International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
NAAS Rating (2025): 5.29
IJABR 2025; 9(10): 631-638
www.biochemjournal.com
Received: 17-07-2025
Accepted: 19-08-2025

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Endosymbiont-based strategies in insect pest control: Innovations and applications

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DOI: https://www.doi.org/10.33545/26174693.2025.v9.i10i.6137

Abstract
Insects maintain close relationships with a diverse range of microorganisms, including bacteria, fungi, viruses, and protozoa. These microbes, which can live on the insect's surface or inside their bodies, often play essential roles in insect development, survival, and adaptability. While traditional microbial pest control has focused on a limited number of insect-pathogenic microbes, recent advancements in sequencing technologies, functional genomics, and gene editing have broadened our understanding of insect-microbe interactions. This has opened new possibilities for pest management by targeting the microbes that influence insect biology and behavior. Endosymbionts, in particular, contribute significantly to insect fitness by enhancing stress tolerance, offering protection against diseases, improving plant interactions, and even conferring pesticide resistance. Some also manipulate insect reproduction through mechanisms like male killing, feminization, and cytoplasmic incompatibility traits that can be leveraged for novel control methods. Emerging strategies, such as paratransgenesis, microbe-based attractants, and RNAi-driven gene targeting, show promise when integrated with existing pest control techniques. These approaches offer a more targeted, sustainable, and adaptable solution for managing pest populations in a rapidly changing environment. This review provides an overview of recent advances in insect-microbe interactions and highlights their potential applications in modern pest control strategies.

Keywords: Endosymbionts, insect pest control, microbial interactions, paratransgenesis

1. Introduction
Insects live in close association with a wide variety of microorganisms including bacteria, fungi, viruses, and protozoa which can reside on their surfaces (ectosymbionts) or within their bodies (endosymbionts). These microbial partners often play vital roles in insect survival, development, and adaptation. Traditionally, pest control strategies involving microbes have focused on a few entomopathogens that act as bioinsecticides. However, recent advances in high-throughput sequencing, functional omics, and gene editing have dramatically expanded our ability to discover and understand the complex roles of microbial communities in insect biology. By manipulating these microbial associations, it's now possible to influence insect behavior and biology in ways that reduce their impact as pests. For example, certain microbes can alter an insect's ability to spread diseases or reduce its reproductive success. Endosymbionts provide a range of benefits to their insect hosts. These include helping insects withstand environmental stress, defending them against pathogens, improving their interactions with host plants, and even offering resistance to pesticides. Additionally, endosymbionts play a role in shaping insect populations and aiding their adaptation to new environments (Qadri *et al.*, 2020) [22].

Some endosymbionts can also influence insect reproduction in fascinating ways. For example, they may cause the death of male offspring (known as male killing), transform genetic males into functioning females (feminization), or create reproductive mismatches through a process called cytoplasmic incompatibility. These natural reproductive disruptions have the potential to be harnessed in innovative pest control strategies.

New approaches, such as using endosymbionts as attractants, employing paratransgenesis (genetically modifying symbiotic microbes), or slowing the buildup of pesticide resistance, show promise for future pest management. These microbe-based strategies can also be used alongside other methods—like biological control, chemical pesticides, incompatible insect techniques, or sterile insect releases—to improve pest control outcomes, especially in genetically diverse pest populations and under changing environmental conditions (Arora and Douglas, 2017) [1] Meeting the challenges of using genetically modified endosymbionts in insect pest control by increasing the specificity of the active agents by RNAi can be designed to target sequences at any level of phylogenetic conservation, including total specificity to the pest species.

2. Classification of Insect Endosymbionts

Endosymbionts in insects are generally classified into two main types: primary (obligate) and secondary (facultative), depending on their role and how closely they're linked to the host. Primary endosymbionts are vital for the insect's survival and development. These microbes are usually found inside specialized cells called bacteriocytes, which are sometimes grouped into an organ known as the bacteriome. Over millions of years, these symbionts have co-evolved with their insect hosts and are consistently found in every individual of the host species. One of their key roles is to produce nutrients like certain amino acids and vitamins—that the insect cannot get in sufficient amounts from its food. A good example is *Portiera aleyrodidarum*, the primary endosymbiont of the whitefly *Bemisia tabaci*, which helps make up for nutritional gaps in the insect's phloem-based diet (Hu and Tsai, 2020) [17]. Primary endosymbionts have co-evolved with their insect hosts over long periods, leading to significant genome reduction. As a result, they cannot survive outside their hosts and are considered obligate, intracellular partners. In contrast, secondary endosymbionts are not essential for the host's basic survival or development under normal conditions, but they often provide important ecological and evolutionary benefits.

Unlike primary symbionts which are typically confined to specialized cells or organs secondary symbionts are more flexible in their location within the host. They can be found in a variety of tissues, including the gut, reproductive organs, or circulating in the hemolymph. Their distribution is inconsistent, meaning they may be present in some individuals or populations but absent in others.

These facultative symbionts contribute to several advantageous traits, such as protection against pathogens and parasitoids, improved resilience to environmental stress (like heat or drought), and the ability to alter host reproduction. Well-studied examples include *Wolbachia*, *Cardinium*, and *Rickettsia*, which can influence reproductive processes through mechanisms like cytoplasmic incompatibility, male-killing, or feminization (Harris *et al.*, 2010) [12]. Such traits can be applied in pest and disease vector control strategies aimed at reducing insect populations.

Moreover, some secondary symbionts have been shown to boost host fertility, assist with dispersal, or even contribute to insecticide resistance by helping break down toxic compounds. Understanding the differences between primary and secondary endosymbionts is essential for creating precise, symbiont-based pest management methods.

