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## Endosymbionts mediated detoxification of insecticides in insects

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### Abstract

Insects face a world saturated with toxins due to widespread pesticide use in various settings. While herbivorous insects primarily rely on their own genomic mechanisms for resistance, recent omics analyses unveil the presence of specific microorganisms in some insects. These symbiotic microbes play an important role in pesticide degrading, offering insights into the genetic basis of symbiont-mediated detoxification. This review underscores the significance of environmental microbiota in serving as a reservoir for microorganisms capable of degrading toxins, explores the ecological and evolutionary implications of such symbiotic associations, and discusses their potential impact on pesticide development and pest control as well as it focuses on how endosymbionts are effectively utilized in pest management.

**Keywords:** Microorganisms, associations, discusses

### Introduction

The growing awareness of environmental issues has heightened concerns regarding the use of pesticides, emphasizing the necessity for sustainable practices. Both consumers demands and policy changes are increasingly focused on promoting environmental stability through the adoption of green chemistries, biological control, and bioremediation (Tilman *et al.*, 2002; Damalas and Koutroubas, 2018; Thomas *et al.*, 2019) [81, 19, 80]. Bacteria, which are involved in microbial transformations, play a crucial role in detoxifying various insecticide classes such as pyrethroids, neonicotinoids, and organophosphates (Sethunathan and Yoshida, 1973; Fisher *et al.*, 1978; Serdar and Gibson, 1985; Chaudhry and Huang, 1988; Bhat *et al.*, 1994; Hayatsu *et al.*, 2000; Kamal *et al.*, 2008; Boricha and Fulekar, 2009; Zhang *et al.*, 2011; Kong *et al.*, 2013; Nayarisseri *et al.*, 2015; Pankaj *et al.*, 2016; Shetti and Kaliwal, 2016; Fernández-López *et al.*, 2017; Gangola *et al.*, 2018; Kumar, 2018; Aswathi *et al.*, 2019; Meng *et al.*, 2019) [75, 22, 73, 16, 7, 27, 34, 8, 91, 40, 60, 65, 76, 54].

Despite over 50 years of continuous research on bacterial pesticide degradation, the understanding of the relationship between these bacteria and insects has been not done completely. While the biotransformation of insecticides by endogenous means in insects is well studied (Panini *et al.*, 2016; Bass and Jones, 2018) [64, 5], the role of bacterial symbionts in insecticide detoxification was not reported until the mid-twentieth century. The concept of bacterial degradation of insecticides associated with an insect host gained renewed attention when the detoxification of organophosphates by a bacterial symbiont of the apple maggot was documented in 1967 (Boush and Matsumura, 1967) [10]. This idea was revitalized decades later when *Burkholderia* bacteria colonizing bean bug midguts were shown to confer resistance to the organophosphate pesticide fenitrothion to their insect hosts (Kikuchi *et al.*, 2012) [36].

In this review, we focus on symbiont-mediated insecticide detoxification within host insects and discussing the implications of this phenomenon. The discovery of both facultative and obligate pesticide-detoxifying symbioses across insect taxa emphasizes the need for more research in this area. Finally, to seek creative ways to reduce the environmental impacts of pesticide use. This concept has made a landmark in our understanding of insect gut microbiota as it relates to pro-insecticide metabolism and gives another opportunity to synergize our understanding of insect-bacteria associations with our need to control pest populations.

### Microbial symbiosis in insects

Endosymbionts, also known as endobionts, are organisms that reside within the body or cells of another organism, often establishing a mutually beneficial relationship, known as endosymbiosis. Examples include nitrogen-fixing bacteria in legume root nodules and bacterial endosymbionts supplying essential nutrients to insects. The concept stems from the endosymbiotic theory, suggesting that bacteria become permanent residents within eukaryotic organisms through engulfment, aligning with observations of organelle development. Mitochondria and plastids, such as chloroplasts, are believed to have originated from endosymbiotic bacterial, highlighting the significance of symbiogenesis (Margulis & Chapman, 2009; Mergaert, 2018) [52].

Virtually all insects form associations with heritable endosymbiotic bacteria, establishing either mutualistic or parasitic relationships with their hosts. These endosymbionts are categorized into primary endosymbionts (P-symbionts) and secondary endosymbionts (S-symbionts). Primary endosymbionts, like *Buchnera aphidicola* in pea aphids and *Wigglesworthia glossinidia* in tsetse flies, are vertically transmitted, providing essential nutrients for host development and survival. The co-evolutionary history between insects and primary endosymbionts spans millions of years, resulting in an obligate relationship where host insects depend on these bacteria for growth and reproduction, and the symbionts, in turn, rely on their hosts for survival (Kikuchi, 2009) [37]. Primary endosymbionts reside in bacteriocytes and specialized cells within the bacteriome, which supply nutrients to the bacteria (Bright and Bulgheresi, 2010) [12].

