



# Gut metagenome in Insects and Its Potential in Agricultural and Industrial Applications

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## Authors' contributions

This work was carried out in collaboration among all authors. Author KSM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KS, AK, MA and SKS managed the analyses of the study. Author TA managed the literature searches. All authors read and approved the final manuscript.

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## ABSTRACT

Metagenomics has greatly improved our understanding of microbial ecology by revealing the metagenomes of uncultured bacteria, including those associated with insects. These bacteria play important roles in insect defence, reproduction, and food absorption, which influences pest

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management tactics. Recent next generation sequencing advances in low-cost nucleotide and user-friendly bioinformatics have improved our ability to investigate microbial diversity in economically significant pests. Meta-omics technologies are now essential for defining microbial ecosystems, bio-surveillance, food safety, and commercial advances. Genome studies on insects and plants provide insights into pest control by discovering resistance genotypes and understanding stress-induced genomic changes. This review is focused on how herbivore specialists, such as the diamondback *Plutella xylostella*, adapt to host plants using gut microbiota. Metagenomic sequencing has discovered important bacteria-*Enterobacter cloacae*, *Enterobacter asburiae*, and *Carnobacterium maltaromaticum*- that help detoxify plant defences, breakdown cell walls, and synthesise amino acids. These findings indicate novel pest management tactics that target gut microbiota interactions. The paper continues with an overview of metagenomic DNA extraction, library creation and screening methods, especially on their applications in biotechnology and bioprocessing.

**Keywords:** Metagenomics; insect pests; genome expression; diamond back moth; herbivores.

## 1. INTRODUCTION

Metagenomics is a sophisticated tool for investigating microbial communities in any environment that involves the metagenome of all microorganisms found in a given environment without the requirement for culturing. This method entails extracting DNA from particular environmental samples, cloning it and then sequencing the resulting library to discover microbial diversity and functions [27,42]. Insects are particularly interesting topics for metagenomic research because of their immense microbial diversity, which includes up to 100 times more microbial genes than their own and ten times more bacteria than cells. The subject of insect genomics began in 2000 with the sequencing of *Drosophila melanogaster*, and over 30 more insect genomes have subsequently demonstrated their wide diversity and complex biology [2,8]. Recent study on the functional profile of Insect's gut microbiome has increasingly focused on insect gut microbiomes, spurred by their important functions in digestion, metabolism, and pest management, including antibiotic resistance genes [52]. Increased crop output has increased insect infestations, necessitating extensive pesticide use, which destroys soil and disturbs natural pest foes. In response, DNA-based technologies, like as meta-omics, provide alternative pest management options. Meta-omics, which includes metagenomics, meta-transcriptomics, meta-proteomics, and meta-bolomics, offers a comprehensive understanding of molecular interactions and functions in microbial communities [47]. This holistic approach has resulted in the development of 'IPM-omics' in Integrated Pest Management, which combines meta-omics and GIS data to improve pest control

strategies [63]. Microorganisms play crucial roles in physical, chemical, and biological processes, influencing host interactions, genetic diversity, and ecological balance in soils [58]. The study of microbial ecology has expanded from human microbiology to include marine, food, and insect sciences [19, 33]. Insect-associated microbial communities are essential for various functions such as nutrient absorption and protection from predators [22]. Meta-omics techniques have advanced our understanding of these microbial communities, revealing insights into gut microbiome profile of insect like *Pentalonia nigronervosa* [57], *Lutzomyia* spp. [26,1] and *Apis mellifera* [55, 56,71,72]. This review highlights how metagenomics and related technologies enhance our knowledge of insect gut microbiomes and their applications in pest management and biotechnology [82,77].

## 2. META-OMICS IN PEST MANAGEMENT

Genomics is revolutionizing pest management by identifying critical insect survival genes, which can be targeted using transgenic and RNAi techniques. It also aids in developing new pesticides and discovering host-plant resistance factors. Additionally, population genomics reveals intra-species variations and origin of pest populations, enabling targeted control measures to prevent their spread. Meta-genomics, first introduced by [26] identifies the complete set of genomes in a sample, revealing the diverse microbial community. Metabarcoding, a refined meta-genomic method, uses short DNA fragments (barcodes) like 16S or COI to reconstruct the taxonomy of these communities and assess the presence of specific genes. Meta-transcriptomics extends this by analysing expressed genes, offering insight into which

biological processes are active. However, changes in gene expression might not always correspond to visible traits, which is where meta-proteomics and metabolomics come in. These techniques help determine the actual functional activities of microbial communities by analysing proteins and metabolites, respectively [76, 31]. agriculture, pests have increased due to intensified farming practices, leading to widespread use of synthetic pesticides despite their environmental and health impacts. Microorganisms play a crucial role in pest biology and their interaction with plants. For instance, certain microbes can improve pest resistance to stress or aid in overcoming plant defences [49, 73]. Examples include the psyllid *Bactericera cockerelli*, which uses symbionts to modulate plant defences [12] and beetles that exploit fungi for dietary needs and plant defence evasion [58,59].

