



GUT SYMBIONTS: HIDDEN PLAYERS OF PESTICIDE RESISTANCE IN INSECTS

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ABSTRACT

Development of resistance to insecticides in insects is a global concern. Understanding the mechanisms is critical for effective plant protection and human health. Challenge in dealing the matter is that, resistance can occur via direct organism response (metabolic, physiological, and target-site changes) or via gut microbiome. Insects are constantly evolving like any other organisms; they are adopting various measures to overcome the chemicals sprayed to control them. Increasing evidence suggest that the gut microbiome can promote pesticide resistance in pests. Possible mechanisms by which gut bacteria play role in insecticide resistance are, direct acquisition of pesticide degrading microbes from the environment, difference in gut bacterial composition and diversity, difference in xenobiotic degrading enzymes and presence of microbial xenobiotic degradation pathways and cross-acclimatization to related insecticides.

Key words: Insects, gut symbionts, interaction, insecticide resistance, toxicity, cross-resistance, insecticide degradation

Insects and microorganisms interplay forming mutualistic associations was first reported by Wigglesworth (1929). There are abundant microorganisms forming a symbiotic relationship with their host insects. These microbes are either maternally inherited or directly acquired from the environment. Based on their role, these are classified as primary (Jing et al., 2020) and secondary endosymbionts (Douglas, 2015). They mediate various functions like; nutritional supplementation (Febvay et al., 1999; Douglas et al., 2001; Salem et al., 2014), colonization resistance, behavioral manipulation, adaptation to the environment, impacting population dynamics, influencing insect-plant interaction, pesticide detoxification, defense against pathogens and parasites (Dillon and Dillon, 2004; Oliver et al., 2005). Insects have developed the ability to withstand toxic chemicals. There are various factors which enable them with these abilities viz., evolutionary changes in insect genome such as modification of drug target sites, up-regulation of degrading enzymes, and increased drug excretion (Roush and McKenzie, 1987; Denholm and Rowland, 1992; Devine, 2009). An additional factor which supplements insects' ability for this adaptation is the presence of largely unknown world of microbes viz. symbionts. This review attempts to highlight the role of gut microbes vis a vis insecticide resistance and it's corollaries for developing new insecticides. We present an overview of some recent studies that contribute to understand acquisition of pesticide degrading microbes from the environment, as well as difference in their composition and diversity.

Knowing the difference in host's xenobiotic degrading enzymes and role of microbial xenobiotic degradation pathways leads to understanding the intricacies of the insect-microbe association and exploit that in future to develop novel pest management strategies.

How symbionts help in insecticide resistance?

Symbionts with pesticide degrading ability are reported mainly from three insect orders viz. Lepidoptera, Diptera, and Hemiptera. Mechanism by which insecticide resistance is rendered varies with insect groups, and the details are given in Table 1 and 2.

Direct acquisition of pesticide degrading microbes from the environment: Soil microbes have been playing an important role in degradation of pesticides in soils heavily applied with insecticides. In such areas, an increased growth of bacteria with pesticide degrading activity and accelerated pesticide degradation is observed. Some insects have the ability to directly acquire the microbes with pesticide degrading ability from the environment. Like in case of *Riptortus pedestris* (Fabricius) and some allied stink bugs. Second nymphal instars of *R. pedestris* have the ability to acquire fenitrothion degrading *Burkholderia* strains through feeding on soybean seedlings grown on fenitrothion enriched soil and establish a beneficial symbiosis within them. These bacteria hydrolyze fenitrothion into 3-methyl-4-nitrophenol and use the degradation product as carbon source for their growth. This confers resistance of the host insects to fenitrothion