In contrast to primary endosymbionts, secondary endosymbionts, often commensal bacteria with more recent associations, they can be transmitted horizontally, vertically, or environmentally. These bacteria located in the insect hemocoel (Wernegreen, 2012) [84]. For instance, *Sodalis glossinidius* acts as a secondary endosymbiont in tsetse flies, transmitted maternally and found inter- and intra-cellularly in various tissues (Balmand *et al.*, 2012) [4]. *Wolbachia* and *Spiroplasma* are common facultative endosymbionts affecting insect reproductive properties, inducing phenomena such as parthenogenesis, feminization, male-killing, and cytoplasmic incompatibility (Werren *et al.*, 2008; Saridaki and Bourtzis, 2010) [85, 71]. To ensure survival and transmission, *Wolbachia* and *Spiroplasma* must evade host immune defenses, while hosts regulate bacterial populations to avoid pathogenic effects or fitness costs. Mutualistic insect-endosymbiont relationships, exemplified by *Wolbachia*, offer reciprocal benefits, enhancing reproductive capabilities or providing protection. In contrast, parasitic relationships, such as with *Spiroplasma*, exploit hosts, manipulating reproduction and causing harm.

### Co-evolution of insect–endosymbionts interaction

Phylosymbiosis arises from enduring and stable microbe-host associations driven by processes like coevolution and cospeciation. Here, "Coevolution" emphasizes reciprocal, simultaneous evolutionary changes in interacting species (Janzen, 1980) [31]. Cospeciation, a consequence of coevolution, occurs when hosts and microbes undergo speciation simultaneously (Hoberg, 1997) [28]. Demonstrating animal-symbiont coevolution in controlled laboratory conditions is challenging due to time constraints.

However, through phylogenetic and genomic analyses, confirm the coevolution between insects and symbionts (Moran, 2008) [57].

Insects, particularly Hemiptera species feeding on phloem sap, host symbionts that compensate for nutritional deficiencies in their food source (Douglas, 2016; Sandström, 1999) [20, 70]. Many hemipteran and their endosymbiotic bacterial maintain intimate associations relying on biosynthetic and metabolic complementarity for essential nutrition (Manzano-Marín *et al.*, 2020; Luan, 2015; Russel, 2013) [51, 46, 68]. For instance, the primary endosymbiont *Buchnera aphidicola* has coadapted with aphids over millions of years (Chong, 2019; Moran, 2001; Moran *et al.*, 1993) [17, 56, 58]. Similar coevolutionary instances have been observed with extracellular gut symbionts facilitating nutrient provisioning, such as *Ishikawaella capsulata* in plataspid stinkbugs (Hosokawa *et al.*, 2006) [30] and *Rosenkranzia clausaccus* in acanthosomatid stinkbugs (Kikuchi *et al.*, 2009) [37].

The limited likelihood of the entire microbial community being faithfully passed from mother to offspring suggests that not all microbial members are likely involved in the evolutionary processes driving phylosymbiosis. Priority effects, where early-arriving species influence the establishment of late-arriving ones during community assembly, play a role (Fukami, 2015) [23]. Coevolution, a crucial process in phylosymbiosis, depends on vertical transmissions to maintain the stable inheritance of "early-arriving species." Heritable symbionts are widespread in herbivorous insects (Moran *et al.*, 2008; Baumann, 2005) [57, 6]. Such as *Buchnera* in aphids, transmitted maternally (Koga *et al.*, 2012) [39], and *Rickettsia* in the green rice leafhopper, transmitted both paternally and maternally (Watanabe *et al.*, 2014) [83]. For social insects like *Acromyrmex* leaf-cutting ants (Marsh *et al.*, 2014) [53] and honey bees (Powell *et al.*, 2014) [67], the social acquisition of beneficial microbes is crucial for the specificity and fidelity of host-bacterial associations. This process establishes initial colonizing symbionts with vertical transmission as keystones or hubs responsible for the host-species-specific microbial community composition, facilitating the occurrence of phylosymbiosis.

### Symbiont-mediated functions in insect hosts

#### 1. Role in Nutrient Provision

The dynamic relationship between bacterial endosymbionts and insects involves two key processes: a shift from selfish to cooperative metabolism and coevolutionary changes. In the first process, bacteria synthesize essential nutrients not only for their own needs but also for the host animal. For instance, *Buchnera aphidicola*, a  $\gamma$ -proteobacterium in aphids, releases over 50% of the essential amino acids it synthesizes, supporting aphid growth and reproduction. Coevolutionary changes involve genome reduction in bacteria compensated by the host. This reduction is influenced by obligate vertical transmission, eliminating the free-living life stage. Different endosymbionts exhibit metabolic interdependencies, and their genomes may change linked metabolic pathways.

**a. Amino Acid Production:** Aphids and *Buchnera* collaborate to synthesize essential amino acids in specialized cells called bacteriocytes. This mutualistic relationship addresses deficiencies in essential amino acids

in the insect's diet, with reciprocal contributions from both partners. In certain symbiotic systems, multiple symbionts play crucial roles. For example, in cicadas, *Sulcia* provides eight essential amino acids, while *Hodgkinia* contributes the remaining two along with vitamins. Secondary symbionts in psyllids complement *Carsonella* in essential amino acid biosynthesis pathways.

**b. Nutrient Recycling:** Symbiotic microbes contribute significantly to nitrogen recycling in various insects. Examples include *Erwinia*-like bacteria in psyllids, hindgut bacteria in termites, and *Blattabacterium* in cockroaches. Carpenter ants and their *Blochmannia* symbionts exemplify nitrogen recycling in ants, with the symbionts capable of synthesizing essential amino acids and converting urea to ammonia. Similar nitrogen recycling mechanisms are observed in beetles, such as weevils, where vertically transmitted fungi contribute to nitrogen concentration in host sap.