Meta-omics has significantly advanced pest management research. For example, sequencing the metagenome of the diamondback moth *Plutella xylostella* revealed that bacterial taxa help detoxify plant defense compounds. Similarly, metagenomics was used to explore the endosymbiont diversity in *Diaphorina citri*, a vector of citrus greening disease [66]. Meta-transcriptomics has linked colony collapse disorder in honeybees to Israeli acute paralysis virus [16] and similar techniques have identified pathogens in the yellow crazy ant *Anoplolepis gracilipes* [15]. Additionally, meta-omics is shedding light on viral diversity in ecosystems, which could enhance biocontrol strategies [17, 54].

### 3. GUT MICROBES

Gut microbes are crucial for insect nutrition, often playing a symbiotic role by enhancing nutrient availability and regulating their allocation [20]. Insects have evolved to leverage microorganisms to adapt to nutrient-limited environments, either by consuming them directly or relying on them for pre-digesting complex diets [21]. These microbes can be transferred vertically between generations, horizontally among individuals, or acquired from the environment [21, 38]. In some cases, disrupting gut microbial communities can significantly impair insect performance [32]. Gut microbes help detoxify plant secondary metabolites, aiding insects in digesting their diets [9]. For example, the gut microbiota of *Hyles euphorbiae* and *Brithys crini* is dominated by *Enterococcus*, which likely assists these insects in feeding on

toxic plants [78,79]. Recent studies have shown that host plant species significantly impact the gut microbiota of polyphagous insects. This has been observed in *Acyrtosiphon pisum* [23], *Phylloxera notabilis* [49], *Melitaea cinxia* [65], *Thaumetopoea pityocampa* [74], and *Ceratitis capitata* [47]. Factors such as diet, life stage, and environment are major drivers of gut microbial community composition [47, 14]. Additionally, insects reared on artificial diets exhibit different gut microbiota compared to those from natural environments [10].

## 4. METAGENOMIC ANALYSIS OF INSECT PESTS

### 4.1 Diamondback Moth (*Plutella xylostella*)

Diamondback moths are infamous pests of cruciferous plants such as cabbage, broccoli, and cauliflower. Metagenomic analyses of its microbiota show a predominance of Proteobacteria, particularly *Enterobacteriales*, and *Firmicutes*, most notably *Lactobacillales*. Resistant strains to chlorpyrifos and fipronil exhibit higher *Lactobacillales* and other uncommon taxa such as *Pseudomonadales*, implying that these bacteria may contribute to pesticide resistance [82].

### 4.2 Whitefly (*Bemisia tabaci*)

Whiteflies are polyphagous pests that harm more than 900 plant species and serve as carriers for a variety of viral illnesses. 16S rDNA sequencing revealed 57 bacterial species spanning 10 phyla in wild *B. tabaci* from cotton plants in southern Punjab, Pakistan, with Proteobacteria, Bacteroidetes, Firmicutes, and Actinobacteria being the most common. PacBio sequencing of full-length 16S rRNA genes has detected endosymbionts such as *Halomonas*, as well as fresh Rickettsia and Arsenophonus strains across distinct whitefly populations [70, 34, 78].

### 4.3 Termites

Termites pose a hazard to agriculture and wooden infrastructure and their gut microbiome is essential for lignocellulose digestion. Firmicutes and Spirochaetes are common in wood-feeding termites such as *Amitermes wheeleri*, but *Nasutitermes corniger* contains Spirochaetes and Fibrobacteres. Functional investigations show that *A. wheeleri* microbiota helps with hemicellulose breakdown and nitrogen fixation, whereas *N. corniger* microbiota focusses

on cellulose degradation and nitrogen fixation [29, 24].

#### 4.4 Fungal Microbiota

Mutualistic relationships between insects and fungi are complex and employ a variety of methods across taxa. Bark beetles, ambrosia beetles, fungus-farming ants, termites, wood wasps, and gall midges are among examples [37, 35]. Bark and ambrosia beetles, in particular, have strong symbiotic associations with fungus. Beetles from international harbours have been linked to both plant diseases and previously unknown fungus, which can spread internationally via wood-product shipping [45].

However, certain fungi have hostile associations with insects. Entomopathogenic fungi, such as *Beauveria* and *Metarhizium*, are utilised as pest biocontrol agents [69, 64]. These fungi can also form unusual multitrophic relationships. *Metarhizium robertsii*, for example, can transport nitrogen from *Galleria mellonella* infected larvae to plants [7], a behaviour similar to that seen with the ectomycorrhizal fungus *Laccaria bicolor* in white pine [40]. Furthermore, gene transfer between fungi and insects, such as aphids, has been demonstrated [51], and plant-fungus interactions can ameliorate the negative consequences of herbivores.