While primary symbionts may serve as stable, conserved targets for disrupting essential physiological functions, secondary symbionts offer more flexibility and diversity for engineering or manipulation to achieve desired pest control outcomes (Rupawate *et al.*, 2023) [25] The exploitation of these microbial partners particularly through genetic modification, paratransgenesis and symbiont-mediated RNA interference presents an emerging frontier in sustainable and species-specific pest management strategies, which will be explored in detail in this review.

2.1 Guest Endosymbionts

Guest endosymbionts are microbial partners that live temporarily or facultatively within the insect host and are not necessarily passed on through generations like obligate symbionts. These symbionts can exert profound effects on the reproductive systems of their hosts, particularly by manipulating the sex ratio—the proportion of males to females within a population. One of the most well-studied and widespread examples of such a symbiont is *Wolbachia*, an intracellular bacterium that can manipulate host reproduction through several mechanisms, including male killing, feminization, parthenogenesis, and cytoplasmic incompatibility (Hochstrasser, 2023) [14]. These reproductive manipulations not only enhance the transmission of *Wolbachia* but also influence the ecology and evolution of insect populations.

2.1.1 Male Killing

Male killing is a type of reproductive manipulation where maternally inherited bacterial symbionts cause the death of male embryos in their arthropod hosts. Since these bacteria are passed from mother to offspring, eliminating males who do not transmit the symbiont can be advantageous. By reducing the number of males, more resources are available for the surviving infected females, enhancing their fitness and reproductive output (Stevens *et al.*, 2001) [26]. *Wolbachia* is one of the most well-known bacteria responsible for male killing, and it has been found across a wide range of insect groups, including beetles (Coleoptera), flies (Diptera), butterflies and moths (Lepidoptera) and even pseudoscorpions. Specific strains of *Wolbachia* have been shown to induce male killing during embryonic development in several species, such as the two-spot ladybird beetle (*Adalia bipunctata*), the butterfly *Acraea encedon*, moths like *Ephestia kuehniella* and *Cadra cautella*, and the fruit fly *Drosophila bifasciata*. This ability to skew sex ratios can significantly impact population structures and presents interesting possibilities for developing new pest management strategies

2.1.2 Feminization

Feminization is a reproductive strategy used by Wolbachia, where it turns genetic males into functioning females. This happens because the bacteria disrupt the formation of male-specific organs, mainly by inhibiting the androgenic gland, which plays a key role in male development. This effect was first observed in isopods like *Armadillidium vulgare* (commonly known as pill bugs or woodlice). In this species, females are heterogametic (ZW) and males are homogametic (ZZ) (Werren, 1998) [32]. When infected with a feminizing *Wolbachia* strain, the host may lose the W chromosome, destabilizing the typical sex-determining mechanism and leading to the development of phenotypic females from ZZ individuals. Feminization has also been observed in various insects, including the leafhopper *Zyginidia pullula*, the butterfly *Eurema hecabe* (Hiroki *et al.*, 2002) [13] and the Asian corn borer *Ostrinia furnacalis*. By increasing the number of female hosts who transmit Wolbachia to their offspring this strategy helps the bacterium spread more effectively through populations.

2.1.3 Thelytokous Parthenogenesis
Thelytokous parthenogenesis is a form of reproduction where females produce only female offspring without needing to mate. In many haplodiploid species like certain wasps, thrips, and mites. Males usually develop from unfertilized (haploid) eggs, while females come from fertilized (diploid) ones. However, Wolbachia can alter this system by triggering a process called gamete duplication during early cell division. In this process, the chromosomes in an unfertilized egg condense as usual during prophase but don't separate during anaphase, causing the cell to become diploid instead of haploid. As a result, what would have developed into a male instead becomes a female, allowing females to be produced asexually (Wang *et al.*, 2020) [31]. This has been observed in insects like *Trichogramma*, *Aphytis*, *Encarsia* and *Musidifurax*. Since males are no longer needed, populations can grow rapidly.

Advantages of Thelytokous *Trichogramma* as Biocontrol
Trichogramma wasps infected with Wolbachia, which allows them to reproduce without mating, offer clear benefits for biological pest control. One major advantage is the ease of mass rearing since males are not needed, managing colonies becomes simpler and more cost-effective. All offspring are female, which is ideal because only females parasitize pest eggs, so resources aren't wasted on non-parasitic males. This leads to faster population growth and greater effectiveness in the field. In addition, females don't have to spend time or energy on finding mates. Research has also found that *Wolbachia* infected *Trichogramma brassicae* females lay more eggs, boosting their value as pest control agents even further.

2.1.4 Cytoplasmic Incompatibility (CI)
Cytoplasmic incompatibility (CI) is the most common reproductive effect caused by Wolbachia and is found in many arthropods, including insects, arachnids, and isopods. It works as a kind of reproductive barrier that prevents successful offspring from certain male-female pairings. Most often, this happens when a male infected with Wolbachia mates with a female who is either uninfected or carries a different strain of the bacteria. As a result, the embryo doesn't develop properly, leading to lower fertility. This is believed to happen because the chromosomes from the sperm don't behave normally after fertilization, causing the embryo to stop developing.

