University of São Paulo
"Luiz de Queiroz" College of Agriculture

Genomics of insecticide-metabolizing bacteria associated with *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential role in host detoxification

Ana Flávia Freitas Gomes

Thesis presented to obtain the degree of Doctor in Science.
Area: Entomology

Piracicaba
2023

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To my parents, Dorinha and Didi
To my brother, Arthur
To my fiancé, Diego

I DEDICATE

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TABLE OF CONTENTS

RESUMO

Genômica de bactérias metabolizadoras de inseticidas associadas a *Spodoptera frugiperda* (Lepidoptera: Noctuidae) e sua potencial contribuição funcional na desintoxicação do hospedeiro

O sucesso evolutivo da classe Insecta pode ser parcialmente atribuído à sua associação com bactérias. A associação com microrganismos não patogênicos garante a manutenção de fenótipos complexos, em que hospedeiro e microbiota respondem juntos a condições ambientais adversas. Em estudos anteriores, demonstramos que linhagens resistentes a inseticidas e populações naturais de *Spodoptera frugiperda* carregam em seu intestino bactérias metabolizadoras de inseticidas (BMI), algumas delas fixadas e com capacidade distinta de metabolizar diferentes inseticidas. Com a hipótese de que *S. frugiperda* e sua microbiota se comportam como um organismo único (holobionte), este trabalho tem como objetivo melhor caracterizar as BMI e verificar seu potencial na desintoxicação e na resposta desse lepidóptero a inseticidas. A caracterização das BMI foi realizada por meio de análises genômicas, metatranscritômicas e metabolômicas. Genes e vias associadas à possível metabolização de compostos tóxicos por bactérias foram identificados, bem como aspectos de sua interação e evolução com o hospedeiro. Estudos taxonômicos baseados no genoma permitiram a reavaliação da identidade taxonômica das BMI, destacando a necessidade de reconsiderar identificações bacterianas baseadas apenas no sequenciamento do gene 16S rRNA. Novas espécies bacterianas associadas ao intestino de *S. frugiperda* foram descritas, denominadas *Enterococcus entomosocium* n. sp., *Enterococcus spodopteracolus* n. sp., e *Pseudomonas fraudulenta* n. sp. As demais BMI foram classificadas como *Acinetobacter soli* ou como diferentes subespécies da recentemente descrita *Pseudomonas bharatica*. As BMI analisadas apresentaram um notável repertório enzimático envolvido na degradação de xenobióticos, incluindo carboxilesterases, dehalogenases e desidrogenases e, para algumas delas, enzimas de desintoxicação, como glutationa-S-transferases, superóxido dismutases e peroxirredoxinas. Constatou-se que os isolados de *Enterococcus, Acinetobacter* e *Pseudomonas* carregam em seus genomas o maquinário molecular para interagir com o hospedeiro, incluindo mecanismos para colonização intestinal, fornecimento de nutrientes, modulação da microbiota intestinal e interações com o sistema imunológico do hospedeiro. No entanto, experimentos para a colonização intestinal de *S. frugiperda* por isolados de *Acinetobacter soli* e *Pseudomonas bharatica* sugerem que, para essas espécies, isso ocorre somente quando o inseto hospedeiro está sob pressão de seleção pelo inseticida. A análise compreensiva da resposta de linhagens suscetíveis e resistentes de *S. frugiperda* aos inseticidas flubendiamide, spinosad e teflubenzuron destacou a complexa interação entre o genótipo do hospedeiro, as comunidades microbianas e a exposição ao inseticida no metaboloma larval e na atividade transcricional da microbiota intestinal. A análise revelou que a exposição ao inseticida e a evolução da resistência induzem mudanças metabólicas que afetam a imunidade do hospedeiro, alteram as interações do hospedeiro com sua comunidade microbiana intestinal e, possivelmente, afetam as associações com vírus associados. Por fim, a análise da associação de *E. entomosocium* e *E. spodopteracolus* com outras espécies do gênero *Spodoptera* revelou que elas não apresentam variação genética suficiente para serem separadas como entidades taxonômicas ao nível de espécie ou subespécie, mostrando que essas bactérias não acumularam variação genética suficiente após o processo de especiação de *Spodoptera,* devido ao processo de co-especiação.

