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Review Article

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Gut bacterial diversity in different life stages of Fall Armyworm, *Spodoptera frugiperda*(Lepidoptera: Noctuidae)

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ABSTRACT

The fall armyworm is an important polyphagous insect that causes widespread damage to many crops, including maize, rice, and sorghum. It poses a major threat to food security in several developing countries due to its rapid spread. Chemical insecticides are used as the main management strategy to control fall armyworms in many parts of the world. However, there have been reports of insecticide resistance developing. Hence, it requires some green control methods to prevent and control fall armyworm populations. Fall armyworm, like other insects, is associated with various microbiota in their different life stages that influence their several characteristics and activities. It is found that the manipulations of gut microbiota are considered as desirable options for fall armyworm management. The Spodoptera frugiperda gut microbiota is generally different from microorganisms in the external environment, including ingested food. It can promote insect fitness by contributing to nutrition, especially by providing essential amino acids, vitamins, etc. It also protects insect hosts against pathogens, parasitoids, and predators by synthesizing specific toxins or modifying the insect immune system. However, so far, the function of gut microbiota in Spodoptera frugiperda remains to be investigated. But, the research hindrance has been overcome with the development of modern approaches such as high-throughput sequencing of the 16S rRNA gene and meta-genome analysis. Keeping all of the above into consideration, this review paper was written to study the structure, evolution, composition, gut microbiota diversity, and microbiome-host interactions, as well as some important approaches to study microbiome-host interactions and the major roles of fall armyworm gut microbiota.

Keywords: *Diversity, Gut microbiota, Resistance, Spodoptera frugiperda, 16S rRNA gene.*

Introduction

The fall armyworm (*Spodoptera frugiperda*, J.E. Smith) is an important polyphagous insect in many crops [1]. This insect causes significant damage to several important crops each year. The primary management strategies, such as the use and spraying of synthetic insecticides and genetically modified crops, are used for controlling FAW insects [2]. However, there are various reports on the resistance developed by FAW against several insecticides [3] [4]. Hence, there is an urgent need for some green control methods to prevent and control FAW populations. The exploitations of *Spodoptera frugiperda* gut microbial communities are considered desirable options for the management of fall armyworms. It plays various roles in the growth and development of the FAW insect. These gut microbiomes may change insect biology, metabolism, and behaviour, thus influencing plant-insect interactions significantly. The symbiotic associations between the insects and their gut bacteria have been studied in detail in other insects such as termites and aphids [5] [6]. And very little is studied about lepidopteran insects and their gut microbial associations. The lepidopteran larvae are alkaline (pH>10) hence, they are in extreme environments for the microorganisms [7]. Previous studies on lepidopteran insects reveal that the lepidopterans harbour midgut bacteria, thus suggesting that these microorganisms provide essential nutrients and also play some important roles in biochemical functions [8] [9]. There are various symbiotic associations of bacteria within the insect gut [10]. These symbiotic associations between the bacteria inside the insect's gut play various roles in regulating the metabolism of the insects and also help to improve digestion for the extraction of maximum energy from the ingested foods. Several studies have been done in the area of microbial diversity and its association with plants and insects, but the precise role of these microbial communities in plants and herbivorous insects remains unclear. However, the advanced sequencing methods and molecular technologies are a boon for us in understanding the role of these microbiomes in plant-insect interactions at the molecular level. There are two approaches, such as culture-dependent and culture-independent which are used to study the microbial associations inside the insect gut. The culture-independent method, which is based on 16S rRNA gene analyses, gives a clear picture of the bacterial communities and a more precise understanding of the microbes living inside the insect gut. Besides it, next-generation sequencing (NGS) technologies also help in assessing the higher diversity and structure of these microbial communities, allowing the finding of the taxonomic diversity of microbes in many ecosystems and environmental conditions. Further, these technologies realize high-throughput sequencing of PCR-amplified taxonomic genes (e.g., 16S rRNA gene for bacteria, 18S rRNA gene for fungi),

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whole meta-genome sequencing, and whole meta-transcriptomics. Thus, a comprehensive knowledge of the bacterial communities of FAW gut is vital for the full understanding of its host's biology and ecology and will provide insight into the development of novel strategies in FAW management. Hence, the manipulations, characterization, and identification of FAW gut-associated microbiomes would be a useful tool for improving control strategies. Therefore, this review paper was written to study the structure, evolution, composition, microbiota diversity, and microbiome-host interactions, as well as some important approaches to study microbiome-host interactions and the major roles of fall armyworm gut microbiota.

Structure, evolution, and composition of gut microbial communities in fall armyworm