## 2. Insect Fitness and Development

Various endosymbionts impact insect fitness and development. *Hamiltonella defensa* protects aphids against parasitoids, and *Sodalis glossinidius* positively influences the longevity of tsetse flies. *Wolbachia*, found in diverse insect hosts, influences behaviors such as sleep, learning, memory, mating, feeding, and aggression. Some studies indicate the effect on mating preferences, mating time, and mating frequency in different insect species.

## 3. Defense against Pathogens and Parasites

Insects commonly face threats from various natural enemies, including parasites, predators, pathogens and parasitoid wasps. Understanding the mechanisms governing resistance to parasites and pathogens is expanding, particularly through the role of secondary symbionts. Recent examples in insects highlight instances of symbiont-mediated protection. Pea aphids, including species like pea aphids, harbor an obligatory endosymbiont (*Buchnera*) alongside several optional endosymbionts (*Hamiltonella defensa*, *Regiella insecticola*, *Serratia symbiotica*, *Rickettsia*, *Rickettsiella*, *Spiroplasma*, and *Arsenophonus*). The vulnerability of pea aphids to predation by *Aphidius ervi* is influenced by the presence or absence of these optional endosymbionts. For example, *H. defensa* and *S. symbiotica* heighten aphid resistance against *A. ervi* while still allowing oviposition, ultimately diminishing the success of parasitoid wasps. Another bacterium, *R. insecticola*, provides robust protection against parasitic wasps, suggesting the development of protective traits in various endosymbiotic bacteria. Research indicates that toxin genes carried by phages, which rely on the *H. defensa* genome, contribute to aphid defense, providing a mechanism for swiftly acquiring new toxins. The ecological role of phages in symbiosis is not fully explored, and further investigations are necessary to comprehend how phages deliver toxins to parasitoid wasps without causing harm to aphid hosts.

*R. insecticola* shields pea aphids from the Entomophthorales fungus *Pandora* (*Erynia*) *neoaphidis*, whereas other symbionts belonging to the genera *Regiella*, *Rickettsia*, *Rickettsiella*, and *Spiroplasma* decrease mortality and fungal sporulation on deceased aphids. This dual safeguard not only ensures the survival of individual insects but also enhances the overall health of the entire host population.

The precise mechanisms underlying the protective role facilitated by *R. insecticola*, which may entail the synthesis of antifungal molecules, are yet to be fully understood.

## 4. Adaptation to Environment

Endosymbionts influence on insect hosts' heat tolerance. Aphids, unable to inherently adapt to high temperatures, witness a decline in primary endosymbiont *Buchnera*'s bacteriocyte count under heat conditions. Conversely, secondary endosymbionts *S. symbiotica* and *H. defensa* enhance heat tolerance, retaining secondary bacteriocytes to counteract primary bacteriocyte effects during heat stress. *S. symbiotica* partially restores *A. pisum* fitness without *Buchnera*, emphasizing its role in aphid heat tolerance. The groEL chaperone, overexpressed in endosymbionts, protects host proteins from heat degradation. *A. pisum* with *S. symbiotica* exhibits improved heat tolerance and fecundity at 25 °C. *Rickettsia*, a secondary endosymbiont in *Bemisia tabaci*, contributes to host heat tolerance by inducing temperature tolerance gene expression under normal conditions.

Body color in pea aphids is an ecologically significant trait affecting their susceptibility to parasites and predators. The aphid exhibits color polymorphism, enhancing its capacity to resist against natural enemies. Studies have demonstrated that red aphids are more susceptible to predation by ladybird beetles on green plants, while green aphids face higher rates of parasitoid wasp attacks (Losey *et al.*, 1997; Libbrecht *et al.*, 2007) [45, 44]. Pea aphids carrying the secondary endosymbiont *Rickettsiella* undergo a color change from red to green due to increased amounts of blue-green polycyclic quinines, influencing prey-predator interactions.

## 5. Pesticide detoxification

Recent years have witnessed an upsurge in discoveries unraveling novel microbial-host relationships. Beyond classic nutritional symbioses, microbes impact host reproduction, mate choice, and provide defense against natural enemies. This review explores the role of symbionts in detoxifying pesticides and the mechanisms that underlie insect resistance to a variety of pesticides. For instance, the bean bug *Riptortus pedestris* (Hemiptera: Alydidae) possesses sac-like tissues known as "crypts" in the posterior region of the midgut. These crypts are primarily inhabited by a symbiotic *Burkholderia* bacterium (*Betaproteobacteria*), which is actively involved in the detoxification of fenitrothion.

### Detoxifying symbionts Localization in insect body

Symbionts exhibit a wide range of localization patterns within host organisms, demonstrating significant variability among insect species. The specific placement of symbionts within host bodies is extensively documented in the case of the olive fruit fly. In this species, symbionts responsible for degrading toxins inhabit the "pharyngeal bulb," a sac-like organ located in the head (Capuzzo *et al.*, 2005) [13]. Similarly, in the cigarette beetle *L. serricornis*, bacteriocytes host yeast-like symbionts involved in toxin degradation (Shen and Dowd, 1991; Dowd *et al.*, 1989; Dowd and Shen, 1990) [62, 63, 69]. Conversely, the majority of symbiotic relationships involved in detoxification are categorized as "gut symbiosis", where symbiotic microorganisms are situated extracellularly within the lumen of the gastrointestinal tract. For example, certain stinkbug species

like *M. cribraria* and the bean bug *R. pedestris* form sac-like tissues known as "crypts" in the posterior region of the midgut, housing symbiotic bacteria (Hosokawa *et al.*, 2007; Kikuchi *et al.*, 2005; Kikuchi and T. Fukatsu, 2014)<sup>[79, 89, 83]</sup>. In *A. gossypii*, the *Sphingomonas* symbiont consistently localizes in the foregut.

