

University of São Paulo
“Luiz de Queiroz” College of Agriculture

Gut microbiota of the rice and corn strains of *Spodoptera frugiperda*: diversity
and function

Nathalia Cavichioli de Oliveira

Thesis presented to obtain the degree of Doctor in Science.
Program: International Plant Cell and Molecular Biology

Piracicaba
2021

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Biologist

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Prof. Dr. **FERNANDO LUIS CÔNSOLI**

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5. Metaboloma 6. Metatranscritoma I. Título

To my parents, Antoniel and Cristina

To my brother Arthur and my sister-in-law Isabella

To my grandparents Walter and Isola

To God

I DEDICATE

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*You are not an encapsulated bag of skin
Dragging around a dreary little ego.
You are an evolutionary wonder,
A trillion cells singing together in a vast chorale,
An organism – environment,
A symbiosis of cell and soul.*

Jean Houston

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RESUMO

Microbiota intestinal das raças milho e arroz de *Spodoptera frugiperda*: diversidade e função

A microbiota intestinal de insetos é um importante fator que contribui para vários aspectos da fisiologia e ecologia do seu hospedeiro. Neste estudo nós testamos a hipótese de que os simbiontes associados ao intestino de *Spodoptera frugiperda*, uma importante praga agrícola, podem estar desempenhando um papel relevante no processo de adaptação às plantas hospedeiras de suas raças geneticamente distintas, as raças “milho” e “arroz”. Para este fim, caracterizamos a microbiota intestinal das raças utilizando sequenciamento de nova geração do 16S rRNA de espécimes coletados em condições de campo para a avaliação do efeito da raça, da planta hospedeira e da origem da população. Nós também analisamos a composição da microbiota intestinal sob condições controladas, incluindo neste último, a análise do intestino posterior. Também procuramos compreender a origem da microbiota associada às lagartas de duas formas: avaliando a microbiota presente nos alimentos ingeridos e buscando investigar a presença de bactérias nos ovos e tecidos reprodutivos de *S. frugiperda* usando FISH e microscopia confocal. Para explorar os aspectos funcionais destas interações, utilizamos uma abordagem metatranscritômica, para definir o perfil de genes diferentemente expressos no intestino das raças de *S. frugiperda* e o perfil transcricional das bactérias associadas ao intestino dessas raças. Finalmente, utilizamos a abordagem metabolômica de forma holística, comparando o perfil metabólico das raças ao fornecê-las diferentes fontes alimentares. Nós não encontramos diferenças na composição e na estrutura da microbiota intestinal das raças. Verificamos que a microbiota intestinal de *S. frugiperda* é principalmente modulada pelo alimento ingerido, mas não é reflexo dele. Adicionalmente nós fornecemos indicações de transmissão vertical de bactérias por meio da detecção de bactérias nos ovos e oócitos de *S. frugiperda*. Ao nível funcional, encontramos uma comunidade bacteriana metabolicamente ativa que funcionou da mesma forma nas raças de *S. frugiperda*, com exceção de quando a dieta artificial foi oferecida com alimento as lagartas. Em contrapartida, verificamos que as raças respondem diferentemente ao nível transcricional ao alimento ingerido. Da mesma forma, o perfil metabólico do intestino médio das raças também diferiu em cada substrato alimentar. Finalmente, nossas descobertas fornecem apoio adicional para *Enterococcus* como membro central da comunidade bacteriana associada ao intestino larval de *S. frugiperda*, pois este gênero foi encontrado metabolicamente ativo e consistentemente associado ao intestino de *S. frugiperda* em todas as condições analisadas, apoiando a hipótese de que estas bactérias mantêm relações verdadeiras de mutualismo com *S. frugiperda*.

Palavras-chave: Simbiose, Lagarta do cartucho, Ecologia microbiana, 16S rRNA, Metaboloma, Metatranscritoma

ABSTRACT

Gut microbiota of the rice and corn strains of *Spodoptera frugiperda*: diversity and function

The insect gut microbiota is an important factor that contributes to various aspects of the physiology and ecology of their host. In this study we tested the hypothesis that the gut-associated symbionts of *Spodoptera frugiperda*, an important agricultural pest, may be playing a relevant role in the process of adaptation to the host plants of genetically distinct host-adapted strains, known as corn and rice strains. To this end, we characterized the gut microbiota composition of the strains using next generation 16S rRNA sequencing by sampling larvae in field conditions, evaluating the effect of strain, host plant and population origin. We also analyzed the composition of the gut microbiota under controlled conditions, including in the latter the analysis of the hindgut. We also sought to understand the origin of the microbiota associated with the caterpillars in two ways: by assessing the microbiota present in the ingested food, and by investigating the presence of bacteria in the eggs and reproductive tissues of *S. frugiperda* using FISH and confocal microscopy. To explore the functional aspects of these interactions, we used a metatranscriptomic approach to profile differentially expressed genes between strains in the gut of *S. frugiperda* and the transcriptional profile of the bacteria associated with the strains. Finally, we also used metabolomics to compare the metabolic profile of strains fed on different food sources. We found that the composition and structure of the gut microbiota between the strains was not different in the tests performed. We found that the gut microbiota of *S. frugiperda* is greatly modulated by the food ingested but is not a reflex of it. Additionally, we provided indications of vertical transmission of bacteria by detecting bacteria in eggs and oocytes of *S. frugiperda*. At the functional level, we found a metabolically active bacterial community that functioned equally in both strains of *S. frugiperda*, except when the food source was the artificial diet. In contrast, we found that the strains responded differently at the transcriptional level to the ingested food. Similarly, the metabolic profile of the midgut of the strains also differed for each food source tested. Finally, our findings provide additional support for *Enterococcus* as a core member of the bacterial community associated with the larval gut of *S. frugiperda*, as this genus was found metabolically active and consistently associated with the gut of *S. frugiperda* under all conditions analyzed, supporting the hypothesis that *Enterococcus* maintain true symbiotic mutualistic relationships with *S. frugiperda*.

Keywords: Fall armyworm, Symbiosis, Microbial ecology, 16S rRNA, Metabolomics, Metatranscriptomics

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1. INTRODUCTION

The fascinating complexity of the interactions between living things has instigated scientists from all fields to seek a better understanding of the causes and effects of these relationships in the organisms and their surroundings. Symbiosis (from the Greek syn 'together' and bios 'life') is a term widely used to describe the most variable interactions between organisms of different species (De Bary 1879). The symbiosis between microorganisms and multicellular organisms is increasingly receiving attention due to the discovery of the essential roles that microbial symbionts play in the physiology and ecology of host (Charroux and Royet 2012; Clemente et al. 2012), and that the diversity of microbes depends on genetic or environmental factors. Thus, a more comprehensive research of the individual and its responses to the environment required a holistic investigation that includes the relationship between the individual and its associated symbionts. Within this context the term holobiont was originated, which defines the individual and its symbionts as a single selection unit (Gilbert et al. 2012; Guerrero et al. 2013; Margulis 1993; Mindell 1992; Zilber-Rosenberg and Rosenberg 2008). This concept that is still controversial and causes intense debate in its definitions (Bordenstein and Theis 2015; Douglas and Werren 2016; Roughgarden et al. 2018; Skillings 2016). However, even if the heritable aspect of the holobiont is disregarded, the microbial community and its functions to the host may still provide time for the host genome to evolve during periods of changes in the environment (Zilber-Rosenberg and Rosenberg 2008).