The alimentary structure of the intestinal system is alike among insects, even though they have a variety of alterations connected with adaptation to diverse feeding styles and environmental conditions (Figure 1). The digestive system of *Spodoptera frugiperda* is divided into three main parts: the foregut, midgut, and hindgut [11]. The foregut and hindgut originate from the embryonic epithelium and are protected from pathogens by an exoskeleton of chitin and integument glycoproteins. This exoskeleton is shed at each ecdysis, separating the gastrointestinal lumen from the epithelia. The hindgut has distinct portions like fermentation compartments and a distinct rectum for retaining faeces during earlier evacuation. In *Spodoptera frugiperda*, the midgut is mainly responsible for absorption and digestion. It lacks an exoskeletal lining and develops from endodermal cells rather than the rest of the body [12]. A protective envelope known as the peritrophic membrane is released by the midgut epithelial cells of *Spodoptera frugiperda*. This envelope is essential for FAW survival. Further, the midgut has two parts. One is endo-peritrophic, and another is ecto-peritrophic space. Microbiomes are generally found in the endo-peritrophic space, which prevents them from coming into direct contact with the epithelium. Peritrophic matrixes are classified into two discrete parts, namely, type I and type II. Type I is called the "whole midgut and is occasionally active when particular foods are consumed, whereas type II is called the "anterior midgut [12]. The peritrophic matrix shields the epithelium against mechanical injury by food elements, toxins in food, invasive microbes, absorbed food, and digestive enzymes [13] [14]. In other circumstances, the peritrophic medium wraps around the undigested food mass as it passes along the digestive tract. Tiny pores in the peritrophic matrix prevent most microbiomes from passing through while allowing enzymes and small molecules to digest food [12]. The malpighian tubules of *Spodoptera frugiperda* are excretory structures that extend from the anterior (Fig. 1). As a result, the hindgut of *Spodoptera frugiperda* comprises a distinct nutritional environment that is well documented for water re-absorption [11], and further hindgut might function as a location of nutrient assimilation. The basic form of *Spodoptera frugiperda* gut has undergone numerous alterations due to adaptations to specialized niches and eating patterns.

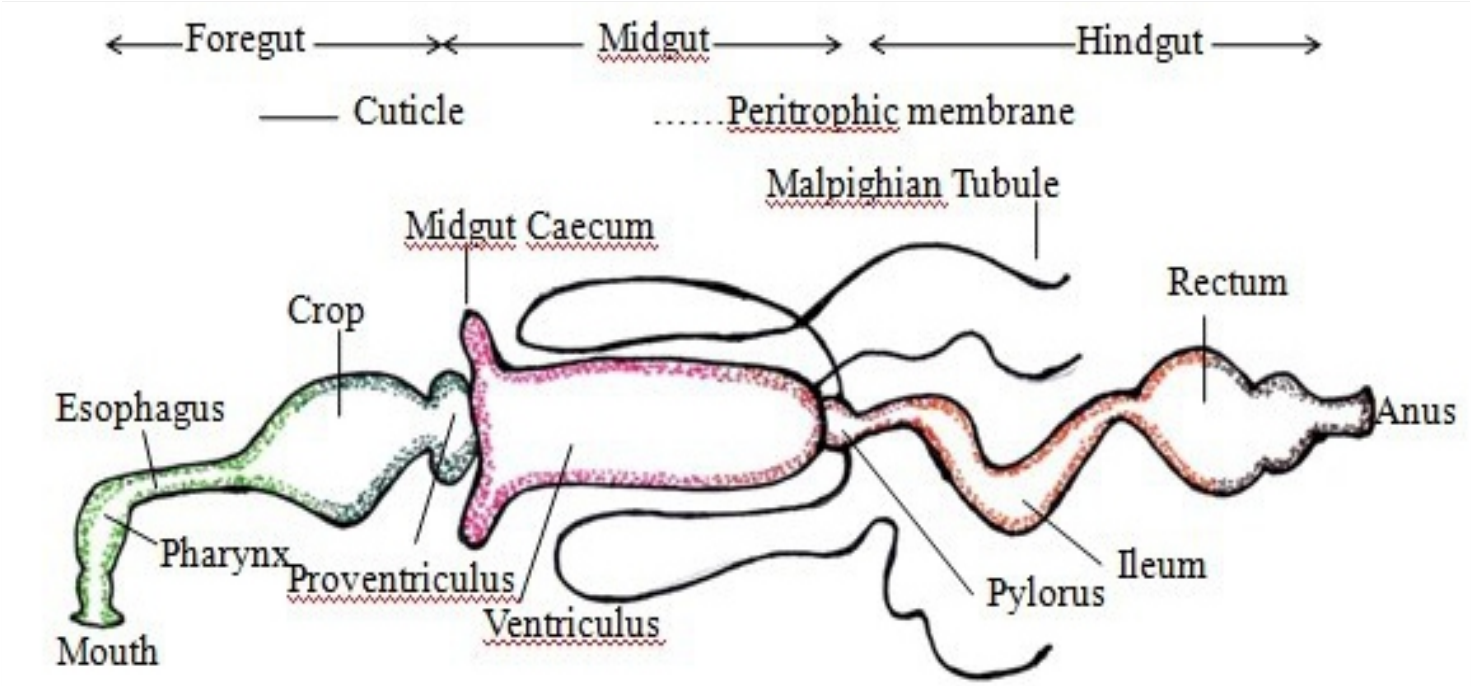

Fig.1. Structure of fall armyworm gut microbiota

Gut bacterial diversity in different life stages fall armyworm

According to Jaffar et al. [15] the application of high throughput and next-generation sequencing provides new insights into microbial ecology. It reveals that the diversity of microbial species can be increased by using independent culture methods, which identify a higher number of microbial communities than traditional culture-based or conventional molecular methods. In another study, using an independent culture technique and adopting molecular approaches such as denaturing gradient gel electrophoreses and 16S rRNA analysis, a high diversity of genus *Gamma proteobacteria* was identified in the gut of the locust *Schistocerca gregaria*. The results of this study suggested that this diversity of bacterial species engaged in a defensive mechanism and enhanced it against external pathogens and toxic chemicals [16]. Recently, Xue et al. [17] investigated the diversity of gut microbial species in various life stages of *Adelphocorissuturalis* by adopting the independent culture technique. They explained that the gut of the first and second instars was highly accomplished with a diversity of bacterial species. Further, they demonstrated that in the phylum, Proteobacteria and Firmicutes were dominant with a ratio of