Despite these findings, research into insect-associated fungal microbiomes is scarce. Collembola [5], Lepidoptera [28], Coleoptera [50, 36] and Diptera [13] are the primary focus of current study. For example, DNA metabarcoding of *Bactrocera oleae*, a prominent pest of olive orchards, revealed correlations with *Colletotrichum* species known to cause olive anthracnose [46].

### 5. RECENT INSIGHTS AND POTENTIAL APPLICATIONS OF INSECT GUT MICROBIOME

#### 5.1 Cellulose and Xylan Hydrolysis

Termites, which are effective wood degraders, have the potential to produce biofuel due to their various cellulose and xylan hydrolysing enzymes. [80] discovered several bacterial genes for these processes in the hindgut of wood-feeding *Nasutitermes* species, showing many unidentified protein families involved in lignocellulose breakdown. Traditionally, cellulose degradation was attributed to microbial gut symbionts, however recent research has

revealed termite-derived cellulase gene transcripts. Additionally, xylanase genes have been found in both lepidopteran and termite intestinal samples. The combined activity of microbial and termite enzymes promotes lignocellulose digestion, which is essential for turning wood into biofuels and lowering greenhouse gas emissions [80, 11, 53] [81].

#### 5.2 Vitamin Production

The genome of *Wigglesworthia* sp., a symbiont of *Glossina brevipalpis*, has been sequenced, revealing genes for synthesizing several B vitamins: pantothenate (B5), biotin (B7), thiamin (B1), riboflavin (B2), pyridoxine (B6), nicotinamide (B3), and folate (B9) [3].

#### 5.3 Nitrogen Fixation, Phenolics Metabolism and Antibiotic Resistance

Insects use symbiotic gut bacteria to fix atmospheric nitrogen, a mechanism absent in eukaryotes but widespread in bacteria. Nitrogen-fixing *Enterobacter* species have been identified from the southern pine beetle, and they, together with some fungi, may improve nitrogen availability for growing larvae [39]. Furthermore, *Rahnella aquatilis*, *Klebsiella* species, and *Pantoea* species are common in these beetles, and *Dendroctonus frontalis* larvae can fix nitrogen in a variety of environments [6]. Bacteria in the beetles gut also play a role in detoxifying conifer defensive compounds, primarily monoterpenes, diterpenes, and phenolics [60, 4] revealed new antibiotic-resistant genes, including  $\beta$ -lactamases, in the gypsy moth's midgut microbial population. These genes, which provide resistance to *E. coli*, indicate that insects may contribute to the spread of important antibiotic resistance genes. Furthermore, insect-associated microorganisms generate a variety of metabolites having biological functions [30, 25] discovered a new metagenomic clone that produces distinct quorum-sensing inducers by analysing the gypsy moth midgut microbiome. This clone carries a gene for a monooxygenase homologue, which opens up a pathway for indole oxidation and the production of a novel quorum sensor chemical.

### 6. APPLICATION DOMAINS

Meta-omics pipelines are becoming more accessible, broadening their applications from insect microbial ecology to industry and

biocontrol. [80] employed metagenomics to discover genes involved in cellulose and xylan hydrolysis in the termite *Nasutitermes ephratae*, while study on protistan communities in *Coptotermes formosanus* and *Reticulitermes flaviceps* underlined their importance in strengthening lignocellulolytic systems [43, 44, 82]. Studies have looked into leveraging wood-feeding beetle microbiome to uncover new enzymes [68, 48, 75] discovered that leaf-cutter ant microbiota enhances cellulose degradation, while [41] examined insect stomach microbiomes for industrial uses. Meta-omics also improves bio-surveillance by analysing complete genetic pools collected from traps to detect invasive species and plant pathogens [62, 61]. Furthermore, modifying insect-microbe connections may improve pest management efforts [18, 67]. DNA metabarcoding aids in the identification of honey sources, reducing fraud through advances in extraction and machine learning [58].

## 7. CONCLUSION

Metagenomics has significantly improved our understanding of microbial communities linked with insects, providing new insights into manipulation of it in agricultural applications. especially pest control and biotechnological manufacturing processes. The thorough examination of insect gut microbiomes revealed crucial roles for microbial diversity in nutrition absorption, detoxification of plant defences, and pesticide resistance. These findings have resulted in novel pest control tactics that target gut microbiota interactions and show the potential of using insect-associated microorganisms for sustainable agricultural practices. Furthermore, the integration of meta-omics with biotechnology and bioprocessing is creating new opportunities for industrial applications such as biofuel production and bio-surveillance. As technology advances, the complete approach given by meta-omics will be crucial in tackling agricultural difficulties, improving pest control, and extending our understanding of microbial ecosystems in both natural and agricultural systems.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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