Insects are excellent models for the study of microbial symbiosis. Due to its great diversity, it is quite easy to find groups with short life cycles, easy management and rich in microorganism associations (Charroux and Royet, 2012). Symbiosis in insects is a vast and very interesting topic. There are symbionts that inhabit specialized host cells and organs, called bacteriocytes and bacteriome, respectively (Moran and Telang, 1998). These are called primary symbionts that have intimate associations with their hosts to the point of being vital for their survival (Bourtzis and Miller 2003; Douglas 2009; Moran and Telang 1998; Wernegreen 2002). Other symbionts are found adhered externally to the host cells or in the hemocoel, the most notable ones are those that inhabit the intestines of insects. In some cases, this association became so intimate that insects developed specialized cells to harbor bacteria, known as bacteriocytes. These cells are free in the hemocoel or assembled into a specific structure called bacteriome (Smith and Douglas 1987).

Symbionts associated with insects are partly responsible for the wide distribution of these arthropods through a range of habitats. Symbionts allowed insect hosts to explore restricted food sources by supplementing them with essential nutrients, considering their availability or not in the host's diet (Gilbert et al. 2018; Shropshire and Bordenstein 2016). In addition, several symbionts protect insect hosts from xenobiotics contained in their food sources (Tartar et al. 2009), from infection by pathogens (Eichler and Schaub 2002; Oliver and Moran 2009) and parasitism by parasitic wasps (Oliver et al. 2012), besides improving insect resistance to other sources of stress (Montllor et al. 2002), allowing the exploitation of different food resources and consequently new habitats. Moreover, symbionts may also serve as a source of new genes for their hosts through horizontal/lateral gene transfer, potentially providing new functions to their eukaryotic hosts (Frost et al. 2020; Wybouw et al. 2014). Additionally, several of them are important from a medical, veterinary, and agricultural point of view. Consequently, the use of insects as models in addition to providing meaningful ecological answers can at the same time provide solutions to economically important issues.

The insect gut is inhabited by a diverse and abundant microbial community. The gut microbiota can vary depending on specialized structures in the gut, pH values, redox conditions, digestive enzymes, food type and host habitat (Yun et al. 2014). The gut microbiota can contribute with food digestion via production of digestive

enzymes (Anand et al. 2010; Tartar et al. 2009), detoxification of allelochemicals from plants and synthetic insecticides (Adams et al. 2013; Almeida et al. 2017; Kikuchi et al. 2012). Besides, gut microbiota can allow insect hosts to explore suboptimal dietary sources through the production and release of vitamins and essential amino acids (Douglas 2006) and the cycling of nitrogen (French et al. 1976; Hongoh et al. 2008; Ohkuma et al. 1996). One of such example is the case of the western corn rootworm, in which the gut microbiota shifted in response to crop rotation increasing the abundance of *Klebsiella* and *Stenotrophomonas*. This change in the microbiota led to an increase of bacterial enzymes in the gut that could aid food digestion and were insensitive to the soybean cysteine protease inhibitors. In addition to their nutritional contributions, gut microbes can influence the process of species differentiation. Gut microbes of *Drosophila melanogaster* were demonstrated to interfere with the mating choice as they induced changes in the cuticular hydrocarbons that serve as contact sex pheromones (Sharon et al. 2011). Another study with the parasitoid *Nasonia* showed the gut microbiota interferes with the lethality of hybrids in this parasitic wasp (Brucker and Bordenstein 2013).

Lepidoptera is considered one of the most widespread and diverse groups of insects. Furthermore, many lepidopterans are considered important agricultural pests causing huge economic losses every year in several crops, as they own a broad range of host plants (Scoble 1992). The gut microbial communities in lepidopterans are simple and dynamic. Even considering that symbionts are important players in the interactions between insects and plants, currently it is controversial the relevance of microorganisms in lepidopterans. Some investigations point out that due to the group simple food tube morphology, without specialized structures, the high alkalinity of the midgut and rapid food digestion, intestinal bacteria are only temporary and non-resident, reflecting the microbiota of the food ingested (Anand et al. 2010; Hammer et al. 2017). In addition, studies have shown that these bacteria are not functionally important, because caterpillars can survive without bacteria in their gut (Hammer et al. 2017). On the other hand, reports have proved the presence of a microbial core gut that is distinct from the food ingested and capable of actively colonize the gut of lepidopterans (Mason et al. 2020; Teh et al. 2016). Some functional roles in the host have also been found in immunity, nutrition, and suppression of plant defense (Shao et al. 2017). In addition, evidence of vertical transmission has also been observed (Freitag et al. 2014; Teh et al. 2016). Therefore, given the importance of this group, more studies are fundamental to a better understand of microbial associations to develop possible new methods of interventions, and it may shed light upon symbiont–host co-adaptation and how insects acquire their microbial partners.

Within Lepidoptera, the genus *Spodoptera* is widely studied due to its broad geographical distribution, as it can be found in almost all continents (Kergoat et al. 2012). *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae), the fall armyworm, is a widespread and well-known agricultural pest in the Western hemisphere (Johnson 1987) and a recent invasive species to Africa, Asia and Oceania (Goergen et al. 2016, Otim et al. 2018, Johnson 1987, Padhee and Prasanna 2019, Piggott et al. 2021). *S. frugiperda* stands out as a highly polyphagous species that can feed on more than 300 host plants (Montezano et al. 2018). *S. frugiperda* is however widely known as a pest of grasses even though it does not possess a suitable buccal apparatus for this type of plant, due to the high quantity of silica in grasses. Mandibles of the *S. frugiperda* has serrate-like processes adapted to the consumption of dicots or monocots that do not accumulate silica (Pogue 2002). Therefore *S. frugiperda* is primitively a polyphagous, but because of the mandible-type it is thought to have started exploiting cultivated grasses as host plants only recently (Kergoat et al. 2021, Kergoat et al. 2012). This species adds another level of complexity as two host-adapted strains, known as "corn" (CS) and "rice" strains (RS) are recognized. The molecular dating analyses suggested these strains diverged more than 2 Myr ago (Kergoat et al. 2021) The strains are morphologically similar but they differ in several aspects such as their genomes and genetic expression, preferences and performance in host plants, sexual behavior and susceptibility to insecticides (Orsucci et al. 2020,

Silva-Brandão et al. 2017, Schöfl, Heckel and Groot 2009, Ríos-Díez and Saldamando-Benjumea 2011, Dumas et al. 2015, Pashley, Hammond and Hardy 1992, Unbehend et al. 2013, Meagher, Nagoshi and Stuhl 2011, Veenstra, Pashley and Ottea 1995, Pashley, Hardy and Hammond 1995).

