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Gut Microbiota of Cotton Leafworm (*Spodoptera littoralis*): A brief Review

***Ahmed M. H. Ali*[*,**] *and Emad M. Abdallah*[*]**

[*]*Department of Laboratory Sciences, College of Sciences and Arts at Al-Rass, Qassim University, Saudi Arabia.*
[**]*Department of Zoology and Entomology, Faculty of Science, Assiut University, Assiut, Egypt.*

(Corresponding author: Ahmed M. H. Ali)
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ABSTRACT: Gut microbiota of insects is essential for their survival. They play an important role in nutrition, modulation of immune responses, protection from pathogens and communication. The cotton leaf worm (*Spodoptera littoralis*) is a serious polyphagous pest in Egypt which attacks a wide range of cultivated plants in Egypt alone. It is difficult to control with the regular insecticides and other control measures. In this brief review, the gut microbiota of *Spodoptera littoralis* is discussed in the context of understanding more about the bioactive interactions and relationships of microorganisms inside this pest for developing new strategies of effective biological control as an alternative to the current chemical control.

INTRODUCTION

Insects are the most successful animals, they are comprises about three-fourths of the total living organisms in the animal kingdom with tremendous benefits and applications in the industry (silkworm, honeybee, dye insect, lac insect, and aesthetic insect), as a food source (grasshoppers, crickets, termites, ants, grubs, moths, caterpillars, and pupae), or as potential predators and parasites of destructive pests (Lepidoptera, Orthoptera and Diptera) (Lokeshwari and Shantibala, 2010). On the other side, insects can serve as vectors of many serious and threatening diseases to humans and domestic animals and as dangerous pests to some economic crops.

A. The gut of insects

The general structure of gut has three major regions: foregut, midget, and hindgut (Fig. 1). The foregut and hindgut are lined with chitin and cuticular glycoproteins. The foregut is developed to temporary store the food. The midgut is the primary site of digestion and absorption and the hindgut including separated sections such as fermentation chambers and a rectum for holding feces before defecation (Chapman *et al*., 2013). Undoubtedly, numerous symbiotic microorganisms (mostly bacteria) entering into different mutualistic relationships with eukaryotic multicellular organisms.

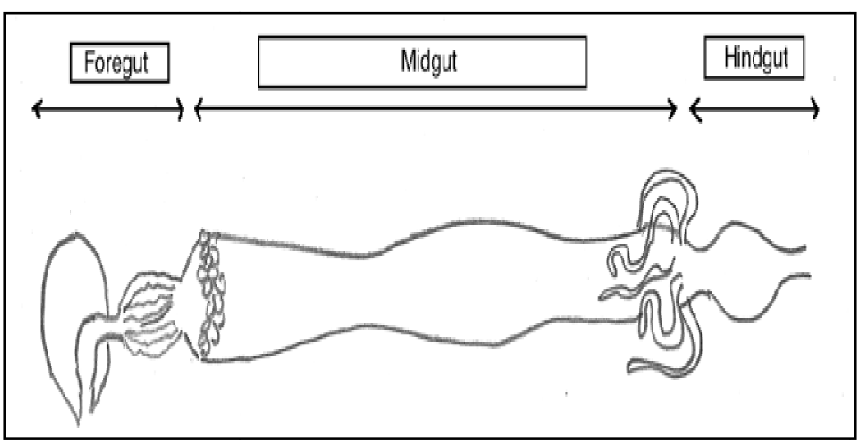

Fig. 1. The general structure of gut of *S. littoralis.*

The alimentary canal of almost all animals is colonized by different types of symbiotic microbes which play a vital role in nutrition, modulation of immune responses, protection from pathogens and communication. Insects as the largest and most diverse animal classes which greatly rely on their gut microbiota for some of its bioactive processes. However, the gut of insects contain few microorganisms compared to guts of higher animals, like mammals, also the characteristics of the insect's microbiota are unstable and vary during insect development (Engel and Moran, 2013). This brief review aimed to discuss the vital role of gut microbiota of *Spodoptera littoralis* in the context of understanding more about the symbiotic interactions and relationships of microorganisms in this pest insect for developing new strategies for pest biocontrol.

B. The impact of microbiota on insects

Although numerous investigations have been performed to understanding the role of gut microorganisms in insects, these symbiotic relationships are still far from fully understood and requires more extensive studies (Pernice *et al.,* 2014). Many integrated factors (biological and environmental factors) influences the microbiota composition in the gut of insects, but diet remains the major factor in all animals (invertebrates or vertebrates) (Lozupone *et al.*, 2012). There is a strong association between insects and different microorganisms such as some protozoans, bacteria and fungi, which play an intrinsic role in digestion of hard or poor diets, improve host immune response, protect insects from heat stress, and detoxify some metabolites produced by plants as defense or repellent agents (Almeida *et al.,* 2017). As an example of the direct effect on insects, an interesting study on the microbiota of mosquito (*Aedes aegypti*) showed that the exposure to some environmental bacteria during larval development can modulate or carry over the ability of adult mosquitoes to transmit human pathogens (Dickson *et al.,* 2017). Recently, some studies claim that resistance of some pests to insecticides could be attributed to insecticide-degrading bacteria live inside the guts of these insects (Almeida *et al.,* 2017).