### Novel Role for endosymbionts in Insect Hosts

Arthropods have evolved diverse mechanisms, including behavioral and molecular adaptations, to withstand pesticides. These mechanisms encompass: (A) Acquisition of resistance through toxin avoidance: Insecticides often struggle to reach target insects beneath leaves, facilitating the development of resistance. (B) Reduction of toxin penetrability by thickening the insect cuticle: A physical adaptation that hinders the entry of toxins into the insect. (C) Pesticide insensitivity caused by mutations in the binding site within the target pest: Genetic alterations that result in decreased susceptibility to pesticides. (D) Pesticide metabolism through molecular machinery: Modifications at the epigenetic level, such as DNA methylation or histone modification, leading to altered gene expression upon pesticide exposure. These epimutations are often heritable. Transcription factors (TFs), like the CncC-Maf mediated xenobiotic response, can modulate xenobiotic response elements. Overexpression of phase I (e.g., Cyt P450s), phase II (e.g., GSTs), and phase III (e.g., ABC transporters) enzymes can lead to the detoxification or excretion of entomotoxic pesticide molecules. (E) Genetic resistance against pesticides facilitated by single or multigene mutations: Evolutionary changes occurs in pest genomes, such as modification of the pesticide target sites, upregulation of pesticide degrading esterases, and improvement of pesticide secretion.

Recent findings shed light on the role of endosymbionts in facilitating the pesticide detoxification. Microbial symbionts play a pivotal role in the development of resistance by either detoxifying harmful compounds or aiding in the encapsulation of toxic molecules, thereby triggering the insect's immune system. Given the widespread use of chemical insecticides for global agricultural pest control, their misuse frequently leads to the emergence of insecticide resistance in diverse pest species. In this context, a mechanism of insecticide resistance involving a bacterial symbiont capable of degrading insecticides, which establishes itself in pest insects. The microbiota within hosts exposed to pesticides, acting as a source of selection pressure, may assist the host in metabolizing these substances. This microbiota serves as a reservoir of genetic variation, resulting in reduced susceptibility to pesticides (Akami *et al.*, 2019)<sup>[2]</sup> and the subsequent development of insecticide resistance in insects.

Pesticide-degrading bacteria are diverse, identified in Lepidoptera, Hemiptera, Diptera, and Coleoptera. *Rhagoletis pomonella*, the apple maggot, historically faced organophosphate pesticide challenges. In the mid-20th century, *Pseudomonas melophthora* associated with *R. pomonella* was studied for potential detoxification mechanisms. It was suggested that *P. melophthora*, initially considered a plant pathogen, could degrade organophosphates through bacterial esterases. However, the direct impact on host insect resistance and the specificity of these esterases for organophosphate degradation remained unclear. Several studies laid the foundation for subsequent

research confirming bacterial esterase involvement in insecticide degradation (Boush and Matsumura, 1967; Fisher *et al.*, 1978; Kamal *et al.*, 2008; Gangola *et al.*, 2018)<sup>[10, 22, 34, 24]</sup>.

### Detoxication of pesticides in lepidopteran insect pests

The diamondback moth (*Plutella xylostella*), a global important pest affecting cruciferous vegetables, has more attention due to its evolving resistance to various pesticides. In a conducted study, *Bacillus cereus*, isolated from the moth's gut, exhibited notable abilities in breaking down and incorporating indoxacarb, an oxadiazine pesticide commonly used against lepidopteran pests. Furthermore, bacteria including *B. cereus*, *Enterobacter asburiae*, and *Pantoea agglomerans* were identified within the insect gut, showcasing their capacity to degrade acephate—an organophosphorus compound that inhibits acetylcholine esterase. Additionally, *Enterococcus* sp., isolated from the guts of *Plutella xylostella*, was found to show insecticide resistance to chlorpyrifos by monitoring the expression of an antimicrobial peptide called gloverin, ultimately leading to the degradation of chlorpyrifos.

The fall armyworm moth (*Spodoptera frugiperda*), a significant pest affecting crops like corn, cotton, and oranges, underwent scrutiny for pesticide resistance. De Almeida *et al.* (2017)<sup>[43]</sup> investigated resistant strains of *S. frugiperda*, revealing over 120 pesticide-degrading bacterial strains isolated from moth lines resistant to various pesticides. These strains, including *Leclercia adecarboxylata*, *Pseudomonas stutzeri*, *Arthrobacter nicotinovorans*, *Pseudomonas psychrotolerans*, and *Microbacterium arborescens*, were found in moth lines resistant to chlorpyrifos, lambda-cyhalothrin, deltamethrin, spinosad, or lufenuron, suggesting the potential role of gut endosymbionts in pesticide detoxification.