Considering the holistic view that a multicellular individual interacts directly and/or indirectly with its associated microbiome and that there are previous reports on the involvement of the gut microbiota in the process of host plant adaptation and speciation in insects, it is likely that symbionts associated with the gut of *S. frugiperda* are playing a role or reflecting the process of strain differentiation in this species. In order to gain a better understanding of this complex system of interactions, we used a variety of molecular methods and techniques.

First, we aimed to characterize the gut microbiota of the strains using 16S rRNA high-throughput sequencing in order to assess whether they harbor a gut microbiota with distinct composition and structure. For this purpose, we evaluated field-collected larvae feeding on different host plants, maize, millet and cotton in the same landscape. Then we compared the microbiota of strains from different populations throughout the Americas on the same host plant, maize, and also on rice plants, and analyzed populations under controlled laboratory conditions using maize and artificial diet as food sources. In the last study, we also sought to better understand the origin of this microbiota in two ways: by characterizing not only the bacteria present in the larval gut but also in the food ingested by them, and by detecting the presence of bacteria in the eggs and reproductive tissues of *S. frugiperda* using FISH and confocal microscopy in order to have clues whether the microbiota was only a reflection of the food ingested or coming from vertical transmission. To explore the functional aspects of these interactions, we used metatranscriptomics to define the transcriptional profile of genes differentially expressed between the strains in the gut of *S. frugiperda* and the transcriptional profile of the associated bacteria. Finally, we used metabolomics to compare the metabolic profile of the strains when feeding on different food sources.

1.1. HYPOTHESIS AND PREDICTIONS

The gut microbiota of *Spodoptera frugiperda* strains is involved in the process of host plant adaptation

1. The gut microbiota will differ from strain to strain when using the same host plant. And changes in the gut microbiota within a strain from one host plant to another will be less conspicuous than changes in between strains in the same host plants;
2. The variation in the composition of the gut microbiota would be higher in between strains than within different populations of a same strain;
3. The functional contribution of the gut microbiota will differ in between strains when using the same host plants;
4. Members of the gut microbiota would be vertically transmitted from mother to offspring.

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2. DYSBIOSIS OF THE LARVAL GUT MICROBIOTA OF *Spodoptera frugiperda* STRAINS ON DIFFERENT HOST-PLANTS

ABSTRACT

The gut microbiota plays important roles in the bioecology of insects, including host plant adaptation and speciation. *Spodoptera frugiperda* has two well-established host-adapted strains with marked differences at the genetic and host plant utilization levels. We investigated whether differences in the gut microbiota would occur between the “corn” (*CS*) and “rice” (*RS*) strains of *S. frugiperda* when feeding on different crops. The gut microbiota of larvae fed on corn and millet was predominantly represented by *Firmicutes* followed by *Proteobacteria*, with an opposite pattern in larvae fed on cotton. No differences were observed between the *CS* and *RS* using PERMANOVA. PCoA analyses resulted in distinct bacterial clusters based on the host plant. Comparisons of strains gut microbiota at the phylum level resulted in differences only for larvae fed on cotton, but differences in the relative abundance of minor representatives at the genus level between strains were observed regardless of the food source used. We also found differences in the potential for functional contribution of bacteria between the strains. In conclusion the gut microbiota of *S. frugiperda* is strongly modulated by the host plant while strains seemed to play a minor role in changing the abundance of members of the gut bacterial community.

Keywords: Fall armyworm; gut microbes; host preference; host utilization; symbiosis, nutritional ecology.

2.1 INTRODUCTION

It is increasingly recognized that microbes, particularly bacteria, play a crucial role in a wide range of aspects of the host physiology, ecology and evolution (Gilbert et al., 2018; E. T. Miller, Svanbäck, & Bohannan, 2018; Shropshire & Bordenstein, 2016). Thus, it is expected that hosts harboring beneficial microbes will have advantages over their peers, and their joint exposure to processes of natural selection will also act on characteristics that will contribute to select the most suitable microbiota to deliver the services required by the host, maintaining therefore the established association (Shapira, 2016). The best symbiont-service providers will increase the fitness of their host and of their own, providing a basis for coevolution (Shapira, 2016). Thus, the host-microbiota coevolution predicts the existence of species-specific gut microbiota composed of beneficial microbes adapted to the host (Shapira, 2016).

The insect gut may harbor a diverse and abundant microbial community. The composition of the gut microbiota is prone to variations depending on the existence of specialized structures in the gut, gut pH, redox conditions, digestive enzymes, antimicrobial peptides, food type, and host habitat among others (Ryu et al., 2008; Yun et al., 2014). The gut microbiota can contribute with food digestion through the synthesis and release of digestive enzymes (Anand et al., 2010; Krishnan et al., 2014), and the detoxification of plant allelochemicals and synthetic insecticides (Adams et al., 2013; Almeida, Moraes, Trigo, Omoto, & Cônsoli, 2017; Kikuchi et al., 2012). Moreover, the gut microbiota can provide the host with vitamins and essential amino acids (A. E. Douglas, 2006; Nikoh, Hosokawa, Oshima, Hattori, & Fukatsu, 2011), as well as recycle waste nitrogen (French, Turner, & Bradbury, 1976; Ohkuma, Noda, Usami, Horikoshi, & Kudo, 1996), allowing the host to establish new associations with suboptimum food sources. In addition to their nutritional contributions, gut microbes can influence the process of species differentiation. Gut microbes of *Drosophila melanogaster* interfere with the mating choice as they influence the hydrocarbon composition of the cuticle that serve as contact sex pheromones (Sharon, Segal, Zilber-Rosenberg, & Rosenberg, 2011)]. Gut microbes were also shown to influence species differentiation by inducing hybrid lethality in the parasitic wasp *Nasonia* (Brucker & Bordenstein, 2013).