87.06 and 9.43%, respectively, while at the genus level, *Erwinia* (28.98%), *Staphylococcus* (5.69%), and *Acinetobacter* (4.54%) were dominant bacteria. Li et al. [18] experimented to study fall armyworm gut bacterial diversity associated with different developmental stages (eggs, larvae, and adults), environmental habitats (field and laboratory), and diets (corn and an artificial diet). They observed that the microbial diversity in the egg stage was the highest, and the microbial diversity decreased dramatically after the eggs hatched into larvae; in the larval stage, L6 had the highest microbial diversity; the adult stage had the lowest community richness. Firmicutes were the most abundant bacterial community in the larval stage; the dominant bacterial phylum in the egg and adult stages was Proteobacteria, followed by Firmicutes. At the genus level, *Ralstonia* was the most abundant bacterium in the egg stage, followed by *Enterobacteriaceae*, including *Enterobacteria*, *Klebsiella*, *Pantoea*, and *Escherichia*; the bacterial community composition of male and female adults was similar to that of the early larvae stage (L1-L2), and OTUs (operational taxonomic units) with abundant content were *Enterococcus* and *Enterobacteriaceae*, including *Enterobacteria*, *Klebsiella,Pantoe*, and *Escherichia.* The bacterial community of L3 consisted mainly of *Enterococcus*; the community composition of the late larvae (L4-L6) harboured high proportions of *Enterococcus*, *Rhodococcus*, and *Ralstonia*. They also observed that the diversity of the gut microbes of the laboratory-raised *S. frugiperda* was lower than that directly collected from the field. Correspondingly, the gut microbial diversity of *S. frugiperda* was also reduced after one year of continuous laboratory rearing. The environment of the field is more complex and variable than that of the laboratory, so *S. frugiperda* may need more symbiotic microorganisms to defend against adverse environments or pathogens. In addition, the leaf microbiome of host plants can be enriched by the environmental microbiome, e.g., by rain splash or wind. Previous studies have shown large differences in microbial titers between field and greenhouse-grown maize leaves [19], which may contribute to differences in gut microbes that were introduced into the gut of *S. frugiperda* through the diet consumed. Previous reports have shown that changing diets can dramatically alter the gut microbiome of the host insect [20]. Mason et al. [19] demonstrated that different diets affect the proliferation of gut microbes in *Spodoptera frugiperda*by counting colony-forming units. Li et al. [18] further observed that 16S rRNA sequencing suggests that the gut microbiota of *Spodoptera frugiperda* fed with maize leaves and artificial diets differs greatly. On the one hand, since the nutritional components of corn leaves and artificial diets are different, the differences in the gut microbial composition of *S. frugiperda* may be related to different nutrient metabolisms. A dynamic gut microbiome facilitates the adaptation of herbivores to a new diet [21]. On the other hand, maize leaves contain microbes, but the artificial diets are sterile, so differences in microbes introduced during feeding may lead to differences in gut microbes. Finally, plant tissues contain large amounts of indigestible and toxic compounds, so herbivorous insects have evolved a range of plant-adaptive strategies, including symbiosis with microbes, to adapt to host plants. Gichuhi and coworkers [22] experimented to study the diversity of the fall armyworm, *Spodoptera frugiperda,* and their gut bacterial community in Kenya. For this, they collected *Spodoptera frugiperda* larvae from four maize-growing fields in Kenya between June and December 2017 and further performed high-throughput sequencing of the bacterial16S rRNA gene. They identified Proteobacteria and Firmicutes as the most dominant bacterial phyla, with lesser proportions of Bacteroidetes and Actinobacteria. They also observed differences in bacterial microbiome diversity between larvae and adults that are likely indications that some prominent larval bacterial groups are lost during metamorphosis. However, several bacterial groups were found in both adults and larvae, suggesting that they are transmitted across developmental stages.