### Degradation of Trichlorphon in oriental fruit fly

The oriental fruit fly (*Bactrocera dorsalis*), a major pest affecting citrus fruits and horticultural crops on a global scale, has developed resistance to the organophosphorus pesticide trichlorphon, presenting a serious challenge. Comparative examinations of the gut microbiota community structure in susceptible and resistant strains of *B. dorsalis* unveiled a higher prevalence of the genus *Citrobacter* (*Gammaproteobacteria*) in resistant insects (Cheng *et al.*, 2017)<sup>[18]</sup>. *Citrobacter*, isolated from the gut of a resistant *B. dorsalis* strain, exhibited the capability to break down the toxic trichlorphon into less harmful byproducts, specifically dimethyl phosphite and chloral hydrate (McFall-Ngai *et al.*, 2013; Moran, 2007)<sup>[49, 58]</sup>. Importantly, when susceptible insects were transplanted with trichlorphon-degrading *Citrobacter*, they demonstrated resistance to trichlorphon, providing compelling evidence of *Citrobacter*'s role in the fruit fly's resistance to trichlorphon (Cheng *et al.*, 2017)<sup>[18]</sup>.

### Fenitrothion detoxication in bean bug.

The bean bug *Riptortus pedestris* (Hemiptera: Alydidae) is known for infesting leguminous crops, particularly soybeans. This insect possesses sac-like tissues referred to as "crypts" located in the posterior region of the midgut, primarily inhabited by a symbiotic *Burkholderia* bacterium (*Betaproteobacteria*) (Kikuchi *et al.*, 2005; Takeshita and Y. Kikuchi 2017; Kim and B. L. Lee 2015)<sup>[79, 89]</sup>. Experimental investigations indicate that *R. pedestris* acquires the

*Burkholderia* symbiont during the nymphal stage from the surrounding soils, without vertical transmission from the mother to offspring (Kikuchi *et al.*, 2007) [179]. Although not indispensable for the host's survival, the *Burkholderia* symbiont contributes to rapid growth, larger body size, and increased fecundity in symbiotic insects, underscoring its pivotal role (Kikuchi *et al.*, 2007) [179]. Recent discoveries suggest that the *Burkholderia* symbiont enhances pesticide resistance in the stinkbug host. Fenitrothion (MEP), an extensively used organophosphorus pesticide, undergoes degradation by soil microbes (Itoh *et al.*, 2018) [126]. *Burkholderia*, a significant soil microbe, is capable of degrading MEP. Bean bugs infected with MEP-degrading *Burkholderia* demonstrated significantly higher resistance to MEP compared to those with *Burkholderia* that does not degrade MEP (Kikuchi *et al.*, 2012) [135].

#### **Buprofezin and Imidacloprid resistance in brown planthopper, *Nilaparvata lugens***

Buprofezin, a widely used chitin synthesis inhibitor against major insect pests, faces resistance issues due to overuse. The brown planthopper, *Nilaparvata lugens*, naturally acquired symbiotic bacteria like *Serratia marcescens* (Bup\_Serratia) from soil and water, providing resistance. Inoculating Bup\_Serratia into susceptible *N. lugens* conferred resistance, while antibiotic treatment rendered them susceptible, revealing the mutualistic role of Bup\_Serratia in host resistance. Genome sequencing identified candidate genes linked to buprofezin degradation, upregulated upon exposure, highlighting *S. marcescens*' capability to metabolize the insecticide and enhance host resistance. Following imidacloprid exposure, there was an observed increase in the abundance of *Wolbachia* in *Nilaparvata lugens*. The removal of this bacterium resulted in a reduction in the enzyme activity of CYP450s, and simultaneously, there was a significant decrease in the transcript level of NICYP4CE1. This outcome indicates that *Wolbachia* plays a role in enhancing the hosts' resistance to imidacloprid by stimulating the expression of NICYP4CE1 (Xia *et al.*, 2017) [186].

#### **Insecticide resistance in oriental fruit fly, *Bactrocera dorsalis***

Symbiotic bacteria have significant implications for insect physiology and ecology, influencing various aspects of insect life, including potential roles in mediating insecticide resistance. A unique mechanism of insecticide resistance has been identified in the tephritid pest fruit fly *Bactrocera dorsalis*, wherein a gut symbiont enhances the resistance to the organophosphate insecticide trichlorfon. The recognized gut symbiont, *Citrobacter* sp. (CF-BD), plays a critical role in the degradation of trichlorfon. Comparative genomics analysis revealed the presence of phosphatase hydrolase genes in CF-BD, displaying increased expression in the presence of trichlorfon. Introducing isolated CF-BD into *Bactrocera dorsalis* resulted in heightened resistance to trichlorfon, whereas antibiotic-treated flies showed reduced resistance, confirming the pivotal role of CF-BD in insecticide resistance. These findings suggest that symbiont-mediated insecticide resistance can readily emerge in *B. dorsalis*, representing a potentially more widespread and pertinent mechanism than previously acknowledged.

#### **Chlorantraniliprole detoxification in *Spodoptera frugiperda***

*Enterococcus casseliflavus*, a significant symbiont in the invasive insect pest *Spodoptera frugiperda*, plays a crucial role in enhancing the host's resistance to chlorantraniliprole through amide bond breaking and dehalogenation-related degradation of the insecticide. This suggests its considerable potential for managing insecticide resistance in Lepidoptera pests. In response to the heightened exposure risk to chlorantraniliprole, *E. casseliflavus* symbionts in insects, as opposed to those in mammals or environmental strains, were notably enriched with putative genes associated with chlorantraniliprole degradation. The study also revealed that *E. casseliflavus* can efficiently transmit horizontally (100% efficiency) through cross-diet and cannibalism, rather than relying on vertical transmission from the mother insect to offspring. Furthermore, the widespread infection of *E. casseliflavus* in field populations not only suggests an ongoing symbiont-host co-evolution process driven by insecticide pressure but also presents a novel therapeutic target for agricultural pests through symbiont-targeted insect control (STIC).