The fall armyworm *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) is a severe, widespread and well-known agricultural pest that was restricted to the Americas (Johnson, 1987), but has recently invaded the Old-World through Africa (Goergen, Kumar, Sankung, Togola, & Tamò, 2016; Otim et al., 2018)], and has now reached the far east Asia (H. Liu et al., 2019; Padhee & Prasanna, 2019)]. There are two well-characterized host-adapted strains of *S. frugiperda* that regardless of the genetic differences (Dumas et al., 2015; Gouin et al., 2017; Roy et al., 2016) and postzygotic mechanisms of reproductive isolation (Kost, Heckel, Yoshido, Marec, & Groot, 2016)] identified so far, are still defined as strains carrying different bioecological traits belonging to a single species. Molecular dating analyses indicate these strains diverged more than 2 Myr ago (Kergoat et al., 2012)]. The two strains are identified as the rice (*RS*) and the corn (*CS*) strains, and they show a high level of genetic differentiation (Gouin et al., 2017)], with differences in host plant utilization (Pashley, Hardy, & Hammond, 1995; Veenstra, Pashley, & Ottea, 1995). The *CS* feeds preferentially on corn, millet, cotton and sorghum, whereas the *RS* on rice and several pasture grasses (Cano-Calle, Arango-Isaza, & Saldamando-Benjumea, 2015; Pashley, 1986; Pashley, Johnson, & Sparks, 1985)]. *CS* and *RS* also have different rates of development and fitness depending on the host-plant used (Busato et al., 2005; Meagher, Nagoshi, Stuhl, & Mitchell, 2004; Pashley et al., 1995; Veenstra et al., 1995). They also differ in mating behavior, such as mating allochronism (Pashley, Hammond, & Hardy, 1992; Schöfl, Dill, Heckel, & Groot, 2011)] and pheromone composition (Groot et al., 2008; Lima & McNeil, 2009)]. A broad study of different populations from Brazil recently recognized several molecular markers and loci under selection when comparing the different strains feeding on different host plants (Silva-Brandão et al., 2018)].

Considering the participation of the gut microbiota in the processes of host adaptation to new food resources and of speciation, we hypothesized the gut microbiota may be involved in the process of host-strain adaptation in *S. frugiperda*. To our hypothesis hold true, we predict that the gut microbiota of the strains differs from each other and therefore we also expect a different functional contribution from each other in exploiting similar host plants. In addition, we predicted that alterations in the gut microbiota within one strain from one host plant to another would be less conspicuous than the changes in the microbiota between strains in the same host plant.

Our investigation addressed field-collected insects, which carry a much higher variation in the gut microbiota than those maintained under controlled laboratory conditions (Gomes, Omoto, & Cônsoli, 2020). Assessing the variation available under field conditions can provide essential information on potential symbionts that could be ecologically important to their hosts in their natural habitats. The selection pressure in natural and laboratory conditions are quite different and can lead to the selection of distinct traits, including the interaction with symbiotic bacteria (Paniagua Voirol, Frago, Kaltenpoth, Hilker, & Fatouros, 2018).

2.2 CONCLUSIONS

In conclusion, our data demonstrate that the larval midgut of *Spodoptera frugiperda* harbor a bacterial community that varies according to the host plant. We also demonstrate that the midgut bacterial community consisted predominantly of Firmicutes followed by Proteobacteria when the larva feeds on corn and millet, with an opposite pattern when the larva feeds on cotton, regardless of the host strain of *S. frugiperda*. Differences at the genus level between the bacterial community of the *CS* and *RS* and predicted functional groups of low abundance were also detected. Studies of the gut microbes of this important agricultural pest can provide new knowledge not only for their control, but also for a better understanding of processes of host adaptation and evolution in insects.

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3. HOST-ADAPTED STRAINS OF *Spodoptera frugiperda* HOLD AND SHARE A CORE MICROBIAL COMMUNITY ACROSS THE WESTERN HEMISPHERE

ABSTRACT

The Fall armyworm *Spodoptera frugiperda* is an important polyphagous agricultural pest in the Western Hemisphere and currently invasive to countries of the Eastern Hemisphere. This species has two host-adapted strains named “rice” and “corn” strains. Our goal was to identify the occurrence of core members in the gut bacterial community of fall armyworm larvae from distinct geographical distribution and/or host strain. We used next-generation sequencing to identify the microbial communities of *S. frugiperda* from corn fields in Brazil, Colombia, Mexico, Panama, Paraguay, and Peru, and rice fields from Panama. The larval gut microbiota of *S. frugiperda* larvae did not differ between the host strains neither was it affected by the geographical distribution of the populations investigated. Our findings provide additional support for *Enterococcus* and *Pseudomonas* as core members of the bacterial community associated with the larval gut of *S. frugiperda*, regardless of the site of collection or strain, supporting the hypothesis that these bacteria hold true mutualistic symbiotic relationships with fall armyworm.

KEYWORDS: Microbial ecology, dysbiosis, symbiosis, Fall armyworm

3.1 INTRODUCTION

The complexity and wide variety of host-microbe interactions are increasingly evident through new molecular techniques and the improvement of bioinformatic analysis tools. The advancement of understanding of this topic has brought support to some hypotheses and challenged others. An example is the discussion about whether the gut microbiota is relevant for all animals (Hammer, Sanders, & Fierer, 2019). The gut is a rich environment for holding a variety of host – microorganism associations, and the gut microbiota has been shown to play crucial roles in a wide range of aspects of host physiology, morphology and ecology. The insect gut microbiota can influence intra and interspecific interactions, such as sexual behavior (Sharon et al., 2010; Sharon, Segal, Zilber-Rosenberg, & Rosenberg, 2011) and the relationship between host plants and natural enemies (Frago, Dicke, & Godfray, 2012). It also plays a key role in insect adaptation to their environment by providing essential nutrients (A. E. Douglas, 2009; Engel & Moran, 2013) and/or boosting the host immune response to parasites and pathogens (Azambuja, Feder, & Garcia, 2004; Cavichioli de Oliveira & Cônsoli, 2020). In addition, microbial symbionts can act in detoxifying xenobiotics as insecticides (Almeida, Moraes, Trigo, Omoto, & Cônsoli, 2017; Chen et al., 2020; Gomes, Omoto, & Cônsoli, 2020; Kikuchi, Hosokawa, & Fukatsu, 2011).

Such range of beneficial contributions has led to the establishment of true mutualistic associations in several groups of hemipterans, dipterans, blattids, and coleopterans, among others (Cheng et al., 2017; Chu, Spencer, Curzi, Zavala, & Seufferheld, 2013; Hosokawa, Kikuchi, Nikoh, Shimada, & Fukatsu, 2006; Kikuchi et al., 2011; Koch & Schmid-Hempel, 2011; Salcedo-Porras, Umaña-Díaz, Bitencourt, & Lowenberger, 2020). Lepidopteran larvae, however, have been thought not to have established mutualistic associations with their gut-associated bacteria. Some studies demonstrated the survival, development time, and weight gain were not affected in antibiotic-fed larvae (Hammer, Janzen, Hallwachs, Jaffe, & Fierer, 2017). Additionally, the lack of special regions in the gut to house microorganisms has been argued as a strong limitation for the establishment of true associations with free-living microbes (Appel, 2017). The harshness of the extremely alkaline conditions of the gut to most microorganisms also represents an unfavorable condition for establishing microbial associations (Dow, 1984). Finally, the high variation in the composition of the microbial community driven by host plants would difficult the occurrence of associations that

could hold through the required evolutionary time in order to allow the selection and establishment of true gut residents (Gayatri Priya, Ojha, Kajla, Raj, & Rajagopal, 2012). Nevertheless, other studies have shown that even in hostile environments as the midgut of lepidopteran larvae, there are evidence of gut colonization by certain bacterial groups (Mason et al., 2020; Mazumdar et al., 2020; Teh, Apel, Shao, & Boland, 2016). In addition, gut-resident bacteria of lepidopteran larvae were demonstrated to play important physiological roles for their hosts (Yongqi Shao, Arias-Cordero, Guo, Bartram, & Boland, 2014; Xia, Lan, Tao, Lin, & You, 2020); besides, the continuous association with their hosts for some of these microbes has been proved as they are horizontally transmitted (Y. Shao et al., 2017).