Palavras-chave: Simbiose, Bactérias metabolizadoras de inseticida, Taxonomia baseada em genoma, Holobionte

ABSTRACT

Genomics of insecticide-metabolizing bacteria associated with *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and their potential role in host detoxification

The evolutionary success of the class Insecta can be partially attributed to its association with bacteria. Association with non-pathogenic microorganisms ensures the maintenance of complex phenotypes, with host and microbiota that respond together to adverse environmental conditions. In previous studies, we have shown that insecticide-resistant strains and natural populations of *Spodoptera frugiperda* carry insecticide-metabolizing bacteria (IMB) in their gut, some of which are fixed and have a distinct ability to metabolize different insecticides. Thus, we hypothesized that *S. frugiperda* and its microbiota behave as a single organism (holobiont), and aim to better characterize the IMB and verify their potential in the detoxification and response of this lepidopteran to insecticides. The IMB characterization was achieved through genomic, metatranscriptomic and metabolomic analyses. Our research has enabled the identification of genes and pathways associated with the potential metabolization of toxic compounds by bacteria, as well as aspects of their interaction and evolution with the host. Genome-based taxonomic studies led to a re-evaluation of the IMB taxonomic identities, highlighting the need to reconsider bacterial identification based solely on 16S rRNA sequencing. New bacterial species associated with the gut of *S. frugiperda* were described: *Enterococcus entomosocium* n. sp., *Enterococcus spodopteracolus* n. sp., and *Pseudomonas fraudulenta* n. sp. The remaining IMB under study were classified as *Acinetobacter soli* or as different subspecies of the recently described *Pseudomonas bharatica.* The analyzed IMB showed a remarkable enzymatic repertoire involved in xenobiotic degradation, including carboxylesterases, dehalogenases, and dehydrogenases and, for some of them, detoxification enzymes such as glutathione-S-transferases, superoxide dismutases and peroxiredoxins. *Enterococcus, Acinetobacter*, and *Pseudomonas* isolates were found to carry in their genomes the molecular machinery for interacting with the host, including mechanisms for gut colonization, nutrient supply, modulation of the gut microbiota, and interactions with host immune system. However, experiments on gut colonization of *S. frugiperda* by isolates of *Acinetobacter soli* and *Pseudomonas bharatica* suggested that these species may require the host maintenance under insecticide selection pressure to secure the colonization of the host gut. The comprehensive analysis of the response of susceptible and resistant strains of *S. frugiperda* to the insecticides flubendiamide, spinosad and teflubenzuron highlighted the complex interplay between host genotype, microbial communities and insecticide exposure on the larval metabolome and on the transcriptional activity of the gut microbiota. The analysis revealed that insecticide exposure and resistance evolution induce metabolic changes that affect host immunity, alter the host's interactions with its gut microbial community, and potentially affect associations with associated viruses. Finally, the analysis of the association of *E. entomosocium* and *E. spodopteracolus* with other species of the genus *Spodoptera* revealed that they do not carry enough genetic variation to be separated as taxonomic entities at either the species or subspecies level, showing that these bacteria did not accumulate enough genetic variation after the *Spodoptera* speciation process due to the process of co-speciation.

Keywords: Symbiosis, Insecticide-metabolizing bacteria, Genome-based taxonomy, Holobiont

1. INTRODUCTION

The evolutionary success of insects can be partially attributed to their complex associations with microorganisms (Engel & Moran, 2013; Sudakaran et al., 2017; Zhang et al., 2022). Interactions between host organisms and their associated microbiota are critical components of ecological and evolutionary processes (Engel & Moran, 2013; Salem et al., 2020; Sudakaran et al., 2017). The outcome of such interactions represents different types of associations – e.g., mutualism, commensalism, and parasitism – ultimately influencing the ecological dynamics and evolution of both host and microbiota (Drew et al., 2021). These associations involve the exchange of metabolic resources, modulation of immune responses, and acquisition of novel traits, among others (Moran, 2007; Russell et al., 2013; Xia et al., 2018). The characterization of symbiotic microorganisms and their potential impact on shaping host phenotypes have been well-documented in various insects (Hughes et al., 2011; Leclair et al., 2016; Voirol et al., 2018), revealing a spectrum of roles from nutrient provision and defense against pathogens to metabolization of toxic compounds (Chen et al., 2020; Lv et al., 2023; Oliver et al., 2003; Russell et al., 2013; Salem et al., 2020).