Zhang et al. [23] explained that the type of diet, host plant, season, population density, and geographic position influence gut bacterial diversity. Feeding can change the gut microbiota community of lepidopteran insects. For instance, mulberry leaves are primarily composed of xylan (10-40%) and cellulose (19-25%), which shows the importance of intestinal microbes for food digestion in silkworms. In 5th-instar larvae of *B. mori* fed on mulberry leaves (the traditional rearing method), the gut microbiota is dominated by *Rhodococcus*, *Escherichia*, and *Enterococcus*. When the diet was changed to lettuce leaves, Bacteroides and Acinetobacter were the predominant species. In addition, the species diversity and richness of the gut microbial communities showed a significant relationship with the *Agrilus planipennisFairmaire* population size [24]. Furthermore, lepidopteran insects are holometabolic, and few studies have reflected the gut microbiota composition throughout development from egg to adult, especially in monophagous species. Gonzalez-Serrano et al. [25] showed that the bacterial composition of *Brithyscrini* was stage-specific and that Rosenbergiella and Serratia were highly abundant in the eggs. Twenty-seven genera (Empedobacter, 23 %, Enterococcus, 10 %) were statistically more abundant in larvae, while only one genus (Serratia, 75 %) was significantly more abundant in adults. More surprisingly, recent work has shown that DNA extraction methodology has the largest effect on the outcome of the metagenomic analysis in *B. mori* gut microbiome studies based on high-throughput 16S rRNA gene sequencing and computational analysis [26]. A taxonomic analysis revealed that the most common phylum was Proteobacteria, which, together with Firmicutes and Actinobacteria, was detected in lepidopteran insects. At the genus level, the dominant bacteria were mainly *Enterococcus*, *Enterobacter*, *Clostridium*, *Acinetobacter*, *Pseudomonas*, *Pantoea*, and *Bacillus*. The composition of the dominant gut microbiota in other insects was different. These differences depend on the diet source and behavioural characteristics of the host insects, which show the relationship between gut symbiotic bacteria and the co-evolution of the host from another perspective [27]. Although a few sequencing-based studies have confirmed the composition of gut bacteria, lepidopteran fungal communities have been largely ignored. However, endosymbiotic fungi are also ubiquitous among lepidopteran insects. Further, the reported fungal gut microbiota of lepidopteran insects, including *Lycaeides melissa*, *A. planipennis*, *A. major*, *D. pyloalis*, and *B. mori,* Basidiomycota and Ascomycota predominated the gut fungal communities, as determined by the sequencing of the fungal internal transcribed spacer (ITS). Most fungal sequences were assigned to the genera Ascomycota and Basidiomycota. At the genus level, most fungal sequences were assigned to the genera *Cladosporium*, *Hannaella*, *Kabatiella*, *Pyrenochaeta*, *Pyrenochaeta*, *Malassezia*, and *Rhodosporidium*[28]. Another experiment is carried out by Wang et al. [29] to study the adaptive evolution in the fall armyworm that is revealed by the diversity of larval gut bacteria. In this experiment, they examined differences in the gut bacterial communities of the

fifth and sixth instar larvae of *S. frugiperda* fed on leaves of different host plants. The 16S rDNA full-length amplification and sequencing method was used to determine the abundance and diversity of gut bacteria in larval intestines. The highest richness and diversity of gut bacteria were found in corn-fed fifth-instar larvae, whereas in sixth-instar larvae, the richness and diversity were higher when larvae were fed other crops. Firmicutes and Proteobacteria were dominant phyla in the gut bacterial communities of fifth and sixth-instar larvae. A similar experiment was conducted by Ugwu and Asiegbu [30] to study the influence of host plants on the diversity of gut microbiota communities of fall armyworms. Their results revealed that gut bacterial composition varied among larvae samples fed on different host plants. Three alpha diversity indices revealed highly significant differences in the gut bacterial diversity of *Spodoptera frugiperda* fed with different host plants. Analysis of molecular variance (AMOVA) and analysis of similarity (ANOSIM) also revealed significant variations in the bacterial communities among the various host plants. Five bacterial phyla (Firmicutes, Proteobacteria, Cyanobacteria, Actinobacteria and Bacteroidetes) were prevalent across the larvae samples. Firmicutes (44.1%) was the most dominant phylum, followed by Proteobacteria (28.5%). Linear discriminant analysis effect size analysis showed that *Spodoptera frugiperda* larvae were enriched by diverse bacterial groups.Additionally, Ugwu et al. [31] experimented to study the microbiomes of the larvae of *Spodoptera frugiperda* from maize plants in Nigeria, and they observed similarities as well as variations in their studies from the reports of previous work on the microbiomes of *Spodoptera frugiperda* from different countries (Table 1). Among studies of six countries, namely, Brazil, USA, Kenya, Argentina, Nigeria, and India, Enterococcus was persistent in the gut of the *Spodoptera frugiperda* larvae. The genus Pseudomonas was recorded in four countries (Brazil, USA, Kenya, and Nigeria), while Enterobacter was persistent in studies in three different countries (USA, Argentina, and Nigeria). Similarly, Chryseobacterium, Comamonas, and Sphingobacterium were recorded in three countries (USA, Kenya, and Nigeria). Recently, Sahani and coworkers [32] worked on a diversity of bacterial communities associated with the gut of *Spodoptera frugiperda* in Eastern India and observed two new genera of bacteria, i.e., Kluyvera and Yokenella, from the gut of *Spodoptera frugiperda*, which had not been reported so far from any other countries. Moreover, Gomes et al. [33] reported that Firmicutes was the predominant bacterial phylum in the gut of *Spodoptera frugiperda* larvae.

Table 1: List of microbiomes (dominant bacteria genera) of Spodoptera frugiperda from different countries (Ugwu et al., 2020)