Controversial topics in the scientific literature are always an invitation to new studies aiming at better understanding and clarification of the topic. The debated existence of true gut-associates in lepidoptera is a subject that needs further clarification due to two important contexts it is placed in. First, its remarkable relevancy to the understanding of how microbial associations can influence host phenotypes (Moya, Pereto, Gil, & Latorre, 2008), and insects have provided simple models for the clarification of fundamental principles in host-microbe interactions (Angela E. Douglas, 2011; Kostic, Howitt, & Garrett, 2013), with a great potential to assist in unravelling complex systems such as in mammalians. Second, lepidopterans are yet the major group of agricultural pests, causing severe losses in food production, posing a serious threat to food security (McCaffery, 1998; Riegler, 2018; Scoble, 1992), and understanding the diversity and function of gut microbes associations can lead to the development of new strategies for herbivore control.

In the present study we have chosen a lepidopteran species that is important both in the ecological and in the economic context to investigate the existence of true gut associates of lepidopteran larvae. *Spodoptera frugiperda* is an important agricultural pest in the Western Hemisphere and is currently invasive to countries in Africa, Asia, and Oceania (Goergen, Kumar, Sankung, Togola, & Tamò, 2016; Johnson, 1987; Otim et al., 2018; Padhee & Prasanna, 2019; Piggott, Tadde, Patel, Gomez, & Thistleton, 2021). *Spodoptera frugiperda* is highly polyphagous, feeding on more than 300 host plants (Débora G. Montezano et al., 2018). This species is actually a complex composed of two distinct strains known as the rice (RS) and corn (CS) strains. The two strains are morphologically identical, with clear differences in host preference, susceptibility to insecticides and transgenic crops (*Bacillus thuringiensis*), composition of sex pheromone and mating behavior (Adamczyk Jr, Holloway, Leonard, & Graves, 1997; Cruz-Esteban, Rojas, Sánchez-Guillén, Cruz-López, & Malo, 2018; Ingber, Mason, & Flexner, 2018; Lima & McNeil, 2009; Orsucci et al., 2020; Pashley, Hardy, & Hammond, 1995; Schöfl, Heckel, & Groot, 2009; Veenstra, Pashley, & Ottea, 1995). Genomic analysis of the host-adapted races of *S. frugiperda* identified several genes involved in the chemodetection of non-volatile molecules and detoxification of xenobiotics showing signatures of positive selection, suggesting their contribution to their host plant preferences (Gouin et al., 2017). Some of these genomic variations between host races of *S. frugiperda* were also detected at the transcriptional level, including those involved in xenobiotic metabolism (Silva-Brandão et al., 2017).

Genetic studies suggest that population structure of *S. frugiperda* in the Western Hemisphere shows more variation within *S. frugiperda* populations than between populations of different locations, indicating a significant gene flow (Clark et al., 2007; Kondidie, 2011). The Mexican populations, on the other hand, have proven to be the most different, suggesting limited migratory interactions with foreign populations (Nagoshi et al., 2015; Tay et al., 2020). The population genetic structure of Brazilian populations of *S. frugiperda* is partially based on host plants, with rice populations, which are basically represent by rice strain individuals, having a strong effect on the overall genetic structure of fall armyworm populations in Brazil (Silva-Brandão et al., 2018).

Therefore, in this study we aim to verify the existence of bacterial groups that remain associated with the gut microbial community of *S. frugiperda* larvae regardless of the geographical region or host plant used. So, we sampled and sequenced the gut microbiota of fall armyworm larvae from corn and rice fields across the American continent. Larvae were genotyped as rice or corn strain, and the structure of the bacterial gut community was checked based on geographical origin of the larvae, host-adapted strain and/or host plant used. Despite the variation expected due to uncontrolled and unforeseen environmental factors, the field conditions may provide essential information on potential symbionts that could be ecologically important to their hosts in their natural habitats.

3.2 CONCLUSIONS

Overall, this study provided an extended view of the fall armyworm gut microbiota and supported the hypothesis that bacterial taxonomic compositions across different localities in the Western Hemisphere are similar to each other, presenting high inter-individual variance, and that there are no significant differences in gut microbiota composition between the host-adapted strains of *S. frugiperda*. Nevertheless, our findings provide further evidence that *Pseudomonas* and *Enterococcus* are true symbionts of *S. frugiperda* as they were identified in the gut microbiota of *S. frugiperda* larvae regardless the host plant and site of collection. Further investigations on the functional contribution of these species as members of the gut bacterial community of fall armyworm larvae is required for a deeper understanding of the nature of this relationship.

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4. DOES THE GUT MICROBIOTA OF THE FALL ARMYWORM STRAINS IS JUST A REFLEX OF THEIR FOOD MICROBIOTA?

ABSTRACT

Insect-associated microorganisms can affect several aspects concerning the physiology and ecology of their hosts. However, the importance of the gut microbiota in Lepidoptera is still controversial. Here we aimed to verify the strength of the association of bacteria to the midgut and hindgut of the corn and rice strains of *Spodoptera frugiperda*, the Fall armyworm (FAW) by comparing the bacterial community hosted in the larval gut with the microbiota present in the offered food, maize leaves and artificial diet. In addition, we investigated whether vertical transmission of bacteria would occur in FAW. We used high-throughput sequencing of the 16S rRNA gene to characterize the composition of the microbiota, and FISH to verify the presence of bacteria in the eggs and reproductive tissues of FAW adults. Diet played an important role in altering the composition of the gut microbiota, but the diversity and structure of the larval gut microbiota was distinctive of the that of the food source. We found no differences in gut microbiota composition between FAW strains when feeding on the same food source, but did observe differences in the bacterial communities between the midgut and hindgut of FAW. Our findings indicate the FAW harbors a microbiota that is independent of the microbiota of the food source used, and that these bacteria is likely inherited through vertical transmission.

KEYWORDS: Symbionts, host plant adaptation, maize, *Spodoptera frugiperda*.