Mechanism of CI
The cellular basis of cytoplasmic incompatibility (CI) involves a mismatch in how the male and female genetic material behaves right after fertilization. During the first cell division, the chromosomes from the female side organize and line up correctly, but those from the male side fail to fully condense. When the cell tries to divide, the female chromosomes separate as they should, while the male chromosomes either lag behind or are left out completely. This causes the loss of the father's genetic contribution, leading to an embryo that's either missing half its DNA or dies altogether. In species with two sets of chromosomes (diploids), this usually means the embryo won't survive. But in species where males naturally develop from unfertilized eggs (haplodiploids), the embryo can still live and become a male, which can shift the balance of males and females in a population.

Mechanism of Rescue
Embryo death caused by cytoplasmic incompatibility (CI) can be avoided if both mating partners are infected with the same strain of Wolbachia. In these cases, the infection in the female can "rescue" the problems caused by the male's sperm, allowing the chromosomes to behave normally and the embryo to develop properly. This rescue effect is specific to the Wolbachia strain, which is why successful reproduction often depends on both partners carrying the same type of infection (Zhang *et al.*, 2016) [37]. This matching not only helps Wolbachia continue spreading in a population but also has practical uses in pest control. For example, it forms the basis of the incompatible insect technique (IIT), which is used to reduce populations of harmful insects or disease-carrying vectors.

3. Metagenomics study of various insect pests
16s rRNA based metagenomics studies reveal that insect gut is resided by number of bacterial genera. Proteobacteria, Firmicutes and Actinobacteria were the most dominant phyla present in the insect gut could be due to their diverse roles in nutrition. In our studied examples it is found that overall, the most abundant bacterial taxa at the phylum level were Proteobacteria except in termites where Spirochaetes are abundant. Phylum Firmicutes secured second position in bacterial composition in insect. Actinobacteria showed less symbiotic association in insect gut (Yong *et al.*, 2017) [34].

4. Endosymbionts of pest insects
4.1 Bacteria as Endosymbionts
While bacteria are the most commonly studied endosymbionts in insect pest systems, recent research highlights that a wide range of microbial taxa—including fungi, viruses, protozoa, and archaea—also play vital roles in influencing insect physiology, development, reproduction, and ecological interactions. For example, *Buchnera aphidicola* is a well-studied primary symbiont in aphids that supplies essential amino acids missing from their phloem-based diet (Gurung *et al.*, 2019) [11]. Similarly, *Baumannia cicadellinicola*, found in the glassy-winged sharpshooter, and Candidatus Erwinia dacicola, in the olive fruit fly, help their insect hosts by producing essential amino acids and vitamins. In bark beetles, certain Enterobacter species contribute to nitrogen fixation, which supports insect growth. In Drosophila flies, Spiroplasma bacteria provide a form of defense by protecting against parasitic nematodes and parasitoid wasps. Beyond these examples, many insect pests harbor a wide range of bacteria, including groups like Gammaproteobacteria, Alphaproteobacteria, Betaproteobacteria, Actinobacteria, Firmicutes (such as *Lactobacillus* and *Bacillus*), Spirochetes, Clostridia, and others. This highlights the remarkable microbial diversity present within insect hosts and the important roles these microbes play in insect biology.

4.2 Fungi as Endosymbionts
Fungal endosymbionts play a vital role in the biology of many insects. For example, bark beetles rely on *Ophiostoma* fungi to help overcome plant defenses, while various yeasts assist these beetles by aiding in the absorption of essential vitamins. In the brown planthopper (*Nilaparvata lugens*), yeast-like fungi help recycle nitrogen-rich waste like uric acid, making nutrient use more efficient. Similarly, in *Drosophila melanogaster*, yeast symbionts contribute to

sterol metabolism, which is essential for maintaining cell membranes and supporting development. In several planthopper species—including *N. lugens*, *Laodelphax striatellus*, and *Sogatella furcifera*—these fungal partners are passed directly from the fat body of adult insects to their eggs, ensuring that each new generation inherits the symbionts. Vega and Dowd (2005) [30] showed that reducing yeast populations through heat treatment leads to serious consequences for these insects. Symptoms such as slower growth, problems with molting, fewer viable eggs, disrupted protein production, and an overall drop in fitness clearly demonstrate how crucial these fungal symbionts are for insect health and survival.

4.3 Virus as Endosymbionts

Viruses, especially those belonging to the Polydnaviridae family, have developed unique symbiotic relationships with certain parasitoid wasps, including species in the Ichneumonidae and Braconidae families. These viruses are located primarily in the reproductive tissues of the wasps, particularly within the ovaries, and are introduced into host insects during egg-laying. Once inside the host, the viruses play a crucial role by weakening the host's immune defenses, which allows the wasp larvae to develop successfully. This intricate three-way relationship—between the parasitoid wasp, the virus, and the host insect—illustrates how viral endosymbionts can contribute indirectly to controlling pest populations (Drezen *et al.*, 2014) [8].

Aphids offer an intriguing example of virus-bacteria-insect symbiosis. The protective capacity of *Hamiltonella defensa* in aphids is enhanced by bacteriophages such as *Acyrthosiphon pisum* secondary endosymbiont (APSE) phages. These phages carry toxin genes that help the host defend against parasitoid wasps. Aphids infected with *H. defensa* harboring APSE exhibit higher resistance to parasitoid attacks compared to those lacking the phage, demonstrating a unique case where the endosymbiont's defensive function depends on viral elements (Ayoubi *et al.*, 2025) [2].

4.4 Protozoan as Endosymbionts

Protozoan endosymbionts also play important roles, especially in wood-feeding insects like termites. These protozoa assist in digesting complex plant materials by producing hydrolytic enzymes, enabling termites to thrive on nutritionally poor substrates such as decaying wood. Additionally, archaea—especially methanogenic and non-methanogenic members of the phylum *Euryarchaeota*—have been identified in the guts of beetles, cockroaches, termites, and millipedes (Protasov *et al.*, 2023) [21]. These archaea contribute to metabolic processes like hydrogen removal and methane production, which can influence gut microbial dynamics and host digestion.