C. *Spodoptera littoralis* and its microbiota

The cotton leaf worm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) is considered as a well-known insect pest that infests solanaceous like cotton plants (*Gossypium hirsutum* L.), as well as tobacco (*Nicotiana tabacum* L.) and corn (*Zea mays* L.), in Mediterranean and Asian regions. Larvae are polyphagous, causing critical losses to various industrial and vegetable crops (Kandil *et al.,* 2003: Martins *et al.,* 2005). Previously, using chemical pesticides such as lufenuron, cyfluthrin, fenpropathrin, mephospholan, and other organophosphorus synthetic pyrethroids as a control is a highly effective method to reduce the pest treat level in many countries. Recently, this approach is not attractive due to residual toxicity on humans, plants, and other animal species, especially predators and parasitoids of important pests (White, 1995; El- Torkey 2008; Rizk *et al.* 2010). Additionally, this serious pest has developed resistance towards almost synthetic and microbial insecticides (Sarfraz *et al.*, 2006: Mosallanejad and Smagghe, 2009). Recently, using pathogens in the microbial control program of insect pests is considered an alternative strategy in the Integrated Pest Management (IPM) (Hamm 1984). Numerous microorganisms such as viruses, bacteria, nematodes, protozoa, and fungi that infect or intoxicate the pest are have been used as biological pesticides (Khetan, 2001). Additionally, several studies have been carried out on microbial control agents as a biological control of *Spodoptera littoralis* (Farag, 2008; Masetti *et al.,* 2008). In general, studies regarding gut microbiota of “order: Lepidoptera” are scant and received less attention, based on the primitive thought that Lepidoptera members have a simple digestive tract and do not harbor diverse microbial load (Pandiarajan and Krishnan, 2018). The lepidopteran larval gut is extremely high in pH (above 10), which make them selective in eco-environment for its microbiome, explaining highly phytophagous properties of the order Lepidoptera (Chen *et al.,* 2016). Table 1, showing some of bacterial species live in the gut of *Spodoptera littoralis*. Also, Çakici *et al.* (2014) cited that the bacterial microbiota of *Spodoptera littoralis* was found to be *Enterobacter* sp., *Klebsiella* spp., *Flavobacterium* sp., *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Staphylococcus* sp. Moreover, studies on 16S rRNA contents revealed that the gut microbiotas of *S. littoralis* are co-developed with the host, bacteria such as *Pantoea* spp., *Citrobacter* spp. and *Clostridium* spp. were among the most active bacteria in early-instar, but with the development of the insect *Enterococcus* spp. become the dominant bacterial species in the gut of *S. littoralis* which could play an important role in nutritional and defensive mechanism the gut (Shao *et al.,* 2014), the understanding and exploration of the gut microbiota of insects could lead to new approaches of pest biocontrol using some genetically engineered gut flora capable of attack the alimentary canal or the internal organs of these pests when eating infected leaves of the target plants.

Table 1: Some bacterial species living in the gut of *Spodoptera littoralis.**

Gram-positive bacteria	Gram-negative bacteria
Bacillus sp.	*Citrobacter murliniae*
Bacillus subtilis	*Klebsiella pneumoniae*
Bacillus licheniformis	*Enterobacter udwigii*
Bacillus thurungiensis	*Enterobacter asburiae*
Enterococcus sp.	*Pantoea agglomerans*
Enterococcus casseliflavus	*Pseudomonas* sp.
Enterococcus gallinarum	*Pseudomonas otitidis*
Enterococcus durans	*Cedecea davisae*
Enterococcus munditii	*Proteobacteria*
Staphylococcus sp.	*Legionella* spp.
Staphylococcus sciuri	*Bacteroidetes*
Clostridium sp.	-
Actinobacteria	-

*Source of data: (Pandiarajan and Krishnan, 2018, Shao *et al.*, 2014).

D. Prospects for biocontrol of Spodoptera littoralis

In searching for biocontrol agents to be used against *Spodoptera littoralis*, some studies were conducted using specific fungi as a biocontrol agent. Ahmed and El-Katatny (2007) recommended two fungal isolates, namely *Trichoderma harzianum* T24 and *Beauveria bassiana* IMI 382302, as effective myco-insecticides against *Spodoptera littoralis*, which showed immune-dependant sensitivity to this pest. Alobaidi and Samir (2011) found that a fungus (*Beauveria bassiana*) showed a direct effect on different stages of *Spodoptera littoralis*, recommended it as a good agent for biocontrol of this pest. Sneh *et al.* (1983) used a combination of bacteria (*Bacillus thuringiensis* subsp. *entomocidus*) and parasitic wasp (*Bracon hebetor*) to get mortality up to 70% of *Spodoptera littoralis* larvae and even the cotton leaf area consumed by the surviving larvae was significantly smaller than that consumed before treatment. Çakici *et al.,* 2014) mentioned that *Bacillus thuringiensis* subsp. *kurstaki* from *Malacosoma neustria* was the most promising bacterial biocontrol agent against all larval stages of *Spodoptera littoralis*. Actually, microbial pest-control such as viruses, bacteria, fungi, protozoa are becoming an important factor in crop protection, which are natural disease-causing microorganisms with less or no side effect on the human consumer (Khetan, 2001). However, the absence of adequate knowledge of the microbial microbiota of these pests will stay a barrier against the development of these microbial biocontrol programs. Accordingly, further studies on the microbiota diversity, mechanism and symbiotic interaction with these pests are required.

CONCLUSION

The cotton leafworm (*Spodoptera littoralis*), is a serious polyphagous pest herbivore that consumes huge amounts of economic plants in a short period of time. However, studies on the gut microbiota of this pest are still not adequate and only a few reports comparing metabolic activities in the microflora of this insect associated with lifecycle stages. Interestingly, these few studies revealed relatively low phylum-level diversity compared to vertebrates. However, the bacterial population of *Spodoptera littoralis* plays an important role in its metabolic activities and defense mechanism against plant phytochemicals and insecticides. Understanding these mechanisms could lead to the development of effective biocontrol strategies.

CONFLICT OF INTEREST

Non declared

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