Acevedo et al. [34] USA	Almeida et al. [35] Brazil	Jones et al. [36] USA	Gichuhi et al. [22] Kenya	Rozadilla et al. [37] Argentina	Ugwu et al. [31] Nigeria	Sahani et al. [32]India/Bihar
Enterobacter	Arthrobacter	Acinetobacter	Achromobacter	Enterobacter	Acinetobacter	Enterococcus
Klebsiella	Delftia	Aquabacterium	Acinetobacter	Enterococcus	Chryseobacterium	Klebsiella
Pantoea	Enterococcus	Arthrobacter	Aeromonas	Janibacter	Clostridium_ sensu_stricto_5	Kluvera
Raoultella	Leclercia	Bradyrhizobium	Camobacterium	Lysobacter	Comamonas	Raoultella
Serratia	Microbacterium	Chryseobacterium	Chryseobacterium	Pediococcus	Delftia	Enterobacter
	Pseudomonas	Comamonas	Citrobacter	Rubrobacter	Enterobacter	Citrobacter
	Staphylococcus	Enterococcus	Comemonas	Vibrio	Enterococcus	Yokenella
		Flavobacterium	Cutibacterium	Xanthomonadaceae	Erwinia	Pantoea
		Leuconostoc	Delftia	Xylanophilus	Faecalibaculum	Leclercia
		Luteibacter	Enterococcus		Klebsiella	
		Methylobacterium	Lysinbacillus		Leucobacter	
		Ochrobactrum	Morganella		Paenibacillus	
		Pseudomonas	Ochrobacterium		Pseudomonas	
		Ralstonia	Pseudomonas		Bradyrhizobium	
		Rhizobium	Serratia		Rhizobium	
		Sphingobacterium	Sphingobacterium		Sphingobacterium	
		Sphingomonas	Stenotrophomonas		Sphingomonas	
		Unclassified Enterobacteriaceae			Sporosarcina	
		Unclassified Enterobacteriaceae			Unidentified_ Chloroplast	
					Vagococcus	

Gut bacterial-host interactions

Insects are the world's most diverse and abundant animals in terms of species diversity and body mass in all ecological habitats [38]. Their numerous interactions with beneficial microbes are essential for survival and diversity. Microbes that are living in the guts of insects play a vital role in the biology and behaviour of their hosts, including assisting in the digestion of recalcitrant food components, upgrading nutrient-poor diets, modulating the immune response, and protecting from predators, parasites, pathogens, and disease vectors. Other functions include facilitating plant specialization, governing mating preference and reproductive systems, and contributing to inter- and intra-specific communication [39] [40] [41] [42]. Many studies describing symbiotic connections between microbes and insects have been published [43] [44] [45]. Most insects are thought to be in symbiotic partnerships with microbes, with estimates ranging from 15 to 20% of the total [46]. The role of microorganisms, particularly gut microbes, in insect function is important from various viewpoints, including agriculture, ecology, and medicine. Few insects are good laboratory models for studying microbe populations and their associations with hosts, especially immunology and metabolic associations [47]. Entomological studies of parasitic and mutualistic connections have focused on social insects like ants,

which have evolved diverse interactions with other species at various levels, including individual and community interactions. These interactions can occur between bacteria and different insects and plants [48]. Symbiotic bacteria can affect the efficacy of disease vectors or their developmental time, making them possible targets for disease control [49] [50]. Microorganisms allied with pollinators and herbivores, and insects that feed on them, are likely to impact crops' health substantially. Insects and their gut microbial populations play vital roles in the nitrogen cycle and the decomposition of plant material in natural and human-impacted ecosystems [12]. A symbiotic relationship with very adaptable bacteria may have opened new ecological niches and unbalanced food sources like plant sap or blood [51]. Mutualism between insects and microbes is unquestionably one of the primary drivers of insect evolution. It is one of the most important factors contributing to the remarkable success of this gigantic group of animals. Mutualism is described as an interaction between various species that is mutually advantageous to both parties [52]. Several fitness traits of insects are heavily influenced by their associated microbiota [53]. The association of insects with microbiota is very important for the evolution of ecological features and feeding habits in which insects exchange nutrients or specific functions, such as protection. Insects associated with various microbes also play an important role in degrading pesticides.

Moreover, insect-microbiota interactions are quite diverse. Insects rely on symbiotic bacteria for a variety of essential activities. Symbiotic bacteria can be critical for host survival and growth. A diverse range of symbiotic microbial species have been produced within the insect gut and have significantly contributed to the regulation of insect metabolism, enhanced food digestion, increased excretion of waste fluids, protecting the host from enemies, developing resistance against toxins, and degrading them into their intermediates. Many studies have reported that insect gut microbiota plays a significant role in developing symbiotic insect interactions facilitated by secondary metabolites [15]. Besides this, they also play an essential role in the detoxification of pesticides, providing a natural defense system, nutrient availability, the development of resistance against toxins and pathogens, the breakdown of food, and asuitable environment for the proper growth of insects. They can also help in the breakdown of food by providing energy, making vitamins, and even shaping the body's natural defenese [54]. Microbial symbionts have been proven to have many consequences for insect health and behaviour. Certain insects have specialized organs that can only house a few symbiont species, while others have a far more diverse and variable flora in their guts and other internal organs. Numerous associations are developed with a few species of microbiota. They might require establishing specialized insect organs and cells to house definite obligate symbionts. In these partnerships, the genetic integral of biochemical processes essential for the persistence of both interrelated groups is frequently observed [55]. Some insect species are more involved in symbiotic associations with bacteria than others. Among the insects, three taxonomic groups are regularly involved. These groups include Blattaria, Coleoptera, Homoptera, and Hymenoptera. Additionally, certain bacteria seem to be particularly adept at symbiotic interactions. The microbiota of other various insects may be more varied and adaptable, as they do not rely on explicit critical symbionts. The gut biota is critical for most insect digestion, fertility, fecundity, and immunity [56] [57] as growing axenic insects can be deadly.