Together, these diverse endosymbionts—including bacteria, fungi, viruses, protozoa, and archaea form complex, often species-specific associations with insect pests, significantly influencing their survival, adaptability, and ecological fitness. Recognizing and understanding this microbial diversity is crucial for the development of novel and targeted pest management strategies that exploit the functional roles of non-bacterial symbionts in conjunction with bacterial ones.

5. Transmission of Endosymbionts

For symbiotic relationships between insects and their endosymbionts to continue across generations, reliable transmission methods are essential. These microorganisms are passed from one generation to the next through several strategies, including transmission directly through the ovaries (transovarial), through specialized capsules, by smearing eggs with symbionts, or by acquiring them from the environment after hatching. Each of these methods showcases the variety of evolutionary solutions that have allowed endosymbionts to remain closely linked with their insect hosts over time.

5.1 Transovarial Transmission

Vertical, or transovarial, transmission is the most widespread and evolutionarily stable way that insects pass endosymbionts to their offspring. In this process, the symbionts are inherited directly through the mother's ovaries, ensuring that each new generation carries them. This method is especially common among primary endosymbionts microbes that are vital for the insect's survival and development. Well-known examples include *Buchnera aphidicola* and *Serratia symbiotica* in aphids, along with *Wolbachia*, *Rickettsia* and *Spiroplasma*, which are found in a range of insect species (Romanov *et al.*, 2020) [24]. These symbionts are often localized within the reproductive tissues and are packaged into the developing oocytes during oogenesis, guaranteeing their stable inheritance.

5.2 Capsule Transmission

Capsule transmission represents a more specialized form of symbiont transfer observed in certain Hemipteran insects such as stinkbugs. A well-documented case is that of *Megacopta punctatissima*, which produces small, brownish capsules during oviposition. These "symbiont capsules" contain high densities of the beneficial bacterium *Ishikawaella capsulate* (Fukatsu and Hosokawa, 2002) [10]. After hatching, the nymphs actively probe and ingest the contents of these capsules, thereby acquiring their essential gut symbionts. This mechanism ensures that symbionts are transmitted externally but remain closely associated with the egg mass, allowing for effective host-symbiont continuity.

5.3 Egg Smearing

Egg smearing is an external form of symbiont transmission, where the parent insect coats the egg surface with beneficial bacteria during egg-laying. This method has been observed in insects such as those in the Acanthosomatidae family and some Lepidopteran species. After hatching, the larvae pick up the symbionts either by ingesting them or through direct contact with the egg surface. While not as direct as transovarial transmission, egg smearing remains an effective way to pass on symbionts, particularly those that are not essential but still offer important benefits to the host (Hosokawa and Fukatsu, 2020) [16].

5.4 Environmental Acquisition

In contrast to vertical transmission methods, some insect hosts acquire their symbionts afresh from the environment with each generation, a process known as environmental acquisition or horizontal transmission. This strategy is prominent in insect families such as Alydidae and Coreidae. For example, the bean bug *Riptortus pedestris* acquires its beneficial *Burkholderia* symbionts from the surrounding soil during the early nymphal stages. The bacteria colonize specific gut regions, such as the midgut crypts, and contribute to host fitness by enhancing growth and resistance to environmental stressors (Takeshita *et al.*, 2018) [27]. Environmental acquisition allows insects to select symbionts from a diverse microbial pool, potentially enhancing adaptability under varying ecological conditions.

6. Diverse Roles of Endosymbionts in Insect Hosts

6.1 Role of Endosymbionts in Pesticide Detoxification

Endosymbionts play a crucial role in breaking down pesticides, offering valuable insights into the genetic mechanisms that underlie symbiont-mediated detoxification. Environmental microbiota act as reservoirs of microorganisms capable of degrading harmful chemicals, shaping the ecological and evolutionary dynamics of insect-microbe partnerships. These associations have significant implications for pesticide formulation and pest management strategies, especially in the context of using endosymbionts as biocontrol tools (Xia *et al.*, 2018) [33].

Resistance to Buprofezin and Imidacloprid in *Nilaparvata lugens* (Brown Planthopper)

Buprofezin, a commonly used chitin synthesis inhibitor, is facing resistance issues due to overapplication. The brown planthopper, *Nilaparvata lugens*, has naturally acquired symbiotic bacteria such as *Serratia marcescens* (referred to as Bup_Serratia) from the environment, particularly soil and water, which confer resistance to the insecticide (Zeng *et al.*, 2023) [35].When Bup_Serratia was introduced into susceptible individuals, resistance was observed. Conversely, treating insects with antibiotics made them vulnerable again, indicating a mutualistic role for this bacterium. Genome analysis revealed genes likely responsible for buprofezin degradation, which were significantly upregulated upon exposure, demonstrating *S. marcescens* metabolic capabilities in breaking down the pesticide.

In the case of imidacloprid, another widely used insecticide, resistance in *N. lugens* was linked to increased levels of the endosymbiont *Wolbachia*. Removal of this bacterium led to reduced activity of cytochrome P450 enzymes and a significant drop in the expression of the resistance-related gene *NlCYP4CE1*. This suggests *Wolbachia* boosts host resistance by enhancing detoxification pathways at the molecular level (Cai *et al.*, 2021) [5].