Table 1. Insect gut symbionts responsible for insecticide resistance

S. No.	Insect pest	Symbiont	Insecticide	Country	Result	Reference
1	<i>Anopheles albimanus</i> Wiedemann	<i>Klebsiella pneumoniae</i>	Fenitrothion	United States	Composition of gut microbiota varies between resistant and susceptible mosquitoes with significant enrichment of organophosphate degrading bacteria in resistant population	Dada et al., 2018
2	<i>Bactrocera dorsalis</i> (Hendel)	<i>Citrobacter freundii</i>	Trichlorphon	China	Presence of Trichlorphon triggered higher expression of phosphatase hydrolase genes in <i>Citrobacter freundii</i> resulting in degradation of the insecticide	Cheng et al., 2017
3	<i>Plutella xylostella</i> L.	<i>Enterococcus</i> sp.	Chlorpyrifos	China	Gut bacteria play an important role in <i>Plutella xylostella</i> insecticide resistance	Xia et al., 2018
4	<i>Riptortus pedestris</i> (F.)	Genus <i>Burkholderia</i>	Fenitrothion	Japan	Direct uptake of bacterial genus, <i>Burkholderia</i> from the soil and establishment of beneficial symbiosis in <i>Riptortus pedestris</i> renders <i>Riptortus</i> and allied stinkbugs resistant to fenitrothion	Kikuchi et al., 2012
5	<i>Plutella xylostella</i> L.	<i>Bacillus cereus</i>	Indoxacarb	India	<i>Bacillus cereus</i> in <i>Plutella xylostella</i> utilized indoxacarb for metabolism and growth and thereby degraded 20% of indoxacarb	Ramya et al., 2016
6	<i>Plutella xylostella</i> L.	<i>Pseudomonas</i> sp., <i>Stenotrophomonas</i> sp., <i>Serratia</i> sp. and <i>Acinetobacter</i> sp.	Prothiofos	Republic of Korea	There is significant difference in the diversity of larval gut bacteria in prothiofos resistant, susceptible and field collected population of diamond back moth	Indiragandhi et al., 2007
7	<i>Bemisia tabaci</i> B-biotype	Rickettsia	Acetamiprid, Thiamethoxam, Spiromesifen and Pyriproxyfen	Israel	Susceptibility of whiteflies to insecticides increased with the presence of rickettsia	Kontsedalov et al., 2008
8	<i>Bemisia tabaci</i> Q-biotype	<i>Arsenophonus</i> , <i>Rickettsia</i> and <i>Wolbachia</i>	Thiamethoxam, Acetamiprid, Imidacloprid, Pyriproxyfen, Spiromesifen and Diafenthiuron	Israel	Increase in insect's ability to detoxify toxic compounds	Ghanim and Kontsedalov, 2009
9	<i>Spodoptera frugiperda</i> J E Smith	<i>Enterococcus</i> sp., <i>Delftia lacustris</i> , <i>Leclercia adecarboxylata</i> , <i>Microbacterium</i> sp., <i>Pseudomonas</i> sp., <i>Arthrobacter nicotinovorans</i> , <i>Staphylococcus sciuri</i>	Lambda-cyhalothrin, deltamethrin, chlorpyrifos ethyl, spinosad and lufenuron	Brazil	Gut of <i>S. frugiperda</i> is an unexplored niche for isolation of insecticide degrading bacteria	Almeida et al., 2017

Table 2. Insect gut symbionts responsible for Bt toxin activity

S. No.	Insect Pest	Symbiont	Bt toxin/ formulation	Country	Result	Reference
1	<i>Helicoverpa armigera</i> (Hübner)	Proteases, Aminopeptidases and Antioxidant enzymes of gut symbionts	<i>Bacillus thuringiensis</i>	India	Biological activity of Bt toxins against <i>Helicoverpa armigera</i> is influenced by the symbionts residing in their midgut	Visweshwar et al., 2015
2	<i>Holotrichia oblita</i> (Falderman), <i>Holotrichia parallela</i> (Motschulsky) and <i>Anomala corpulenta</i> (Motschulsky)	Predominant phylum; Proteobacteria	<i>Bacillus thuringiensis</i>	China	Antibacterial activity of gut symbionts of the studied insects contribute to their relatively low susceptibility to <i>Bacillus thuringiensis</i>	Shan et al., 2014
3	<i>Lymantria dispar</i> L.	<i>Enterobacter</i> sp.	<i>Bacillus thuringiensis</i>	USA	Resident gut microbiota are necessary for the insecticidal activity of Bt	Broderick et al., 2006
4	<i>Vanessa cardui</i> (L.) <i>Manduca sexta</i> (L.) <i>Lymantria dispar</i> L. <i>Pieris rapae</i> L.	<i>Lactococcus lactis</i> and <i>Klebsiella</i> sp. <i>Enterobacter</i> sp. and <i>Klebsiella</i> sp. <i>Enterobacter</i> sp. NAB3 and <i>Pseudomonas putida</i> <i>Enterobacter</i> sp. and <i>Pantoea</i> sp.	DiPel (<i>Bacillus thuringiensis</i>)	USA	The insecticidal activity of DiPel is affected by gut bacteria	Broderick et al., 2009
5	<i>Manduca sexta</i> (L.)	<i>Enterococci</i>	<i>Bacillus thuringiensis</i> (DiPel and Cry1Ac)	United Kingdom	Gut bacteria are not required for the insecticidal activity of Bt	Johnston and Crickmore, 2009
6	<i>Pectinophora gossypiella</i> Saunders	<i>Enterobacter</i> and <i>Pantoea</i>	Cry1Ac, Cry2Ab and DiPel®	India	Gut bacteria play role in Bt toxicity	Chaitra and Kalia, 2020

(Kikuchi et al., 2012). In subtropical Asia, Oriental chinch bug, *Cavelerius saccharivorus* Okajima is among the major pests of sugarcane (Schaefer and Panizzi, 2000) it harbours *Burkholderia* symbiont in the midgut (Kikuchi et al., 2011). Analysis of posterior midgut of this pest collected from the sugarcane fields with intensive fenitrothion usage showed that, 8% of the collected adults had remarkable fenitrothion degrading activities in their posterior midgut (Kikuchi et al., 2012). Development of resistance by acquiring the pesticide degrading microbes is sudden unlike, selection pressure leading to mutation and resistance development over the years.