#### **Detoxification of imidacloprid in *Apis gossypii***

Neonicotinoid insecticides, constituting approximately 25% of global insecticide sales by 2015, are potent agents targeting insect nicotinic acetylcholine receptors (nAChRs), effectively controlling various pests. Imidacloprid, the pioneering neonicotinoid launched in 1991, gained prominence for its low mammalian toxicity and widespread use, although instances of resistance, such as the case of the cotton whitefly *Bemisia tabaci* in Spain, have been reported. Mechanisms of resistance involve cytochrome P450s (CYP6CY22 and CYP6CY13), uridine 5-diphosphate glucuronosyltransferases (UGTs) overexpression, and mutations in the nAChR  $\beta$ 1 subunit (R81T, L80S, V62I, K264E). Gut symbiotic bacteria, particularly *Pseudomonas* species and other genera like *Stenotrophomonas maltophilia*, *Klebsiella pneumoniae*, *Bacillus alkalinitrilicus*, and *Mycobacterium* sp., have been identified to metabolize imidacloprid, potentially contributing to enhanced resistance in insect hosts.

#### **Detoxification of Atrazine in *Nasonia vitripennis***

The intestinal bacteria *Serratia marcescens* and *Pseudomonas protegens* within *Nasonia vitripennis* demonstrated resistance to atrazine. After multiple generations of exposure to atrazine, there was a notable increase in the bacterial densities of *S. marcescens* and *P. protegens* in *N. vitripennis*. These strains exhibited atrazine degradation rates of 20% and 10%, respectively, and the results of whole-genome sequencing confirmed the presence of atrazine metabolism genes (Wang *et al.*, 2020) [182].

#### **Transmission mechanism of detoxifying endosymbionts**

The vertical transmission of nutrient and defensive symbionts from one generation to the next in host insects has been thoroughly elucidated (Bourtzis and Miller, 2003; Kaltenpoth, 2009) [19, 33]. Similarly, some of the symbionts involved in toxin degradation follow a strict vertical transmission pattern from mother to offspring. Olive flies and related fruit flies that harbor symbiotic bacteria in the pharyngeal bulb and gut transmit these symbionts through contamination of the egg surface (Capuzzo *et al.*, 2005;

Hosokawa *et al.*, 2007; Estes *et al.*, 2009; Guo *et al.*, 2017) [79, 15, 14, 90]. The kudzu bug *M. cribraria* exhibits a distinctive and intricate mechanism known as "capsule transmission" for symbiont transfer. In this process, mother bugs deposit particles filled with symbionts, referred to as "symbiont capsules," in proximity to eggs. Hatchlings then probe these capsules to acquire the symbiont (Fukatsu and Hosokawa, 2002; Hosokawa *et al.*, 2006) [78, 30]. Generally, symbionts are transferred from the mother to offspring through sophisticated mechanisms of vertical transmission, including transovarial transmission, egg-surface contamination, and coprophagy (Itoh *et al.*, 2014) [25].

Unlike the extensively studied cases mentioned above, the mechanisms of symbiont transmission in other detoxifying symbioses have received limited attention. Given that the majority of toxin-degrading microorganisms are linked to the gut and can be readily cultured independently of their hosts, these microorganisms likely have a free-living life stage and are sustained through horizontal transmission, specifically environmental acquisition and transmission.

Indeed, in the instance of the bean bug *R. pedestris*, the primary source of the fenitrothion-degrading *Burkholderia* symbiont is the surrounding soil. A combination of symbiont transmission types has been observed in the chinch bug, *Cavelerius saccharivorus*, where the MEP-degrading *Burkholderia* symbiont is predominantly acquired from environmental soil but is also partially transmitted from mother to offspring through egg-surface contamination. A similar mixed transmission pattern has been documented in the trichlorophon-degrading *Citrobacter* of the oriental fruit fly, *B. dorsalis*. Insects can acquire toxin-degrading microorganisms from their environment, primarily the soil, leading to rapid toxin tolerance. This aspect is crucial and intriguing in comprehending the evolution and maintenance of these detoxifying symbioses within the insect body.

### Genes and enzymes involved in detoxification

The analysis of genomes and transcriptomes, complemented by physiological validations, has unveiled essential microbial genes involved in detoxifying symbiosis across diverse systems. For instance, in the oriental fruit fly, *Bactrocera dorsalis*, genes related to pesticide degradation bear significant resemblance to those found in the trichlorophon-degrading *Citrobacter*, particularly the organophosphorus hydrolase (OPH) genes. Similar patterns are found in the fenitrothion-degrading *Burkholderia* present in stinkbugs. These *Burkholderia* strains exhibit a varied repertoire of pesticide-degrading genes, including the methyl parathion-degrading enzyme (mpd), organophosphorus hydrolase B (ophB), and oph.

Enzymes that hydrolyze organophosphates play a crucial role in mitigating pesticide toxicity and contributing to environmental cleanup, among other functions. These enzymes, extensively studied and categorized into opd (Organophosphorus degradation enzyme) and mpd groups, are essential for various applications. Phylogenetic analyses focusing on genes responsible for SaxA and MEP-degrading enzymes show frequent transmission occurring between different bacterial species and lineages. Notably, these genes are often encoded in plasmids, suggesting that horizontal gene transfer serves as a significant mechanism driving detoxification symbiosis.