Chlorantraniliprole Detoxification in *Spodoptera frugiperda*

In the fall armyworm, *Spodoptera frugiperda*, the gut symbiont *Enterococcus casseliflavus* contributes significantly to resistance against the insecticide chlorantraniliprole. It achieves this through mechanisms such as breaking amide bonds and dehalogenation, which degrade the compound. Genes associated with these detoxification processes were found to be particularly enriched in the insect-associated strains of *E. casseliflavus*, in contrast to strains from mammals or the environment (Zhang and Ju, 2024) [36]. Notably, this symbiont can be horizontally transferred with high efficiency through shared feeding and even cannibalism rather than through maternal inheritance. Its widespread presence in natural populations suggests an ongoing co-evolution driven by pesticide exposure and positions *E. casseliflavus* as a promising target for symbiont-based pest control strategies (STIC: Symbiont-Targeted Insect Control).

Pesticide Degradation by Endosymbionts in *Plutella xylostella*

In the diamondback moth, *Plutella xylostella*, endosymbionts help mitigate the harmful effects of insecticides via two key mechanisms. One involves the direct enzymatic degradation of pesticides through co-metabolism or complete mineralization. The second mechanism centers around the interaction between the insect's immune system and its symbionts, leading to cooperative resistance. For instance, gut bacteria of the genus *Enterococcus*, in combination with compounds like vitamin C and acetylsalicylic acid, protect the insect against the immune-suppressive effects of chlorpyrifos. These bacteria also have the capacity to hydrolyze the organophosphate fenitrothion into less toxic byproducts such as dimethyl thiophosphate and 3-methyl-4-nitrophenol, thereby reducing its insecticidal impact.

6.2 Role of endosymbionts in fighting plant defence systems

The removal of *Wolbachia* bacteria from the host, *Phyllonorycter blancardella*, an apple tree leaf miner, led to significant changes in the plant's physiology. Specifically, the loss of these endosymbionts resulted in the disappearance of cytokine-induced green islands on apple leaves and a decrease in cytokinin levels in the larvae (Dubey *et al.*, 2024) [9]. These findings suggest that Wolbachia plays a crucial role in modulating the phytohormonal profile of the leaves, likely by delivering cytokinins synthesized within the insect. Cytokinins are vital for inhibiting senescence, maintaining chlorophyll content, and regulating nutrient flow in plants, indicating a complex interaction between the insect and its plant host mediated by these bacteria (Body *et al.*, 2013) [3].

6.3 Role of Endosymbionts in protection against external biotic threats

Acyrthosiphon pisum acquires benefits from Rickettisella infection, which protects the insect from the attack of parasitoids as well as predators (Tsuchida *et al.*, 2024) [29]. Usually, this aphid is present in nature in red color, and predators such as ladybird beetles mainly feed on red-colored pea aphids. As ladybird beetle numbers rise in the field, *Rickettsia*-like symbionts in aphids trigger the production of blue-green quinone pigments, turning the aphids green. This color change helps them blend in with surrounding green crops, offering protection from beetle predation. However, green aphids become more visible to parasitoid wasps, which prefer that color. When parasitoid attacks increase, the symbionts shift the aphid's color from green to red, making them less attractive to wasps and improving their chances of survival.

7. Structural Characterization of Endosymbionts: Next Generation Sequencing Approaches

Next Generation Sequencing (NGS) has revolutionized the study of endosymbiotic microbial communities, particularly in insect systems where many symbionts cannot be cultured.

This culture-independent approach enables the identification and analysis of microbial diversity by directly sequencing DNA extracted from host tissues, such as the gut, where many endosymbionts reside (Chellappan and Ranjith, 2022) [7]. The process typically begins by carefully removing the insect's gut under sterile conditions, followed by the extraction of genomic DNA from the tissue. Before sequencing, the DNA is checked for quality, ensuring it's free from protein contamination and has a sufficient yield. Once the DNA passes these quality checks, it's prepared for sequencing through a step called library preparation.

Rather than sequencing entire genomes, researchers often use amplicon sequencing to target specific regions of the 16S rRNA gene—usually the hypervariable V3-V4 regions—using specialized primers. These amplified DNA fragments are then broken into smaller pieces, generally between 100 and 200 base pairs in length, and sequenced in parallel using high-throughput platforms. Each sample is tagged with a unique barcode, allowing multiple samples to be processed together and later separated during analysis.

The resulting DNA sequences are grouped into what are called Operational Taxonomic Units (OTUs), which serve as stand-ins for bacterial species or genera. These OTUs are formed based on sequence similarity—typically using a 97% match threshold—and compared against reference databases to identify the microbes present. This method offers a detailed view of the microbial diversity and abundance within a sample, all without the need to culture the organisms.

One example of this technique in action is the study of midgut bacteria in *Pectinophora gossypiella* (the pink bollworm). Using NGS, researchers have been able to explore how gut microbes might play a role in the insect's resistance to Bt cotton and conventional insecticides (Chaitra *et al.*, 2022) [6]. These findings are valuable for improving pest management strategies and understanding how insect-associated microbes contribute to survival under chemical stress

8. Endosymbiont-Based Insect Pest Management

Endosymbionts offer promising avenues for sustainable insect pest control through innovative biotechnological strategies. Four major approaches include Symbiont Elimination or Replacement, Genetic Modification (paratransgenesis), Microbial Semiochemical Utilization and Incompatible Insect Technique (IIT).