4.1 INTRODUCTION

The advances in molecular and sequencing techniques have greatly expanded our understanding of the microorganisms associated with multicellular organisms and with the environment. The incredible diversity and abundance of these microorganisms and their numerous and pivotal roles in the digestion, defense, behavior, ecology and evolution of their hosts (Charroux and Royet, 2012, Clemente, *et al.*, 2012, Malard, *et al.*, 2021), has led us to shift from a primarily host-centric view to a more holistic perspective that considers the host and its symbionts as a single entity (Gilbert, *et al.*, 2012, Roughgarden, *et al.*, 2018). Insects stand out among the models used to study these interactions because of their great genotypic diversity, easy management, short life cycles, and rich associations with microorganisms (Charroux and Royet, 2012, Ludington and Ja, 2020). Moreover, several of them are important from a medical, veterinary, and agricultural point of view. Consequently, using insects as models provide meaningful ecological information and solutions to economically important issues.

Lepidoptera is considered one of the most widespread and diverse groups of insects and are also considered important agricultural pests (Powell, 2009, Scoble, 1992). The gut microbial communities of lepidopterans are simple and dynamic. Even considering that symbionts are important players in insect-plant interactions, the relevance of microorganisms in larval lepidopterans is currently controversial. Some investigations pointed the simple morphology of the food canal, with the lack of specialized regions to harbor bacteria, and the high alkalinity of the midgut and rapid food transit, would only allow the hosting of temporary, non-resident bacteria, leading to a microbiota that would be similar to the microbiota of the food ingested (Anand, *et al.*, 2010, Hammer, *et al.*, 2017). In addition, lepidopterans-gut inhabiting bacteria were reported to provide non-essential contributions to the larval host, once larvae survived without bacteria in their gut (Hammer, *et al.*, 2017). In controversy, others proved lepidopterans larvae hosted a microbial core community in their guts that is distinctive from that obtained from the food source (Mason, *et al.*, 2020, Teh, *et al.*, 2016). Later investigations demonstrated gut microbes of lepidopteran larvae contribute to host immunity, nutrition, and suppression of plant defenses (Shao, *et al.*, 2017), and can be vertically transmitted (Freitag, *et al.*, 2014, Teh, *et al.*, 2016). However, given the importance that lepidopteran species play as pests of agricultural crops and their

products and the potential for biotechnological exploitation of the associations with their gut microbiota in order to develop new methods of intervention, there are still limited studies aimed at understanding the existence of true symbionts associated with lepidoptera.

Spodoptera frugiperda (Lepidoptera, Noctuidae) is a highly polyphagous (Montezano, *et al.*, 2018) and currently represented by two morphologically indistinct host adapted races, the corn (CS) and the rice (RS) strains, but with distinctive ecology and mitochondrial and chromosomal genome information (Dumas, *et al.*, 2015, Orsucci, *et al.*, 2020, Pashley, *et al.*, 1992, Pashley, *et al.*, 1995, Schöfl, *et al.*, 2011, Silva-Brandão, *et al.*, 2018, Veenstra, *et al.*, 1995). *Spodoptera frugiperda* is native to the Americas, but in the last 6-years has spread to Africa, Asia, and Oceania, becoming a serious worldwide threat to food security (Goergen, *et al.*, 2016, Johnson, 1987, Otim, *et al.*, 2018, Padhee and Prasanna, 2019, Piggott, *et al.*, 2021) due to its polyphagia and capacity to evolve resistance to organic pesticides and Bt-transgenic crops (Carvalho, *et al.*, 2013, Huang, 2020, Ríos-Díez and Saldamando-Benjumea, 2011, Yu, 1991).

Thus, given the economic and ecological relevance of *S. frugiperda*, and the potential of exploitation of the associated microbiota in developing strategies for pest control (Almeida, *et al.*, 2017, Crotti, *et al.*, 2012), it is important to investigate the existing controversy on the true association of gut microbes to lepidopteran larvae for this species. Here we characterized the microbiota associated with the midgut and hindgut of *S. frugiperda* larvae of the RS and CS strains under controlled laboratory conditions and compared with the microbiota associated with food sources used in larval rearing, maize leaves, and artificial diet. In addition, in order to provide additional information on the close association of bacteria and *S. frugiperda*, we also investigated the occurrence of bacterial vertical transmission

4.2 CONCLUSIONS

Our results with the larval gut microbiota of host-adapted strains of *S. frugiperda* do not support the claim that the larvae of lepidopteran do not carry true bacterial associates in their gut and that the gut community is simply a reflection of the bacteria associated with the food source larvae exploit. We also demonstrated bacteria is vertically transmitted in *S. frugiperda*, but future studies are still require to demonstrate these bacteria are indeed the prevalent bacteria inhabiting the larval gut lumen. Our results also highlight that when using the artificial diet in experiments, it is important to consider the high simplification of the caterpillar gut microbial diversity, which does not reflect the condition of this community when the host feeds on a natural diet such as maize. The function of these gut microbes in *S. frugiperda* strains need to be evaluated, as well as the ecological implications of this variability to *S. frugiperda* larval development.

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5. METATRANSCRIPTOME OF THE FALL ARMYWORM HOST STRAINS REVEALED DIFFERENCES IN MIDGUT BUT NOT IN GUT BACTERIAL TRANSCRIPTIONAL ACTIVITY

ABSTRACT

The challenges herbivores face in plant feeding may be attenuated by herbivore-associated microorganisms that aid in the process of host plant adaptation. We assessed the gene expression responses of two host-adapted strains of *Spodoptera frugiperda*, the corn and the rice strains, to different natural and artificial food sources. Assessment of differential gene expression was based on mRNA-based Illumina metatranscriptome sequencing and analysis of the larva midgut and associated gut microbiota. Our analyses revealed the transcriptional activity associated with the gut epithelium of *S. frugiperda* differ between strains in all food sources investigated, with most of the differential expressed genes detected belonging to processes of detoxification, nutrient assimilation and immune response. However, few differences in transcriptional activity of the gut bacteria were detected between the rice and the corn strain to most of the food sources investigated. The only exception in the transcriptional activity of the gut microbiota of both races was the bean-based artificial diet. The gut microbiota of the host-adapted races of *S. frugiperda* responded very differently when larvae fed on the artificial diet. We conclude that the *S. frugiperda* strains have different adaptations to deal with the ingested food, while the associated microbiota plays basically the same role in both strains regardless the natural food sources tested. We also point out that the artificial diet generates a considerable difference in the functioning of the gut microbiota of the strains, mainly on *Enterococcus mundtii*

KEYWORDS: *Spodoptera frugiperda*, symbiosis, gut bacteria, host plant adaptation

5.1 INTRODUCTION

Plant feeding by herbivores represents a tough challenge. Plants utilize a myriad of structural and biochemical defense mechanisms to prevent their attack (Chen 2008, Strauss and Agrawal 1999). Therefore, insects that succeed in herbivory require a series of adaptations to overcome plant defenses and to acquire the nutritional components necessary for their survival and proper development. These adaptations include enzymatic detoxification, followed by excretion or sequestration, physiological tolerance, and/or behavioral avoidance (Zunjarrao et al. 2020). New mechanisms of detoxification have also been achieved through gene recruitment, neofunctionalization, and horizontal gene transfer (Zunjarrao et al. 2020, Wybouw et al. 2014, Heidel-Fischer et al. 2019, Heidel-Fischer and Vogel 2015).