Recent advances in genomics, metatranscriptomics, and metabolomics have allowed a comprehensive characterization of microorganisms and their complex relationships with insect hosts, as well as the assessment of signatures of host/symbiont genome coevolution (Douglas, 2018; Mao et al., 2017; Wilson & Duncan, 2015). These approaches enabled the elucidation of the genetic, metabolic, and transcriptional mechanisms that drive host adaptation and tolerance in a range of insects (Li et al., 2022; Wang et al., 2020; Wu et al., 2020), providing a foundation for understanding the dynamics between insects and their microbial partners in face of adversity (DiGuistini et al., 2011; Wang et al., 2023). Notably, some of the host-associated microorganisms exhibit remarkable metabolic capabilities, encompassing the breakdown of diverse toxic compounds, including insecticides (Almeida et al., 2017; Gomes et al., 2020; Lv et al., 2023). This trait significantly contributes to the survival and adaptation of their host insects (Chen et al., 2020; Cheng et al., 2017; Kikuchi et al., 2012).

This research explores the intricate and multifaceted relationship between insects and their insecticide-metabolizing bacteria (IMB). The intricate host – symbiont associations has brought the concept that organisms are a representation of their own genome and of the whole associated microbiota, working together as a single evolutionary entity – the holobiont (Zilber-Rosenberg & Rosenberg, 2008). *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a notorious agricultural pest that carries a diverse gut microbiota represented by several bacterial members capable of degrading a range of pesticides (Almeida et al., 2017; Gomes et al., 2020). Some of the IMB obtained from the gut of *S. frugiperda* are considered core members of the gut microbial community, but the ability of these bacteria to metabolize insecticides was only detected in insect larvae that were selected for insecticide resistance under laboratory conditions and/or from field-collected larvae that have been exposed to a range of insecticides. No IMB were obtained from susceptible *S. frugiperda* larvae, indicating that the selection pressure that the host was exposed to and that resulted in the evolution of insecticide resistance also acted on the associated bacteria to select IMB (Almeida et al., 2017).

Thus, this study aims to investigate the complex interactions between *S. frugiperda* larvae and its microbial community by 1) conducting functional genomic analysis of the main IMB identified so far associated with the gut of resistant strains and field-collected larvae of *S. frugiperda;* 2) investigating the metabolomic alterations between resistant and susceptible strains of *S. frugiperda* to flubendiamide, spinosad, and teflubenzuron, when larvae are fed or not with these insecticides; 3) conducting comparative metatranscriptomic analysis of the gut microbiota of resistant and susceptible strains of *S. frugiperda* that were exposed or not to flubendiamide, spinosad, and teflubenzuron. In addition to that, we aimed to investigate the history of association of *Enterococcus* with the complex of *Spodoptera* species available in Brazil by conducting the isolation and functional genomic analysis of *Enterococcus* isolates obtained from *Spodoptera albula, Spodoptera cosmioides, Spodoptera eridania,* and *S. frugiperda*. By taking a holistic approach, we expect to better understand the complex dynamics of host-microbiota interactions and thereby shed light on the challenges of insecticide resistance and pest management. The findings promise to influence future pest management strategies by exploiting symbiotic relationships.

1.1. Hypothesis and predictions

The aim of this study was to better characterize the gut symbionts of *Spodoptera frugiperda* and to evaluate their functional contribution for the metabolization of insecticides and in the detoxification of insecticide-exposed hosts. We hypothesized that *S. frugiperda* and its associated microbiota act as a holobiont. Thus, we investigated the potential role of bacteria in the response of this pest to insecticides, and tried to better understand how this symbiotic relationship is established. To accept our hypothesis and based on previous studies indicating that the gut microbiota of *S. frugiperda* is also under the effect of insecticide selective pressure (Almeida et al., 2017; Gomes et al., 2020), we predicted that the insecticide-metabolizing bacteria under study will have genes and pathways associated with the potential metabolization of toxic compounds, as well as with aspects of their interaction with the host (Chapters 2, 3 and 5). Also, it is expected that (i) the microbiota of insecticide resistant and susceptible *S. frugiperda* larvae will respond differently when the host is exposed to such compounds due to (ii) differential gene expression of the hologenome and (iii) differential production of metabolites by the holobiont (Chapter 4). Finally, we hypothesized that *Enterococcus* symbionts, IMB in *S. frugiperda* and core member of its gut microbial community (Almeida et al., 2017; Oliveira et al., 2023), may have a close evolutionary history with their host; thus, it is expected that they carry enough genetic variation to be distinguishable from *Enterococcus* strains associated with other hosts within the genus *Spodoptera* (Chapter 5).