8.1 Symbiont Elimination or Replacement

Disrupting essential symbiotic relationships can impair insect development and reproduction. In *Amblyomma americanum* (lone star tick), treatment with antibiotics such as rifampin and tetracycline significantly delayed oviposition and reduced egg hatchability and larval viability. These antibiotics interfere with bacterial protein synthesis, altering symbiont function and negatively affecting tick reproduction. In a host-switch experiment, eggs of the pest *Megacopta punctatissima* were given symbiont capsules from the non-pest *M. cribraria*. The pest species showed reduced egg hatch rates and high nymphal mortality, confirming the crucial role of species-specific symbionts in early development and viability (Hosokawa *et al.*, 2007) [15].

8.2 Genetic Modification of Symbionts or Paratransgenesis

Paratransgenesis is an emerging and promising strategy in endosymbiont-based pest control that involves the genetic modification of insect-associated symbiotic microorganisms to deliver bioactive molecules within the host. Paratransgenesis offers a promising alternative to traditional genetic modification methods in insects, with advantages like improved efficiency, ecological safety, and simpler deployment. Unlike conventional insect transgenesis which involves complicated procedures like injecting insect embryos with foreign DNA to modify their germline paratransgenesis focuses on genetically altering the microbes that naturally live in or are consumed by the insect (Brown, 2021) [4].

In standard transgenic approaches, scientists inject insect eggs with a donor plasmid carrying the target gene and a reporter gene, along with a helper plasmid that provides the enzyme needed to insert the transgene into the genome. Successful modifications are typically confirmed by observing visible traits, such as fluorescent markers, and further breeding with unmodified insects helps verify stable gene integration. The end goal is often to get these insects to produce certain proteins like in the midgut to interfere with disease transmission or disrupt their biology.

Paratransgenesis, on the other hand, sidesteps the need for direct genetic manipulation of the insect. Instead, it involves modifying the insect's symbiotic microbes such as bacteria or viruses to produce proteins that can block disease transmission or affect the host insect's physiology. These engineered microbes can be introduced into insect populations through feeding, injection, or exposure to treated surfaces. Once inside the insect, the microbes produce functional molecules like antimicrobial peptides, RNA-based gene silencers, or enzymes that can either harm disease-causing pathogens or alter the insect's internal processes.

This approach has been successfully used in real-world cases. For instance, in *Rhodnius prolixus*, a bug that spreads Chagas disease, scientists modified its gut bacterium, *Rhodococcus rhodnii*, to produce the antimicrobial peptide Cecropin A, which targets the parasite *Trypanosoma cruzi*. Delivered using a product called CRUZIGARD (artificial feces), the engineered bacteria were able to greatly reduce parasite levels. Similar strategies have also been used in other insects like reduviid bugs and mosquitoes, where engineered symbionts were made to produce anti-parasitic proteins to block diseases caused by *Plasmodium* and *Leishmania* species (Maffo *et al.*, 2021) [19].

8.3 Incompatible Insect Technique (IIT)

The Incompatible Insect Technique (IIT) involves the use of gnotobiotic insects with controlled microbiomes to reduce vector-borne disease transmission. A landmark trial in 2011 near Cairns, Australia, released *Aedes aegypti* mosquitoes infected with the *wMel* strain of *Wolbachia*, originally from *Drosophila melanogaster*. This strain significantly reduces the mosquitoes' ability to transmit dengue virus. Monitoring using ovitraps and PCR assays showed *Wolbachia* infection rates exceeded 70% within weeks, and a follow-up in 2014 confirmed stable establishment (Rajendran *et al.*, 2024) [23].

By late 2022, infection rates remained around 95%, correlating with a 96% reduction in dengue cases. These *Wolbachia*-infected populations now serve as natural reservoirs for future releases, minimizing the need for lab-based rearing.

Symbiotic bacteria like *Arsenophonus* influence insect traits, including insecticide resistance. In *Nilaparvata lugens*, replacement of the native N-type *Arsenophonus* with the S-type strain significantly reduced resistance to imidacloprid. This effect was linked to down-regulation of detoxification genes and altered host metabolism. These findings highlight the potential of symbiont manipulation as a novel pest control strategy (Pang *et al.*, 2018) [20].

8.4 Antibiotics used against the symbiotic bacteria to check insect survival

Using antibiotics to disrupt symbiotic bacteria in insects has proven to be a powerful method for uncovering the roles these microbes play in host biology and resistance mechanisms. Studies have shown that targeting symbionts with specific antibiotics can significantly alter insect physiology and behavior. For example, antibiotic treatments in *Nilaparvata lugens* led to the collapse of dominant bacterial symbionts such as *Wolbachia*, *Arsenophonus*, *Acinetobacter*, *Lactobacillus*, and *Klebsiella*, resulting in increased susceptibility to insecticides. This effect was linked to the downregulation of detoxification enzymes like cytochrome P450 and glutathione S-transferase (GST), which are key to the insect's resistance mechanisms (Tang *et al.*, 2021) [28]. These findings highlight the potential of microbiome-targeted approaches as novel strategies for enhancing pest control and managing insecticide resistance.

9. Symbiont-Mediated RNAi: A Novel Approach for Insect Pest Management

The discovery of RNA interference (RNAi) through the injection of double-stranded RNA (dsRNA) in *Caenorhabditis elegans* has revolutionized gene-silencing techniques. RNA interference (RNAi) is a natural biological mechanism found in a wide range of organisms, including plants, fungi, and nematodes—and it serves important functions in insects as well. In these insects, RNAi helps regulate gene expression, defend against viruses, and control mobile genetic elements. Scientists have found that RNAi can be activated in insects using carefully designed molecules like hairpin RNA (hpRNA), artificial microRNA, or double-stranded RNA (dsRNA), each tailored to target and silence specific genes in pest species.