Approaches to studying fall armyworm gut microbiota

To study the link between community composition and the function of gut microbiota in *Spodoptera frugiperda*, it would be very helpful if we could experimentally mix and match microbes [58]. For this purpose, we can use two approaches: culture-dependent and culture-independent. In another sense, the identification and characterization of *Spodoptera frugiperda* gut microbiomes are investigated mainly by culture-dependent or culture-independent techniques [59] [60]. In the case of culture-dependent bacteria, we can generally culture the gut microbiome outside of their fall armyworm hosts. Most of the microbes are difficult to propagate or cannot be cultured. In this case, we can use culture-independent approaches such as metagenomics. Metagenomics is based on the taxonomic (amplicon sequencing) and functional (shotgun sequencing, meta-transcriptomics) characterization of microbial communities. The former approach allows us to characterize who is there (species identification), whereas the latter approach allows us to characterize the variety of genes they possess or are expressing, which signifies 'what they are doing' within the host. Thus, we can say both methods indicate potential links between the microbiome and the fall armyworm host. However, the culture-dependent method usually produces biased results. It relies on various parameters and techniques, while in the culture-independent method, a lot of omics and molecular approaches are applied, such as 16S rRNA and BLAST analysis, which provide a better and more comprehensive picture of the microbial communities located in insect guts [61] [62] [63]. The application of high throughput and next-generation sequencing provides new insights into microbial ecology [64]. It reveals that the diversity of microbiomes in *Spodoptera frugiperda* was studied using independent culture methods, which identified a higher number of microbial communities than traditional culture-based and conventional molecular methods [65].

The major role of fall armyworm gut microbiota

The gut microbiota of *Spodoptera frugiperda* play very important roles, such as in insect fitness, by providing essential amino acids, vitamins, lactic acids and sterols, enhancing the immune system, food digestion, excretion of waste fluids, increasing host fertility, increasing resistance to toxins and external pathogens, and degrading pesticides and allelochemicals into less toxic products by the production of different hydrolytic enzymes (fig. 2). Therefore, the major roles of the fall armyworm gut microbiota are as follows:

Development of resistance/tolerance against insecticides:

Pesticides have been applied to manage pests and diseases since the start of agriculture for the production and protection of crops. However, the unwise use of pesticides accumulates in the ecosystem and contaminates plants, air, water, and soil [66]. The storage of pesticides in plants can develop resistance or tolerance against various pests [67]. Various functional parts of an insect's gut microbes, such as enzymes and genes, are responsible for developing pesticide resistance in insects. For example, the FAW gut microbiota develops resistance against organophosphate, carbaryl, and methyl parathion insecticides (Table 2). Further, a lot of studies have demonstrated that resistance is also developed due to the reduction of toxicity of a compound, the introduction of a new pesticide group, target site mutation or over-expression, pre-date or wrong selection of pesticide, repetition of the same chemical, environmental changes, and the degradation of parent compounds into their

metabolites by insect gut microbiota and their detoxifying enzymes [68] [69] [15]. An investigation was carried out by Gomes et al. [33] to study the role of gut microbiota in developing resistance against various insecticides in the laboratory and in open field conditions in the larvae of *Spodoptera frugiperda*. For this purpose, they collected insect pests from various corn fields in five Brazilian states. In their meta-genomic experiment and 16S rRNA analysis, the isolation of bacterial species from insect gut in the selective medium was achieved. The maximum growth of microbial species in insecticides was observed, and it was found that all microbes utilized it as a sole source of carbon and energy. This study indicated that bacteria isolated from field larvae grew better and degraded insecticides more efficiently than those collected from laboratory-selected strains. However, this study concluded that due to the high efficiency and diversity of insect gut microbes in the field, larval insects are more capable of degrading pesticides and show high resistance.

Table 2: Development of resistance against pesticides by gut microbiomes of various insects including Spodoptera frugiperda [15]

