tumors. Curiously, *F. perrara*, which is restricted to the honey bee gut ileum, encodes a closely homologous locus that produces a similar molecule, which has the same cytotoxic effects in cell cultures [37]. The role of *F. perrara* and colibactin in honey bees is unknown, but one possibility is that *F. perrara* may have negative consequences for hosts, despite being found in guts of most workers.

Among genes that vary in presence among strains of *G. apicola* and *S. alvi*, some are likely involved in inter-strain competition. For example, *S. alvi* strains contain Type 6 Secretion Systems, mechanisms for delivering anti-bacterial toxins to other bacteria; the effectors are among the most variable genes sets in *S. alvi* [17,18].

Diversity-Good or bad?

Although it is commonly believed that a diverse microbiota is a good microbiota [47], evidence for this is limited. In bees, species-level diversity is low, while strain diversity is high; however, whether more diversity is good or bad for hosts has not been experimentally addressed. Potentially strain diversity provides more metabolic functions that benefit hosts; for example, *G. apicola* strains vary in ability to use particular sugars or other carbohydrates [17,37]. Some minor species that are erratically present are closely related to insect pathogens, such as *Serratia marcescens* and *Hafnia alvi*; these may represent opportunistic pathogens that can dominate if the typical gut community is compromised. For example, in the first few days following emergence and in workers deprived of normal routes of colonization, gut communities consist of erratic mixes of atypical bacteria, including some potential pathogens [15,27]. Likewise, in bumble bees, individual workers sometimes have few of the typical *S. alvi* and *G. apicola* gut symbionts, and instead have higher representation of possible enteric pathogens [40].

Implications of the bee microbiome for pollinator management and conservation

Very likely, the gut microbiota confers some benefits to honey bees, as genome sequencing suggests roles in digestion and nutrition, and experiments indicate protective effects in bumble bees. Some strains of bee-associated bacteria have antimicrobial properties, suggesting the possibility of inhibition of potential pathogens [21,42-45]. Given this suggestive evidence for a beneficial role of the gut microbiota, interfering with the normal gut community is likely to be detrimental. Long term antibiotic applications to colonies in the United States has resulted in ubiquitous presence of tetracycline resistance loci, present in most of the typical gut bacteria in US honey bees but absent from bees from countries where antibiotics have not been used

[48]. Antibiotics lower diversity in the communities in which only some strains have resistance. Speculatively, the Food and Drug Administration approval of use of a novel antibiotic (tylosin) in 2005 played a part in lowering stress tolerance and in the unprecedented colony mortality that occurred in US honey bees during the winter of 2006-2007 [48]. Thus, it would seem prudent to avoid overuse of antibiotics, as this could have detrimental consequences for colony health, just as chronic use of antibiotics might affect human health by continually perturbing resident gut communities

Another possibility is the use of honey bee gut bacteria as probiotics [17] or as agents for delivering gene products to the bee gut [49,50]. Potentially strains that are particularly useful in bolstering colony health under particular environmental or dietary conditions could be introduced to colonies. However, it is not clear that supplementing the typical bacteria already present will improve colony health. Potentially, the strains present in particular colonies have already been selected to perform well under local conditions.

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Figure Legend

Figure 1. Major components of the honey bee microbiota and their locations in the bee gut or in the hive.

Table 1. Major bacterial species or species clusters associated with honey bees. The first 8 are the dominant clusters in the adult hind gut. See text for citations.

Species or species cluster	Other designations	Bacteria phylum or division	Primary locations	Host species
<i>Gilliamella apicola</i>	Gamma1, older sequences labeled "Pasteurellaceae" or "Serratia" erroneously	Gammaproteobacteria	Adult midgut, hindgut (ileum lumen)	Apis and Bombus species
<i>Frischella perrara</i>	Gamma2	Gammaproteobacteria	Adult hindgut (proventriculus, ileum)	Apis mellifera
<i>Snodgrassella alvi</i>	Beta	Betaproteobacteria	Adult hindgut (ileum wall)	Apis and Bombus species
<i>Lactobacillus mellis</i> , <i>L. mellifer</i>	Firm4	Firmicutes	Adult hindgut (rectum)	Apis and Bombus species
<i>Lactobacillus helsingborgensis</i> , <i>L. melliventris</i> , <i>L. kimbladii</i>	Firm5	Firmicutes	Adult hindgut (ileum, rectum)	Apis and Bombus species
<i>Bifidobacterium asteroides</i> , <i>B. actinocoloniiforme</i> , <i>B. bohemicum</i>	Bifido	Actinomycetes	Adult hindgut (rectum)	Apis and Bombus species
Alpha1	Bartonellaceae (Rhizobiales)	Alphaproteobacteria	Adult gut, variably present	Apis mellifera
<i>Parasaccharibacter apium</i>	Alpha2, Acetobacteraceae	Alphaproteobacteria	Larval gut, adult crop, nectar, honey, hive, some in adult hindgut	Apis and Bombus species
<i>Lactobacillus kunkeei</i>	Fructophilic lactic acid bacteria	Firmicutes	Larval gut, adult crop, nectar, honey, hive, absent from adult hindgut	Apis and Bombus species

Figure

Crop (honey stomach)
Few bacteria, including hive-dwelling bacteria such as *Lactobacillus kunkeei*, *Acetobacteraceae*, such as *Parasaccharibacter apium*

Ileum:
Large dense bacterial community dominated by 3 characteristic species: *Snodgrassella alvi* (on gut wall), *Gilliamella apicola* (layered on top of *S. alvi*), *Frischella perrara* (largely in proventriculus region)
Also some hindgut-adapted *Lactobacillus*

Midgut:
Lacks a stable cuticular lining and has few bacteria, mostly near ileum and resembling ileum in composition.

Rectum:
Large dense bacterial community dominated by 2 hindgut-adapted *Lactobacillus* clusters and *Bifidobacterium asteroides*.

Hive and Larvae:
Beebread, nectar, larval guts share several environmental bacteria, including *Lactobacillus* species and *Acetobacteraceae*