**Insect Gut Microbiome: Untapped Resource for Biotechnological Use**

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**Abstract**

The study of insect-associated microbial communities is a field of great importance in agriculture, principally because of the role insects play as pests. Additionally, a recent emphasis has been placed on the potential of the insect gut microbiome in fields like biotechnology, given that some microorganisms produce molecules with biotechnological and industrial applications, and in fields like biomedicine, given that some bacteria and fungi are a reservoir of antibiotic resistance genes (ARGs).The diversity of insects and their microbial symbionts is still unexplored, and the names and purposes of the enzymes and compounds produced by insect symbionts have not been identified and clarified. Thus, exploring the untapped insect microbiome using multi-omics and synthetic biology technologies is of great interest.

**Introduction**

Insects are one of the oldest group of animals on our planet that are ubiquitous in nature and have been adapted to several environmental conditions represent the most diverse group of animals on earth. The insect gut is estimated to contain 10 times more microbes than total cells of the insect and 100 folds more microbial genes than animal genes.[1] Microorganisms colonize the insect gut through food and plays a significant role in digestion and metabolism. While most of the gut microbes are parasites, some of them are known to play beneficial role for their hosts. Like other eukaryotes, most insects possess specialized gut microbiota participating in host physiology, contributing to health status and also having important nutritional roles.[2] The success of insects is in the association with microbial mutualists, which consequently give rise to the emergence of even more diverse insect traits, such as feeding on recalcitrant plant material, protection against pathogens, and the enhancement of inter- and intraspecific communications. Insights into the insect microbiome represent a particularly promising source for bioactive compounds, natural products for environmentally-friendly pest control, and other industrial biotechnology applications.

 The microbiota of insects, especially the gut microbiota, is as complex and rich as the phylogeny and ecology of insects. The insect intestine is inhabited by microorganisms from all domains, including bacteria, fungi, archaea, protozoa, and viruses, with bacteria being the most abundant.[3] As a result of their co-evolutionary histories with the host, these microorganisms play essential roles in host physiology. Some of the most common bacterial phyla in insects are Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Tenericutes. The gut microbiota complexity and richness throughout the insect phylogeny have awakened an interest in microbial ecology. The analysis of insect-microbiota interactions contribute to a better understanding of insect ecology and to explain their success in nature.

 Recently, both basic and applied research in biotechnology is focused on the identification of novel genes and proteins/ enzymes from various natural sources. Metagenomic approaches allow us to access the genomes of all microorganisms referred as the microbiome. Metagenomics makes it possible to relate potential function of the specific microorganisms within the gut communities. This review describes insect gut metagenomic methodologies, approaches in novel protein/ enzyme discovery and their potential industrial applications.

**Why is the insect gut microbiota important?**

The study of the intestinal microbiota of insects is of great interest, both in medical research and for economic exploitation in the field of agricultural production.[4] Thus, studies into the intestinal microbiota of insects have elucidated new ways to control crop pests, by proving that changes in different conditions, like phylogeny or diet, can modulate insect populations and affect host fitness. The gut microbiota plays a crucial role in developing and maintaining the insect immune system, so studying these communities is essential to better understand the pathogens they carry and transmit or even find ways to prevent them. For example, the gut microbiota of the mosquitoes can prevent them from becoming infected with Plasmodium, the malaria parasite.[5]

 **Insect gut microbiota through marker gene analysis**

Marker gene analysis is based on the sequencing of a gene-specific region to describe the composition of specific taxonomic groups. This approach has been extensively used to study fractions of microbial communities coupled with high-throughput sequencing technologies. Common marker genes are the 16S rRNA gene (archaea and bacteria), the internal transcribed spacer (ITS) (fungi) or the 18S rRNA (eukaryotes).[6] Sequencing of the 16S rRNA gene enables the characterization of the diversity of several types of microbial ecosystems, including environmental and hostassociated microbial communities. Besides, it is the most popular technique to explore associated-microbiota in insects and has been applied to many insect orders such as Blattodea, [7 & 8] Hymenoptera, [9] Lepidoptera [10] or Diptera.[11]

**Metagenomics in insect microbiome**

 Metagenomics is a culture-independent method that allows the identification and characterization of organisms from a myriad of sample types. Through this technique, it is possible to sequence all genomes of a sample and to predict the functional capabilities they encoded. Thus, the diversity and functions of complex microbial ecosystems can be analyzed at once. As the whole DNA is recovered, the full diversity of a sample, including archaea, bacteria, eukaryotes, viruses, and plasmids, can be explored. The combination of metagenomics and high-throughput sequencing technologies has revolutionized the field of microbiology, highlighting the areas of microbial ecology and clinical microbiology.