Name of pesticide	Insect common name	Insect scientific name	Gut microbiota
Carbary	Fall armyworm	*Spodoptera frugiperda*	*Bacillus thuringiensis* and *Varimorphanecatrix*
Methyl parathion	Fall armyworm	*Spodoptera frugiperda*	*Bacillus thuringiensis* and *Varimorphanecatrix*
Organophosphate	Fall armyworm	*Spodoptera frugiperda*	*Bacillus thuringiensis* and *Varimorphanecatrix*
Prothiofos	Diamondback moth	*Plutellaxylostella*	*Pseudomonas* sp., *Stenotrophomonas* sp., *Acinetobacter* sp., and *Serratia marcescens*
Tebuconazole	Brown planthopper	*Nilaparvatalugens*	*Acinetobacter*sp
DDT	Diamondback moth	*Plutellaxylostella*	*Bacillus thuringiensis* and *Saccharopolyspora spinosa*
Imidacloprid	Honeybee Fruit fly Fruit fly Whitefly Bed bug	*Apis mellifera* *Bactrocera tau* *Drosophila melanogaster* *Bemisiatabaci* *Cimex hemipterus*	Bifidobacterium sp., *Lactobacillus* sp., *Klebstellaoxytoca*, *Pantoeaagglomerans*, *Staphylococcus* sp. *Lactobacillus* sp., *Rickettsia* sp., *Frischella* sp. *Wolbachia* sp., *Yersinia* sp., *Bacillus* sp., and *Acetobacter* sp.
Fenitrothion	Bean bug	*Riptortuspedestris*	*Burkholderia*sp
Pyriproxyfen	Silkworm Whitefly	*Bombyx mori* *Bemisiatabaci*	*Burkholderia* sp., *Rhizobia* sp., *Rickettsia* sp., *Caulobacter* sp., *Sphingobacteria* sp., and *Enterobacteria* sp.
Chlorpyriphos	Diamondback moth	*Plutellaxylostella*	*Enterococcus* sp., *Enterobacter* sp., and *Serratia* sp.
Acetamiprid	Honeybee Whitefly	*Apis mellifera* *Bemisiatabaci*	*Snodgrassellaalvi, Bartonella apis, Frischellaperrara, Lactobacillus kunkeei, Bifidobacterium asteroids, Gilliamellaapicola*, and *Rickettsia* sp.
Spinosyns	Diamondback moth	*Plutellaxylostella*	*Bacillus thuringiensis* and *Saccharopolyspora spinosa*
Pendimethalin	Ground beetle	*Pterostichus melas*	*Enterobacter* sp., *Pseudomonas* sp., *Pantoea* sp., and *Paracoccus* sp.
Sulfoxaflor	Cotton aphid	*Aphis gossypii*	*Buchner* sp. and *Arsenophonus* sp.
Avermectin	Gypsy moth	*Lymantria disparasiatica*	*Weissella* sp., *Lactobacillus* sp., *Pseudomonas* sp., *Candida* sp., *Tausonia* sp., *Chaetomium* sp., *Diutina* sp., and *Alternaria* sp.
Buprofezin	Small brown planthopper	*Laodelphaxstriatellus*	*Wolbachia* sp. and *Rickettsia*sp
Boscalid	Honeybee	*Apis mellifera*	*Gilliamella* sp. and *Lactobacillus* sp.
Spiromesifen	Whitefly	*Bemisiatabaci*	*Rickettsia* sp.
Glyphosate	Colorado potato beetl	*Leptinotarsa decemlineata*	*Agrobacterium* sp., *Ochrobactrum* sp., *Rhodobacter* sp., *Rhizobium* sp., and *Acidovorax* sp.
Fenitrothion	Bed bug	*Cimex hemipterus*	*Wolbachia* sp., *Yersinia* sp., and *Bacillus* sp.
Spiromesifen	Whitefly	*Bemisiatabaci*	*Rickettsia*sp
Atrazine	Jewel wasp	*Nasoniavitripennis*	*Serratiamarcescens* and *Pseudomonasprotegens*
Thiamethoxam	Whitefly Honeybee	*Bemisiatabaci* *Apis mellifera*	*Delftia* sp., *Rickettsia* sp.,*Bifidobacterium* sp., *Lactobacillus* sp. *Alphaproteobacteria* sp., and *Gammaproteobacteria*sp.
Deltamethrin	Diamondback moth Mosquitos Cotton aphid	*Plutellaxylostella* *Anopheles albimanus* *Aphis gossypii*	*Enterococcus mundtii*, *Carnobacteriummaltaromaticum*, *Bacillus* sp., *Buchner* sp., *Pseudomonas* sp., *Pantoeaagglomerans* and *Pseudomonas fragi*
Malathion	Fruit fly	*Bactrocera tau*	*Klebstellaoxytoca*, *Pantoeaagglomerans*, and *Staphylococcus* sp
Carboxamide	Honeybee	*Apis mellifera*	*Alphaproteobacteria* sp. and *Gammaproteobacteria* sp.
Phosphine	Red flour beetle	*Triboliumcastaneum*	*Bacillus subtilis*, *Staphylococcus* sp., *Enterobacter* sp., Lysinibacillus*fusiformis*, *Klebsiella pneumonia*.

Pesticide biodegradation: In FAW, resistance to pesticides has been confirmed. It has been found that they are very beneficial for degrading toxic compounds due to their digestion abilities [70] [57]. The degradation of pesticides depends on various factors such as microbial remediation and the chemical hydrolysis process, which are additionally correlated with many physiological properties such as pH, temperature, organic matter, and moisture content. However, the FAW gut provides a favourable environment for developing diverse microbial communities. Hence, they efficiently deliver many promising facilities to their host [71]. Symbiotic microbial species isolated from FAW gut can perform in extreme environmental conditions to degrade pesticides and other emerging pollutants [72] [15].

Initiating a leaky gut syndrome: Mason et al. [73] developed axenic and gnotobiotic methods for *Spodoptera frugiperda*and tested how particular members present in the gut community influence interactions with plant defenses that can alter peritrophic matrix (PM) permeability. Further, many plant defenses that deter insect herbivory target the attacker's digestive system. They found that plant defenses against the fall armyworm created opportunities for resident gut microbes to penetrate protective gut barriers, invading the body cavity and exacerbating the negative impacts of plant defenses on the insect. These interactions triggered insect immune responses and collectively overwhelmed the insect's ability to cope with multiple stressors. However, the effects varied between bacterial taxa, indicating that variation in the caterpillar microbiome can alter their phenotype. Their results reveal a previously unrecognised and likely widespread mechanism allowing the plant to use the insect's gut microbiota against it in collaboration with the plant's own defenses.