Recent findings from insect genomes underscore the prevalence of toxin degradation genes, primarily falling within the glutathione S-transferases (GSTs), cytochrome P450 monooxygenases (P450s), and carboxylesterases (COEs) gene families. In contrast, bacterial metabolic pathways responsible for detoxification exhibit notable diversity, highlighting their capacity for metabolic adaptation. Notably, oxalate detoxification genes or pathways are infrequently reported in insects, suggesting that tolerance to oxalate is more reliant on mechanisms such as behavioral avoidance, upregulated excretion, or symbiont-mediated detoxification.

### Evolutionary significance of symbiont-mediated detoxification

Diverse mechanisms contribute to insect resistance against synthetic toxins, including metabolic detoxification, behavioral avoidance, and target-site mutation. Conventionally, these mechanisms were thought to be encoded within the insect genomes. The emergence of resistance traits through genomic mutation or rearrangement following repeated pesticide exposures is a gradual process, necessitating multiple pest generations for widespread dissemination within the population. Remarkably, symbiont-mediated toxin degradation stands out evolutionarily for several reasons: (1) resistance attainment by insects without genomic alterations, (2) the trait can fastly proliferate through symbiont infection or acquisition within a single insect generation, and (3) such resistance traits may be transmitted horizontally among individual insects and/or different species.

In genetic studies of insects, it has been observed that genes involved in toxin degradation are confined to a few gene families, primarily the P450 and GST gene families. This observation implies that detoxification traits in insects have predominantly evolved by the gene duplication. In contrast, bacteria display remarkable diversity toxin degradation pathways, indicating greater flexibility in the evolution of detoxification mechanisms. Through symbiosis, microorganisms can inhabit or exchange novel metabolic genes or pathways via horizontal gene transfer. Consequently, this suggests that insects may gain an evolutionary advantage in the ongoing arms race with agrichemical companies.

In natural settings, toxic compounds are not uniformly distributed but rather exist in a mosaic fashion. To adapt to this fluctuating toxic environment swiftly and flexibly, symbiotic associations that do not involve vertical transmission should be selectively favored. Moreover, such changes in partners within and between generations could potentially facilitate host-plant-switching in herbivorous insects. Conversely, the vertical transmission of symbionts from mother to offspring may be favored when the host consistently encounters a specific toxic compound from generation to generation. This consideration arises due to potential risks associated with horizontal symbiont transmission, such as the failure to acquire symbionts and susceptibility to infection by unfavorable microbes like parasites and pathogens.

The possibility emerges from this discussion that detoxifying symbiosis may exert an influence on the gene level evolution of host insects. Bacterial symbiont have the potential to serve as a heritable source for the genome evolution of insect, as detoxification genes from these

associated bacteria could integrate into the host genome. Notably, several detoxification genes in lepidopteran insects have origins in entomopathogenic bacteria, although functional confirmations are still required.

### Endosymbionts in pest management

Endosymbionts have important role in Insect pest management in many ways. Here, three ways of exploiting endosymbionts pest management are explained in detail with examples

1. Endosymbiosis disruption affects the insect pest Physiology.
2. Introduction of endosymbionts into a novel host to disrupt the traits contributing to the insect pest status.
3. Manipulation of detoxifying endo-symbionts.

#### 1. Endosymbiosis disruption affects the insect pest physiology

Endosymbiotic organisms play crucial roles in various physiological activities across the insect life cycle. This approach of harnessing endosymbionts for insect pest management is elucidated through relevant examples.

##### a) Effects of endosymbiont disruption on the developmental biology of the West Indian sweet potato weevil, *Euscepes postfasciatus*

The sweet potato weevil hosts the endosymbiont *Nardonella* sp. When the larvae are raised on an artificial diet with antibiotics, they exhibit a significantly lighter body weight, leading to a reduced growth rate compared to control insects. The subsequent generation of insects treated with antibiotics lacks the *Nardonella* endosymbiont, displaying lighter body weight, smaller size, paler coloration, and a diminished growth rate. This suggests that the *Nardonella* endosymbiont plays a critical role in the overall growth and development of the host weevil and can potentially be employed for its management (Kuriwada *et al.*, 2010) [42].

##### b) Effects of gut endosymbiont disruption on the Nutritional Dynamics of the Pea Aphid (*Acyrtosiphon pisum*):

The pea aphid has relation with the primary endosymbiont, *Buchnera aphidicola*. Using the antibiotic Rifampicin, *Buchnera* was disrupted, resulting in infertility, greater loss in body mass, and a notable decline in the overall levels of soluble sugars, proteins, and glycogen. Concurrently, observed a significant increase in total lipids and neutral fats. These findings indicate the indispensable role of *Buchnera* in the pea aphid, suggesting a potential avenue for effective pest control.

c) **Effect of endosymbiont disruption on Immunity in Olive Fruit Fly (*Bactrocera oleae*):** Candidatus *Erwinia*, an obligate endosymbiont, is vertically transmitted in *B. oleae*. It enables the fruit fly to adapt to the olive plant and generates the defensive compound Oleuropein. This symbiotic relationship presents a potential target for disrupting the symbiosis, which could render the fruit fly population susceptible to the antibiosis effect of the olive plant, as suggested by Nobre (2019) [61].

d) **Selective Destruction of Symbiotic Protozoa for Termite Control:** Worker termites, perform the function of foraging and feeding the colony, hindgut protozoan

symbionts aid in cellulose digestion. Husseneder and Collier (2014) [74] genetically modified yeast (*Kluyveromyces lactis*) to produce a lytic peptide called Hecate. Feeding termites with Ligand-Hecate yeast bait led to the expression of lytic peptides, causing the death of gut protozoa within four weeks. This resulted in inefficient lignocellulose digestion, leading to termite mortality within six weeks.