Although RNAi offers high precision in gene targeting, several challenges have slowed its widespread adoption in agriculture. These include the high cost of RNA production, difficulties in delivering RNA molecules effectively to the pests, and concerns over the use of genetically modified (GM) crops. A promising workaround involves using microbes to deliver dsRNA. For example, in the pest beetle *Henosepilachna vigintioctopunctata*, researchers observed that feeding larvae leaves coated with dsRNA—produced by bacteria and aimed at a gene called *Snf7*—resulted in significant mortality among the larvae.

Interestingly, RNAi doesn't just affect the pest's genes; it can also interact with their gut microbiome. In *Plagiodera versicolora*, for instance, dsRNA disrupted the insect's natural gut bacterial balance. Moreover, the breakdown products of dsRNA appeared to fuel the growth of *Pseudomonas putida*, a typically harmless gut bacterium. This unintended bacterial overgrowth contributed to the death of the larvae, revealing another layer of complexity in RNAi's effects.

RNAi has also shown potential in managing aphid populations, which are major crop pests. To reduce costs and improve delivery, researchers are exploring symbiont-mediated RNAi (SMR)—a technique that uses genetically engineered bacteria already living inside the insect to produce dsRNA from within. Aphids are particularly well-suited for this method because they harbor a range of symbiotic bacteria, such as *Buchnera aphidicola* and *Candidatus Serratia symbiotica*. *S. symbiotica* is especially valuable because it can be cultured outside of the insect, making it easier to modify in the lab. Overall, using symbiotic microbes to deliver RNAi offers a promising, cost-effective, and environmentally sustainable solution for insect pest control—an approach with strong potential for future agricultural practices (Li *et al.*, 2022) [18].

10. Conclusion

Multiple opportunities are available for insect pest control based on manipulation of insect associated microorganisms. The status of experimental proof-of-principle under defined laboratory conditions like targeting of obligate microbial partners. To products suitable for field application of paratransgenesis of Rhodnius vector of Chagas disease and pierce disease of grapes. Ongoing field trials in multiple countries like Eliminate Dengue, exploiting heterologous associations between mosquitoes and Wolbachia for Suppression of vector competence of insects. Microbial-based techniques can be applied in conjunction with other methods, including biological control strategies, chemical insecticides, insect incompatible technique and sterile insect technique to optimize control of genetically variable insect pests under variable environmental conditions.

Meeting the challenges of using genetically- modified endosymbionts in insect pest control by increasing the specificity of the active agents by RNAi can be designed to target sequences at any level of phylogenetic conservation, including total specificity to the pest species. The ongoing advances and recent technologies have enabled us to further conduct in-depth analyses of microbiomes in various agroecosystems, including insects, plants, and other natural resources. It signifies an exciting era to discover new microbes or microbiome functions with potentials to be applied for insect pest management.

References

1. Arora AK, Douglas AE. Hype or opportunity? Using microbial symbionts in novel strategies for insect pest control. J Insect Physiol. 2017;103:10-7.
2. Ayoubi A, Talebi AA, Fathipour Y, Hoffmann AA, Mehrabadi M. Symbiont-mediated insect host defense against parasitism: insights from the endosymbiont *Hamiltonella defensa* and the insect host Myzus persicae. Pest Manag Sci. 2025.
3. Body M, Kaiser W, Dubreuil G, Casas J, Giron D. Leaf miners co-opt microorganisms to enhance their nutritional environment. J Chem Ecol. 2013;39:969-77.
4. Brown JJ. Microbiomes in the context of insect communities. 2021.
5. Cai T, Zhang Y, Liu Y, Deng X, He S, Li J, *et al.* Wolbachia enhances expression of NlCYP4CE1 in