Difference in gut bacterial diversity: In some insects, there is compositional change in the gut microbiota in resistant and susceptible insects of the same species. Toxic compounds like insecticides play a discriminatory role in enriching the bacterial population that can degrade xenobiotics (Genta et al., 2006). This change in structure of bacteria may be

attributed to the selection pressure due to insecticidal exposure and adaptive responses in biota to the new chemical environment in the midgut, resulting in shift of normal gut microbial composition to those which have the ability to utilize the pesticide as energy source. Rather, in response to selection pressure there is supplementation of bacterial taxa with a competitive advantage over others (Dada et al., 2018). Marked difference in structure of gut bacterial communities is reported between resistant (RS) and susceptible (SS) populations of *Bactrocera dorsalis* (Cheng et al., 2017) resistant to trichlorophon and *Anopheles albimanus* resistant to fenitrothion (Dada et al., 2018). *Citrobacter freundii* (CF-BD) was found to be the dominant gut bacterium in resistant *B. dorsalis* with new phosphatase genes as compared to other *Citrobacter* species. These genes were more similar to organophosphorous hydrolase genes of other bacteria. Exposure of CF-BD to trichlorophon resulted in significantly higher expression of phosphatase genes. This proved their role in organophosphorous degradation. GC-MS analysis

showed degradation of trichlorphon into chloral hydrate and dimethyl phosphite by CF-BD, which are significantly less toxic than trichlorphon.

Resistant *A. albimanus* were enriched with *Klebsiella pneumoniae*, an organophosphate degrading bacterium. Both *C. freundii* and *K. pneumoniae* belonged to the same class, Gammaproteobacteria. This phenomenon of difference in midgut bacterial composition is also reported in chlorpyrifos and fipronil susceptible and resistant lines of diamondback moth (DBM). Composition of two bacterial phyla, Firmicutes and Proteobacteria which were dominating the midgut of susceptible DBM changed to higher composition of Firmicutes and lesser composition of Proteobacteria in resistant lines (Xia et al., 2013). Indiragandhi et al. (2007) hypothesized that due to the selection pressure caused by insecticide prothiofos, the bacteria in resistant and susceptible DBM populations differed significantly. Antibiotic assays have proved the role of gut microbiota in insecticide resistance in *Spodoptera litura* against flubendiamide, indoxacarb and chlorpyrifos. Higher LC₅₀ values were recorded in larvae with gut microbiota as compared to those devoid of gut microbes (Gadad and Vastrad, 2016). Metagenomic analysis of brown plant hopper, *Nilaparvata lugens* indicated that, susceptible strains were dominated by Proteobacteria (99.86%) in their gut microbiota however, resistant strains consisted of Firmicutes (46.06%), followed by Bacteroidetes (30.8%) and Proteobacteria (15.49%). Taxonomic to phenotypic mapping also showed that resistant population was enriched with bacteria involved in detoxification functions (Malathi et al., 2018).

Difference in xenobiotic degrading enzymes and presence of microbial xenobiotic degradation pathways: Production of extracellular enzymes with various functions is common in bacteria. Certain bacteria produce enzymes that are known to degrade organophosphate compounds viz., phosphotriesterases, methyl parathion hydrolases and organophosphorus acid anhydrolases (Ramya et al., 2016). *Bacillus cereus* in *Plutella xylostella* larvae degraded up to 20% of the indoxacarb and showed high esterase activity. Growth of the bacteria was enhanced when the media contained 100 ppm of indoxacarb and retarded when cultured in media devoid of indoxacarb (Ramya et al., 2016). In *A. albimanus* resistant to fenitrothion, there was significant enrichment in bacterial enzyme families involved in organophosphorus degradation viz. carboxylesterases and phosphomonoesterases (Dada et al., 2018). Bacteria isolated from the insect gut when

exposed to insecticides generate enzymes which results in new metabolic pathway for insecticide degradation (Ramya et al., 2016).

Cross-acclimatization to related insecticides:

Cross resistance of insects to insecticides belonging to same mode of action is a well-known phenomenon. Similarly, the bacterial strains, SFA1, KM-A and KM-G of *Burkholderia* having degrading activity of fenitrothion can also be able to degrade organophosphate insecticides like diazinon, *O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate (EPN) and isoxathion to some extent suggesting a broader impact of the fenitrothion degrading bacteria on resistance of their host to other insecticides also (Kikuchi et al., 2012). Fenitrothion resistant strains of *A. albimanus* consisted of gut bacterial genera viz., *Acinetobacter*, *Bacillus*, *Enterobacter*, *Escherichia* and *Klebsiella* which had organophosphorus degrading ability and were also documented to metabolize other classes of pesticides (Dada et al., 2018).