Moreover, many insects benefit from the contribution of associated microorganisms to be successful in the process of adaptation to host plants (Acevedo et al. 2016, Hammer and Bowers 2015). In this context, gut bacteria can play an important role in this process of adaptation because they are present in the organ where food is initially processed and, therefore can respond relatively quickly to environmental changes, such as to plant defense molecules (Hammer and Bowers 2015). It has been reported that gut bacteria can improve the fitness of lepidopterans by protecting their host against pathogens (Shao et al. 2017) and by processing waste nitrogen, particularly ammonia (Rozadilla et al. 2020). The gut microbiota of lepidopterans has also been shown to facilitate digestion and nutrient acquisition (Indiragandhi et al. 2008, Xia et al. 2017), producing enzymes and amino acids, but also aiding in the detoxification of plant-derived (Ceja-Navarro et al. 2015, Zhang et al. 2020) and synthetic (Gomes, Omoto and C onsoli 2020) xenobiotics.

The Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is currently one of the most devastating agricultural pests in the western hemisphere that has recently spread to West and Central Africa, Asia and

Oceania (Li et al. 2020, Goergen et al. 2016, Zarkani, Wibowo and Sipriyadi 2020). The FAW is highly adaptable and polyphagous, feeding on many plants of agricultural importance (Deshmukh et al. 2021, Ashley et al. 1989, Casmuz et al. 2017, Montezano et al. 2018). Additionally, it has been reported to evolve resistance to many classes of pesticides (Carvalho et al. 2013, Gutiérrez-Moreno et al. 2019) and *Bacillus thuringiensis* (Bt)-crops (Chandrasena et al. 2018, Farias et al. 2014, Storer et al. 2010).

The FAW is a species composed of two morphologically identical, but genetically distinct host-adapted strains (Nagoshi and Meagher 2008). They are asymmetrically distributed among host plants in the field. The "corn strain" (CS) infests corn, sorghum, and cotton plants more commonly, while the "rice strain" (RS) is more often collected on rice and pasture grasses (Pashley 1989, Prowell 1998, Silva-Brandão et al. 2018, Juárez et al. 2014). Several differential effects of the host plants on the viability and development of the two strains have been described (Pashley, Hardy and Hammond 1995, Veenstra, Pashley and Ottea 1995, Meagher, Nagoshi and Stuhl 2011, Juárez et al. 2014, Pashley 1986, Meagher et al. 2004, Silva-Brandão et al. 2017). Additionally, the host-adapted strains also differ in their transcriptional activity, particularly in the expression of genes related to xenobiotic metabolism and mitochondrial genes, pointing to a role in driving strain divergence (Orsucci et al. 2020, Silva-Brandão et al. 2017). Moreover, the RS and CS also differ in their susceptibility to organic pesticides (Veenstra et al. 1995, Adamczyk Jr et al. 1997, Ríos-Díez and Saldamando-Benjumea 2011, Yu 1991) and Bt-plants (Adamczyk Jr et al. 1997, Ingber, Mason and Flexner 2018).

There are still few studies that address the functional role of the gut microbiota when compared to studies describing the taxonomic profile of the microbiota in lepidopterans, especially in the FAW host-adapted strains (McCarthy, Cabrera and Virla 2015, Rozadilla et al. 2020). The analysis of the composition of the microbiota is not sufficient to elucidate the real role of the microbiota of any particular host. The similarity in the taxonomic composition may reflect in different contributions to the host when different food substrates are available in the environment, which may alter the production of molecules by the microbiota (Graf et al. 2015). Moreover, some species that are very abundant in the gut community may have poor metabolic activity, and vice-versa (Chen et al. 2016, Shao et al. 2014).

Therefore, considering the limited current knowledge on the functionality of the gut microbiota of lepidopteran larvae (Paniagua Voirol et al. 2018) and the great potential that the gut microbiota has to be used in developing insect pest control methods, it is of paramount importance to deepen the knowledge on the ecology and functional activity of the gut microbial community of FAW. In this study we report the metatranscriptome profiling of the larval midgut of the CS and RS of FAW in order to elucidate the differential activity of the gut epithelium and the microbiota of both host-adapted strains feeding on different food substrates. An important and novel aspect of this study is that we not only identified the metabolically active gut microbiota, but also gained insight into what genes were being expressed.

5.2 CONCLUSIONS

In conclusion, the gut microbiota of *S. frugiperda* is metabolically active and its functioning is similar between rice and corn strains when feeding on the same food, except when larvae fed on artificial diet, where the rice strain showed a large number of upregulated genes when compared to the corn strain. On the other hand, the gut transcriptome of the host *S. frugiperda* was shown to be quite distinct between the strains when feeding on the same diet. Further studies are needed to explore more in details the complex interplay between the host and its microbiota functioning and our analysis provides a suitable framework for the development of further experiments.

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6. NON-TARGETED METABOLICS REVEAL DIFFERENCES IN THE METABOLIC PROFILE OF THE FALL ARMYWORM STRAINS WHEN FEEDING DIFFERENT FOOD SOURCES

ABSTRACT

The moth *Spodoptera frugiperda*, the fall armyworm (FAW), is an important polyphagous agricultural pest feeding on nearly 350 host plants. *Spodoptera frugiperda* is undergoing incipient speciation with two well-characterized host-adapted strains, the "corn" (CS) and "rice" (RS) strains, which are morphologically identical but carry several genes under positive selection for host adaptation. We used a non-target metabolomic approach based on gas chromatography/mass spectrometry to identify differences in metabolite profiles of the larval gut of CS and RS feeding on different host plants. Larvae were fed on artificial diet, maize, rice, or cotton leaves from eclosion to the sixth-instar, when they had their midgut dissected for analysis. This study revealed that the midgut metabolomic profile of FAW differs on the each host plant depending on the host-adapted strain. Additionally, we identified several candidate metabolites that may be involved in the adaptation of CS and RS strains to their host plants. Our results shed light on our understanding of gut metabolic activities of the FAW, which associates the metabolomics of FAW with the metabolomics of the associated microbiota.

KEY WORDS: Metabolite, herbivore, gut microbiota, insect-plant interaction, nutritional ecology.

6.1 INTRODUCTION

Spodoptera frugiperda, the fall armyworm (FAW) (Lepidoptera, Noctuidae) has a remarkable number of host plants, feeding on approximately 350 different vegetal species from 76 families (Montezano et al., 2018). Despite this wide range of host plants, FAW is best known as one of the most important agricultural pests of grasses (maize, millet, rice and sorghum) and some cultivated dicots such as cotton (Barros, Torres, Ruberson, & Oliveira, 2010). The FAW is native from the New World, but in the last few years has invaded Africa and further spread to Asia and Oceania (Goergen, Kumar, Sankung, Togola, & Tamò, 2016; S. J. Johnson, 1987; Otim et al., 2018; Padhee & Prasanna, 2019; Piggott, Tadle, Patel, Gomez, & Thistleton, 2021). Therefore, FAW is currently considered of a global concern due its polyphagy and capacity for rapid evolution of resistance to pesticides and Bt-crops (F. Huang, 2020; Jakka et al., 2016), representing an imminent threat to food security and a source of significant economic losses.