Nilaparvata lugens in response to imidacloprid stress. Insect Sci. 2021;28(2):355-62.
6. Chaitra HS, Singh A, Pandiyan K, Kalia VK. Sex-biased variance in the structural and functional diversity of the midgut bacterial community of last instar larvae of *Pectinophora gossypiella* (Lepidoptera: Gelechiidae). Microb Ecol. 2022;83(4):1112-22.
7. Chellappan M, Ranjith MT. Metagenomic approaches for insect symbionts. In: Microbial Approaches for Insect Pest Management. 2022. p. 271-313.
8. Drezen JM, Chevignon G, Louis F, Huguet E. Origin and evolution of symbiotic viruses associated with parasitoid wasps. Curr Opin Insect Sci. 2014;6:35-43.
9. Dubey A, Saiyam D, Kumar A. Microbial endophytes as warriors for plant defense against abiotic and biotic stress. In: Advances in Plant Microbiome Research for Climate-Resilient Agriculture. 2024. p. 23-49.
10. Fukatsu T, Hosokawa T. Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. Appl Environ Microbiol. 2002;68(1):389-96.
11. Gurung K, Wertheim B, Falcao Salles J. The microbiome of pest insects: it is not just bacteria. Entomol Exp Appl. 2019;167(3):156-70.
12. Harris HL, Brennan LJ, Keddie BA, Braig HR. Bacterial symbionts in insects: balancing life and death. Symbiosis. 2010;51(1):37-53.
13. Hiroki M, Kato Y, Kamito T, Miura K. Feminization of genetic males by a symbiotic bacterium in a butterfly, Eurema hecabe (Lepidoptera: Pieridae). Naturwissenschaften. 2002;89(4):167-70.
14. Hochstrasser M. Molecular biology of cytoplasmic incompatibility caused by Wolbachia endosymbionts. Annu Rev Microbiol. 2023;77(1):299-316.
15. Hosokawa T, Kikuchi Y, Shimada M, Fukatsu T. Obligate symbiont involved in pest status of host insect. Proc R Soc B Biol Sci. 2007;274(1621):1979-84.
16. Hosokawa T, Fukatsu T. Relevance of microbial symbiosis to insect behavior. Curr Opin Insect Sci. 2020;39:91-100.
17. Hu FY, Tsai CW. Nutritional relationship between *Bemisia tabaci* and its primary endosymbiont, *Portiera aleyrodidarum*, during host plant acclimation. Insects. 2020;11(8):498.
18. Li T, Wei Y, Zhao C, Li S, Gao S, Zhang Y. Facultative symbionts are potential agents of symbiont-mediated RNAi in aphids. Front Microbiol. 2022;34:4710.
19. Maffo CGT, Sandeu MM, Fadel AN, Tchouakui M, Nguete DN, Menze B, *et al*. Molecular detection and maternal transmission of a bacterial symbiont *Asaia* species in field-caught Anopheles mosquitoes from Cameroon. Parasites Vectors. 2021;14(1):539.
20. Pang R, Chen M, Yue L, Xing K, Li T, Kang K, *et al*. A distinct strain of Arsenophonus symbiont decreases insecticide resistance in its insect host. PLoS Genet. 2018;14(10):e1007725.
21. Protasov E, Nonoh JO, Kästle Silva JM, Mies US, Hervé V, Dietrich C, *et al*. Diversity and taxonomic revision of methanogens and other archaea in the intestinal tract of terrestrial arthropods. Front Microbiol. 2023;14:1281628.
22. Qadri M, Short S, Gast K, Hernandez J, Wong ACN. Microbiome innovation in agriculture: development of microbial-based tools for insect pest management. Front Sustain Food Syst. 2020;4:547751.
23. Rajendran D, Vinayagam S, Sekar K, Bhowmick IP, Sattu K. Symbiotic bacteria: Wolbachia, midgut microbiota in mosquitoes and their importance for vector prevention strategies. Microb Ecol. 2024;87(1):154.
24. Romanov DA, Zakharov IA, Shaikevich EV. Wolbachia, Spiroplasma, and Rickettsia symbiotic bacteria in aphids (Aphidoidea). Vavilov J Genet Breed. 2020;24(6):673.
25. Rupawate PS, Roylawar P, Khandagale K, Gawande S, Ade AB, Jaiswal DK, *et al*. Role of gut symbionts of insect pests: a novel target for insect-pest control. Front Microbiol. 2023;14:1146390.
26. Stevens L, Giordano R, Fialho RF. Male-killing, nematode infections, bacteriophage infection, and virulence of cytoplasmic bacteria in the genus Wolbachia. Annu Rev Ecol Syst. 2001;32(1):519-45.
27. Takeshita K, Tamaki H, Ohbayashi T, Meng XY, Sone T, Mitani Y, *et al*. *Burkholderia insecticola* sp. nov., a gut symbiotic bacterium of the bean bug Riptortus pedestris. Int J Syst Evol Microbiol. 2018;68(7):2370-4.
28. Tang T, Zhang Y, Cai T, Deng X, Liu C, Li J, *et al*. Antibiotics increased host insecticide susceptibility via collapsed bacterial symbionts reducing detoxification metabolism in the brown planthopper, *Nilaparvata lugens*. J Pest Sci. 2021;94:757-67.
29. Tsuchida T, Koga R, Fujiwara A, Fukatsu T. Phenotypic effect of “Candidatus Rickettsiella viridis,” a facultative symbiont of the pea aphid (*Acyrthosiphon pisum*), and its interaction with a coexisting symbiont. Appl Environ Microbiol. 2014;80(2):525-33.
30. Vega FE, Dowd PF. The role of yeasts as insect endosymbionts. In: Insect-Fungal Associations: Ecology and Evolution. 2005. p. 211-43.
31. Wang GH, Berdy BM, Velasquez O, Jovanovic N, Alkhalifa S, Minbiole KPC. Changes in microbiome confer multigenerational host resistance after sub-toxic pesticide exposure. Cell Host Microbe. 2020;27:213-24.
32. Werren JH. Wolbachia and speciation. In: Endless Forms: Species and Speciation. 1998. p. 245-60.
33. Xia X, Sun B, Gurr GM, Vasseur L, Xue M, You M. Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). Front Microbiol. 2018;9:25.
34. Yong HS, Song SL, Chua KO, Lim PE. Microbiota associated with *Bactrocera carambolae* and B. dorsalis (Insecta: Tephritidae) revealed by next-generation sequencing of 16S rRNA gene. Meta Gene. 2017;11:189-96.
35. Zeng B, Zhang F, Liu YT, Wu SF, Bass C, Gao CF. Symbiotic bacteria confer insecticide resistance by metabolizing buprofezin in the brown planthopper, *Nilaparvata lugens* (Stål). PLoS Pathog. 2023;19(12):e1011828.
36. Zhang Y, Ju F. Uninheritable but widespread bacterial symbiont *Enterococcus casseliflavus* mediates detoxification of the insecticide chlorantraniliprole in the agricultural invasive pest *Spodoptera frugiperda*. J Agric Food Chem. 2024;72(33):18365-77.
37. Zhang J, Zhang Y, Wang Y, Yang Y, Cang X, Liu Z. Expression induction of P450 genes by imidacloprid in *Nilaparvata lugens*: a genome-scale analysis. Pestic Biochem Physiol. 2016;132:59-64.