Gut microbiota and *Bacillus thuringiensis*: Gut microbes act in coordination in many insects when it comes to *Bacillus thuringiensis* (Bt). They act synergistically and produce positive effect viz., Bt toxicity is more in the presence of gut bacteria. There are several reports on gut microbes playing synergistic role in Bt pathogenicity and in the absence of gut microbes (Table 2). Bt toxicity reduces like in case of *Lymantria dispar* (Broderick et al., 2006) *Vanessa cardui*, *Manduca sexta*, *Pieris rapae*, *Heliothis virescens* (Broderick et al., 2009) *Helicoverpa armigera* (Paramasiva et al., 2014; Visweshwar et al., 2015), *Spodoptera littoralis* (Caccia et al., 2016) and in *P. gossypiella* (Chaitra and Kalia, 2020). Symbiotic bacteria move from midgut epithelial cells to hemolymph through the pores created by Bt toxin and multiply to become abundant in hemolymph (Broderick et al., 2006; Li et al., 2020). Studies also revealed that, they impact on immune system of the insect and thereby increase the efficacy of Bt (Broderick et al., 2010). However, role of gut bacteria in Bt efficacy is contradictory in *M. sexta* (Johnston and Crickmore, 2009), *P. gossypiella* (Broderick et al., 2009) and *L. dispar* (van Frankenhuyzen et al., 2010). Based on the present knowledge, conflicting results involving the same insect can be attributed to; dominant bacterial species at the time of experimentation than on host species per se (van Frankenhuyzen et al., 2010). Difference in experimental results can also be due to difference in stage of the insect used for bioassay and difference in experimental methodology. Johnston and

Crickmore (2009) noticed no role of gut microbes in Bt toxicity in *M. sexta* and they hypothesized that, the earlier reports can be because of the unnoticed ill effects caused by the antibiotic treatment on the health of larvae.

Potential toxic effects of higher doses of antibiotic treatment are also seen in antibiotic treated *P. xylostella* where higher larval mortality, lower pupal weight, malformation and death of prepupae were observed compared to control (Lin et al., 2015). Chaitra and Kalia (2020) also reported that higher concentration of antibiotic treatment (100 µg/gm of diet) against five day old larvae of *P. gossypiella* resulted in slow growth of larvae, malformed pupae, and adults as compared to lower concentration (50 µg/gm of diet) and control. Broderick et al. (2009) reported the presence of gram-negative bacteria viz., *Enterococcus* as the sole bacteria inhabiting the *P. gossypiella* and reported no role of gut microbes in Bt toxicity in *P. gossypiella*. However, Chaitra and Kalia (2020) reported the presence of gram-negative bacteria in the mid gut of *P. gossypiella* and showed their role in Bt efficacy. Similarly, *Enterobacter* sp. NAB3 and *Pseudomonas putida* formed the gut bacteria of *L. dispar* and these had role in Bt efficacy (Broderick et al., 2009). However, in the experiment reporting the contradictory results, *L. dispar* midgut consisted of *Enterococcus* and *Staphylococcus* and lacked *Enterobacter* (van Frankenhuyzen et al., 2010). In *Choristoneura fumiferana*, midgut bacteria do not have any obligatory role in Bt efficacy (van Frankenhuyzen et al., 2010). Likewise, gut bacteria do not have any role in Bt efficacy in *P. xylostella* (Raymond et al., 2009).

CONCLUSIONS

In nature, there may be other insects with the same ability of direct acquisition of pesticide degrading bacteria from the surroundings and becoming resistant. The main disadvantage here is the speed of resistance development. Insects become resistant just by acquiring the pesticide degrading bacteria from soil even though, their ancestors were susceptible. Once acquired by the insect, there are also chances of spreading of these insecticide degrading bacteria horizontally within the population and also over a large area when the insects disperse or migrate. Thus, during the implementation of novel pest control strategies, there is need to consider role of gut symbiotic bacteria, as resistance in insects may develop more rapidly via symbionts. Add to this, generation time of bacteria is considerably shorter than their hosts there by reducing the time required

for resistance development. Understanding of new metabolic pathways leading to insecticide degradation will help in synthesis of insecticides with new mode of action or with different target sites. Targeting the gut microbiota especially primary endosymbionts can be a novel pest control approach because there is no other source of those microorganisms other than their parents. Further studies including molecular techniques and *in vitro* studies are necessary for establishing the facts.

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