Modulate plant defense responses: Acevedo et al. [34] experimented to study how fall armyworm-associated gut bacteria modulate plant defense responses. Mechanical damage caused by insect feeding, along with components present in insect saliva and oral secretions, is known to induce jasmonic acid-mediated defense responses in plants. They investigated the effects of bacteria from the oral secretions of the fall armyworm *S. frugiperda* on herbivore-induced defenses in tomato and maize plants. By using culture-dependent methods, they identified seven different bacterial isolates belonging to the family Enterobacteriaceae from the oral secretions of field-collected caterpillars. Two isolates, *Pantoeaananatis* and Enterobacteriaceae-1, downregulated the activity of the plant defensive proteins polyphenol oxidase and trypsin proteinase inhibitors (trypsin PI) but upregulated peroxidase (POX) activity in tomatoes. *Raoultella* and *Klebsiella* sp. downregulated POX but upregulated trypsin PI in this plant species. Conversely, all of these bacterial isolates upregulated the expression of the herbivore-induced maize proteinase inhibitor (Mpi) gene in maize. Plant treatment with *P. ananatis* and Enterobacteriaceae-1 enhanced caterpillar growth on tomato plants but diminished their growth on maize plants. Their results highlight the importance of herbivore-associated microbes and their ability to mediate insect-plant interactions differently in host plants fed on by the same herbivore.

Gut microbiota dysbiosis influences metabolic homeostasis: According to Chen et al. [74], gut microbiota dysbiosis influences metabolic homeostasis in *Spodoptera frugiperda.* They fed *S. frugiperda* larvae an artificial diet with an antibiotic mixture (penicillin, gentamicin, rifampicin, and streptomycin) to perturb the gut microbiota, and then examined the effect of gut microbiota dysbiosis on *Spodoptera frugiperda*gene expression by RNA sequencing. Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were the most dominant phyla in *Spodoptera frugiperda*. Further, they found that the composition and diversity of the gut bacterial community changed in *S. frugiperda*after antibiotic treatment. Firmicutes were decreased, and the abundance of Enterococcus and Weissella genera was dramatically reduced. Finally, they concluded that dysbiosis of gut microbiota caused by antibiotic exposure affects energy and metabolic homeostasis in *Spodoptera frugiperda*, which helps better understand the role of gut microbiota in insects.

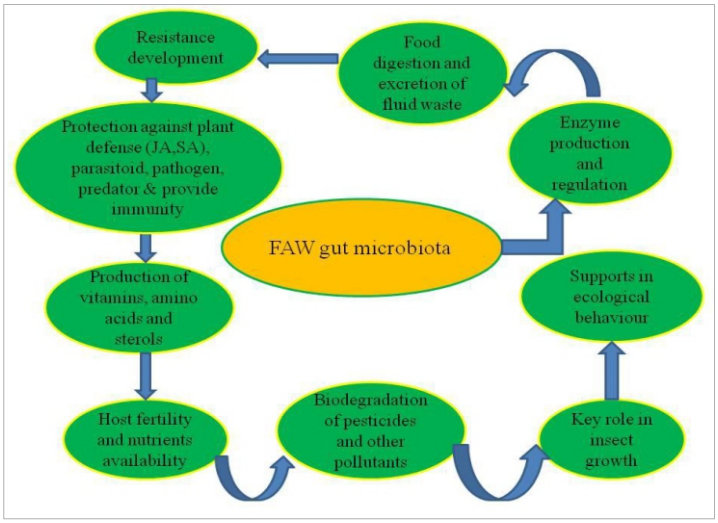

Fig. 2. Major roleof fall armyworm gut microbiota

Conclusion

Cultivation of genetically modified crops has increased annually, leading to a decrease in the use of pesticides, yet there are numerous problems associated with their approval due to a lack of safety issues regarding human health. Further, the functionality of biopesticides is slow as compared to chemical pesticides, therefore insect pest control methods with faster application are needed. The microbiome of the insect gut plays a crucial role in shaping the host insect's physiology. The advent of metagenomics and transcriptomics paved the way to identify the precise role of these symbionts so that one can target the microbiome to impede the development of the paricular host. The information gathered via these techniques would allow us to explore different ways of exploiting the metabolism of symbionts for pest control.

Further, the gut microbiota associated with *Spodoptera frugiperda*in different life stages plays an important role in insect success and adaptability by regulating insect metabolism, food digestion and absorption, excretion of waste fluids, boosting immunity, giving protection against pathogens, predators, and parasitoids, enhancing host fertility, developing resistance against pesticides, and further degrading pesticides into less toxic products. However, so far, the function of gut microbiota in *Spodoptera frugiperda* remains to be investigated. But, with the development of modern approaches such as high-throughput sequencing of the 16S rRNA gene and meta-genome analysis, the research hindrance has been overcome. In another sense, recent advances in independent culture methods such as next-generation sequencing, BLAST analysis and 16S rRNA analysis have provided new insights into understanding the gut microbial diversity and their functions with *Spodoptera frugiperda*.

Future scope of the study

So far, the function of gut microbiota in *Spodoptera frugiperda* remains to be investigated. The detailed investigation of the gut microbiota of *S. frugiperda* provides a basis for future research. Since the plasticity of insect gut microbes helps insects utilize different foods and enhances adaptation of insects, a comprehensive understanding of *S. frugiperda's* gut microbiome will help in future the development of novel pest control strategies for preventing this invasive pest. These review article provides a base and some hints to further investigate the roles of the gut microbiota in the growth, development and reproduction of insects.

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