The FAW is the only species of *Spodoptera* that usually feeds on grasses without having adapted, suitable mandibles. Larvae that feed on grasses typically have specialized mandibles with chisel-like edges adapted to the consumption of silica rich leaves, which causes adverse effects to larval mandibles (Brown & Dewhurst, 1975; Djamin & Pathak, 1967; Pogue, 2002; Smith, 2005). Mandibles of the FAW have serrate-like processes adapted to the consumption of dicots or monocots that do not accumulate silica (Pogue, 2002). FAW is primitively a polyphagous, but because of the mandible-type it is thought to have started exploiting cultivated grasses as host plants only recently (Kergoat et al., 2021; Kergoat et al., 2012).

Another interesting aspect of the FAW is the identification of two distinct strains known as the rice (RS) and corn (CS) strains (Gouin et al., 2017; Pashley, 1986). There are indications that this divergence occurred about 2 Myr ago (Kergoat et al., 2021; Kergoat et al., 2012). These strains differ in their performance and preference for host plants, and the correct classification of these two strains of the FAW is still controversial. Some authors refer to them as "sibling species" (Drès & Mallet, 2002; Dumas et al., 2015), "host strains" (Pashley, 1986; Prowell, McMichael, & Silvain, 2004), and "host form" (Juárez et al., 2014). The lack of consensus is due to the fact these strains co-exist in sympatry and still hybridize, but also due to inconsistencies in the associations with the named host plants. At the adult

stage, both corn and rice strains showed weak evidence of preference for their expected host-plant in choice and non-choice laboratory experiments (Robert L. Meagher, Nagoshi, & Stuhl, 2011; Orsucci et al., 2020). Despite the fact that the corn strain is often associated with maize, sorghum and cotton, while the rice strain with rice and pasture grasses, some reports show the rice strain larvae developed better on corn and sorghum than the corn strain larvae (R. L. Meagher, Nagoshi, Stuhl, & Mitchell, 2004). Moreover, both strains poorly performed when feeding on rice (Silva-Brandão et al., 2017). Therefore, further studies are still needed to understand how the process of host plant adaptation is taking place in FAW.

Every novel acquisition of host plants by herbivores constitutes a new niche adaptation program that opens several evolutionary possibilities, but not without associated costs. In order to exploit a novel host, insects have to become adapted to deal with new defensive secondary metabolites, such as phenolics and terpenoids, and the nutritional quality of the new host plant (Singer, 2008). However, the mechanisms behind the best performance of a given host-adapted strain on a given plant are poorly understood so far. Different approaches can be used to address this question. One alternative is to access the insect metabolome, the set of all low molecular weight metabolites that are produced during cell metabolism (Sun & Hu, 2016). Ultimately, the metabolome is a product of genomic, transcriptomic, and/or proteomic perturbations (C. H. Johnson & Gonzalez, 2012). Among the approaches that can be used, non-targeted metabolomics provide a holistic view of the insect's metabolic profile. It makes no assumptions about which metabolites are important in distinguishing sample types (Sévin, Kuehne, Zamboni, & Sauer, 2015). This approach provides a direct functional measurement of cellular activity and physiological state, reflecting environmental changes such as new host plants as well as aspects related to their genome, as different host-adapted strains (Sun & Hu, 2016). Therefore, the non-targeted study of metabolomes is a good tool to point chemical candidates involved in insect-plant interactions (Maag, Erb, & Glauser, 2015). Particularly, the assessment of the insect midgut may be useful, bearing in mind that it is a permeable, metabolically active tissue, in which most digestion and almost all nutrient absorption takes place (Dow, 1987). However, approaches focused on the assessment of the gut metabolomics of insect herbivores are not common, and little is known on how host plants interfere with the profile of primary metabolites of the gut of herbivores.

The gut microbiota is also a key player in the metabolic processes of their hosts. Gut microbes can play important roles in several metabolic functions, including vitamin production (Chen et al., 2016; Salem et al., 2014), amino acid synthesis (Ayayee et al., 2016; X. Xia et al., 2017), and detoxification of secondary plant compounds and synthetic insecticides (Almeida, Moraes, Trigo, Omoto, & Cônsoli, 2017; Ceja-Navarro et al., 2015), for example. Among the numerous factors that influence the gut microbiota (Dillon & Dillon, 2004; Yun et al., 2014), diet has received considerable attention due to its strong effect on the composition of the microbial community (Mason et al., 2020; Wongsiri & Randolph, 1962; Yun et al., 2014). Diet provides the substrates to produce a plethora of small molecules that can be converted by the gut microbiota and which are not produced by the host (Krishnan, Alden, & Lee, 2015; S. Wang et al., 2020). Therefore, the gut microbiota may also facilitate adaptation to new host plants by regulating or participating in the host's metabolic processes (Hammer & Bowers, 2015; Zhang et al., 2020). Microbial contribution will depend on substrate availability and on microbial gene diversity and activity (Wu et al., 2016). Thus, taxonomic or metagenomic information of the gut microbiota is limited in predicting the metabolome of a microbial community, as it may under or overestimate the functional contribution of associated gut microbiota depending on the nutritional conditions the host is exposed to (Wu et al., 2016).

The FAW is a good model to study adaptation of phytophagous insects to agricultural plants. Moreover, the metabolic processes underlying host shifts or differentiation in this species are not well understood. In terms of

metabolome, we would expect different metabolic profiles to reflect new adaptations. This knowledge can help in understanding the processes lepidopteran larvae employ to face challenges as dietary nutritional deficiencies and host plant switches, considering the insect as a unit with their microbes associated. The aim of the present research is to investigate if the gut metabolome of FAW is determined by the diet and/or by host genotype. Highlighting the metabolic differences in the midgut of the FAW strains has the potential to indicate: 1) how different host plants affect insect nutritional metabolism and 2) how larvae of the two host strains differ in their utilization of diverse host plant nutrients.

6.2 CONCLUSIONS

In conclusion, our study demonstrates the effects of host strains and dietary effects on the metabolome of the FAW midgut. we have provided us with an overview of these changes occurring in their metabolomes of the strains on different diets and identified a wide range of marker metabolites that may help us to better understand the mechanisms involved in the host adaptation process. Our results shed light on our understanding of metabolic activities in the FAW, being a unit composed of its own metabolome and the metabolome of the associated gut microbiota. Further analyses are essential to reveal the links between gut microbiota composition and host metabolic phenotype, thus providing a holistic understanding of the functionality and adaptability of strains to host plants.

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