### Introduction of endosymbionts in novel host to disrupt the traits responsible for insect's pest status

Introduction of endosymbiont organisms in a novel host to disrupt traits contributing to an insect's pest status is crucial in insect pest management. This method is exemplified through various *Wolbachia*-mediated control techniques:

i. ***Wolbachia*-Mediated Control Techniques:** *Wolbachia*, obligate symbionts residing in host cells' vacuoles, are transmitted maternally. The spread infections in the host population if they enhance offspring production by infected females (Hoffmann and Turelli, 1988) [29].

ii. **Using *Wolbachia* in Male-Killing Technique:** Male-killing *Wolbachia* eliminate males during embryonic development, ensuring only females survive to adulthood. This technique is applicable across insect orders and families, including Coleoptera (Coccinellidae, Tenebrionidae), Diptera (Drosophilidae), and Lepidoptera (Nymphalidae), offering a potential tool for population disruption (Stevens *et al.*, 2001) [77].

iii. **Using *Wolbachia* to Control Medfly (*Ceratitis capitata*) Population:** Uninfected medfly populations were transfected with *Wolbachia* from a related fly species (*Rhagoletis cerasi*). This induced complete Cytoplasmic Incompatibility (CI) in newly infected flies, offering an effective means to control the medfly population (Hoffmann *et al.*, 2014) [29].

iv. ***Wolbachia* as a Potential Tool to Control *Spodoptera litura*:** Proposed for the mass release of laboratory-reared CI males, *Wolbachia*-infected individuals can decrease successful mating in field populations of *Spodoptera litura*. Leveraging the tremendous reproductive success of *Wolbachia*-infected individuals provides a strategy to replace a pest species with a less lethal strain carrying *Wolbachia* ftsZ B (Mandla *et al.*, 2018) [50].

### Manipulation of detoxifying endosymbionts

Pest control represents a dynamic challenge with pesticide developers, farmers, agribusiness, and scientists, striving to protect crops and enhance production. However, this confronts the inherent biological drive of pests to thrive and reproduce. The extensive use of insecticides exerts selective pressures, leading to potential control failures, cross-resistance, and unintended ecological impacts. Understanding the role of insect gut microbiota in pro-insecticide metabolism provides an opportunity to synergize insights into insect-bacteria associations with the imperative to manage pest populations. Leveraging knowledge about symbiont-mediated detoxification opens avenues for the development of targeted pesticides, exploiting the intricate symbiotic relationships between hosts and symbionts. Research by the Australian Grains Pest Innovation Program (AGPIP) reveals that *Aphis gossypii* samples with higher

*Buchnera aphidicola* levels exhibit increased resistance to pesticides like imidacloprid and sulfoxaflor. The association of *A. gossypii* with antibiotics reduces *B. aphidicola* concentration, enhancing sensitivity to imidacloprid and mitigating insecticide resistance. AGPIP's ongoing research focuses on key aphids such as the green peach aphid (*Myzus persicae*), the Russian wheat aphid (*Diuraphis noxia*), and the oat aphid (*Rhopalosiphum padi*).

### Constraints faced by endosymbionts in pest management

Endosymbionts can face several constraints when considered for use in pest management strategies. Here are some challenges and limitations associated with utilizing endosymbionts in pest management:

1. Research on symbionts has long been regarded as a topic of interest only to taxonomists and physiologists, and its applied significance of symbiosis has been neglected.
2. Research on symbionts is mainly through a culture-dependent approach, so a vast portion of the endosymbionts are still untapped.
3. The high initial cost of research and lack of funding.
4. High-precision instruments to carry out symbiosis research are not widely available.
5. Lack of standardized procedures for artificial elimination of symbionts from different insect species.
6. Lack of trained human resources to carry out breakthrough research in this aspect.
7. Lack of multidisciplinary approaches and inter-institutional collaboration

### Conclusion

Mutualistic interactions between microbes and insects significantly influence the growth and development of insects, particularly those relying on nutrient-poor food sources like plant sap. Recent studies highlight the protective role of symbionts against pesticides, suggesting that exposure of bacteria to insecticides in agricultural environments could rapidly select for bacterial strains with enhanced detoxification capabilities, potentially leading to more potent resistance in pest insects. While the specific contributions of microbial players in detoxifying symbiosis remain challenging to unravel, the evolving nature of microorganisms, coupled with insects' ability to rapidly acquire novel metabolic functions through symbiotic relationships, opens avenues for pest management improvements. In agriculturally significant insects pest, the microbiome holds potential exploring novel pest management methods. Despite ongoing developments in paratransgenesis, which involves genetic modification, and the routine use of the sterile insect technique, research efforts are directed toward manipulating insecticide-detoxifying endosymbionts for pest management. This minireview compiles relevant studies on detoxifying symbioses in agriculturally important pests, presenting various experimental strategies to further explore the role of microbes in detoxification symbiosis and its applications in pest control.

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