**Recent insights and potential applications of insect gut microbiome**

* **Cellulose and xylan hydrolysis**

Termites are an extremely successful group of wood degrading organisms and hence they are the potential sources of catalysts for efforts aimed at converting wood into biofuels. Degradation of lignocellulose does not occur by a single enzyme but due to the interaction of many macromolecular complexes that lead to its degradation. These macromolecular complexes have been termed as cellulosomes and are partially known in several microbes. The cellulose degradation of termite was long thought to rely only on microbial gut symbionts.[12]

* **Vitamin production**

The genome of Wigglesworthia sp., the mycetocyte symbiont of *Glossina brevipalpis* has been sequenced and the annotation has revealed the presence of genes encoding for the synthesis of pantothenate (Vitamin B5), biotin (Vitamin B7), thiamin (Vitamin B1), riboflavin FAD (Vitamin B2), pyridoxine (Vitamin B6), nicotinamide (Vitamin B3) and folate (Vitamin B9).[13]

* **Nitrogen fixation and phenolics metabolism**

Insects can absorb the atmospheric nitrogen only through the symbiotic association with gut associated bacteria because the ability to fix nitrogen is widely available among bacteria but apparently absent from all eukaryotes. Nitrogen fixing Enterobacter species have been isolated from the southern pine beetle, which together with some fungal associates, may concentrate nitrogen on developing larvae.

* **Antibiotic resistance**

Studies have reported that gypsy moth midgut microbial community harbors hitherto unknown antibiotic resistant genes. In particular, novel β-lactamases from gypsy moth midgut metagenome were identified. These genes were found to confer resistance in *E. coli*, illustrating that insects might play a role in disseminating important antibiotic resistance genes.[14]

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| **Insect Gut Source** | **Enzyme/Gene** | **Potential applications** |
| *Reticulitermes flavipes* | RfBGluc-1 beta- glucosidase | Lignocellulose digestion |
| *Rotschildia lebaeu* | Xylanase | Xylan degradation |
| *Nasutitermes ephratae* | Glycosyl hydrolase | Lignocellulose digestion |
| Termites*Nasutitermes takasagoensis* | Bacterial glycosidase genes |  Polysaccharide degradation |
| *Reticulitermes flavipes* | Esterase | Hemicellulose solubilisation |
| Gypsy moth*Lymantria dispar* | Quorum-sensing compound | Communication within the microbial communities |
| Termites*Reticulitermes flavipes* | Beta-glucosyl ceramidase | Cellulose |
| Trehalase | Trehalose |
| Alpha-mannosidase | Mannose |
| Endo-beta-N-acetylglusosaminidase | Oligosaccharides |
| *Limnoria quadripunctata* | glycosyl hydrolase genes | Lignocellulose digestion |
| *Coptotermes formosanus* | β-glucosidase | Cellulose degradation |

List of enzymes/genes from the insect gut microbiome by functional screening. (Source: Krishnan, 2014)15.

**Microbiota of domesticated silkworm**

Silkworm *Bombyx mori* (Lepidoptera: Bombycidae) is a domesticated silk moth. It is a typical representative of the lepidopteran insects and has great importance in agriculture and the economy. The silkworm as a promising model organism in life science due to its short generation time, rich genetic resources, clearly sequenced genetic background and a considerable number of genes that are homologous to silkworm that makes it suitable for various life science studies. Although the silkworm *B. mori* exhibits rich diversity of microbial flora, very few of them have been identified and poorly characterized (Pandiarajan & Krishnan, 2018)16. Khyade and Marathe (2012)17 indicated the presence of cellulolytic bacteria in the midguts of *B. mori* that helps in food digestion, absorption of nutrients and growth. Several studies have also shown that the gut microflora of *B. mori* aids in fighting infectious diseases in the silkworm host. Studies have showed the presence of *Bacillus* species in the guts of silkworm that can be used as probiotics for silkworm disease management. The research on microflora of silkworm gut is biased towards the gut bacteriome and very little is known about the fungal gut communities. Chen *et al*. (2018)18 reported the presence of fungi belonging to the phyla Ascomycota and Basidiomycota along with bacteria belonging to the phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes. Furthermore, it has been stated that “though the large amount of information on the biology and physiology of the silkworm B. mori is known, very few studies have been carried out on silkworm gut microbiota,” indicating that the research on gut microflora of silkworm *B. mori* is still limited.

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