References

Almeida, L. G., De Moraes, L. A. B., Trigo, J. R., Omoto, C., & Cônsoli, F. L. (2017). The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. *PLoS ONE*, 12(3): e0174754. https://doi.org/10.1371/journal.pone.0174754

Chen, B., Zhang, N., Xie, S., Zhang, X., He, J., Muhammad, A., Sun, C., Lu, X., & Shao, Y. (2020). Gut bacteria of the silkworm *Bombyx mori* facilitate host resistance against the toxic effects of organophosphate insecticides. *Environment International*, 143: 105886. https://doi.org/10.1016/j.envint.2020.105886

Cheng, D., Guo, Z., Riegler, M., Xi, Z., Liang, G., & Xu, Y. (2017). Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). *Microbiome*, 5: 13. https://doi.org/10.1186/s40168-017-0236-z

DiGuistini, S., Wang, Y., Liao, N. Y., Taylor, G. et al. (2011). Genome and transcriptome analyses of the mountain pine beetle-fungal symbiont *Grosmannia clavigera*, a lodgepole pine pathogen. *Proceedings of the National Academy of Sciences of the United States of America*, 108(6): 2504–2509. https://doi.org/10.1073/pnas.1011289108

Douglas, A. E. (2018). Omics and the metabolic function of insect–microbial symbioses. *Current Opinion in Insect Science*, 29: 1–6. https://doi.org/10.1016/j.cois.2018.05.012

Drew, G. C., Stevens, E. J., & King, K. C. (2021). Microbial evolution and transitions along the parasite–mutualist continuum. *Nature Reviews Microbiology*, 19(10): 623–638. https://doi.org/10.1038/s41579-021-00550-7

Engel, P., & Moran, N. A. (2013). The gut microbiota of insects - diversity in structure and function. *FEMS Microbiology Reviews*, 37(5): 699–735. https://doi.org/10.1111/1574-6976.12025

Gomes, A. F. F., Omoto, C., & Cônsoli, F. L. (2020). Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. *Journal of Pest Science*, 93(2): 833–851. https://doi.org/10.1007/s10340-020-01202-0

Hughes, G. L., Ren, X., Ramirez, J. L., Sakamoto, J. M., Bailey, J. A., Jedlicka, A. E., & Rasgon, J. L. (2011). *Wolbachia* infections in *Anopheles gambiae* cells: Transcriptomic characterization of a novel host-symbiont interaction. *PLoS Pathogens*, 7(2): e1001296. https://doi.org/10.1371/journal.ppat.1001296

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K., & Fukatsu, T. (2012). Symbiont-mediated insecticide resistance. *Proceedings of the National Academy of Sciences of the United States of America*, 109(22): 8618–8622. https://doi.org/10.1073/pnas.1200231109

Leclair, M., Pons, I., Mahéo, F., Morlière, S., Simon, J. C., & Outreman, Y. (2016). Diversity in symbiont consortia in the pea aphid complex is associated with large phenotypic variation in the insect host. *Evolutionary Ecology*, 30(5): 925–941. https://doi.org/10.1007/s10682-016-9856-1

Li, Y., Li, Y., Wang, G., Li, J., Zhang, M., Wu, J., Liang, C., Zhou, H., Tang, J., & Zhu, G. (2022). Differential metabolome responses to deltamethrin between resistant and susceptible *Anopheles sinensis*. *Ecotoxicology and Environmental Safety,* 237: 113553. https://doi.org/10.1016/j.ecoenv.2022.113553

Lv, N., Li, R., Cheng, S., Zhang, L., Liang, P., & Gao, X. (2023). The gut symbiont *Sphingomonas* mediates imidacloprid resistance in the important agricultural insect pest *Aphis gossypii* Glover. *BMC Biology*, 21: 86. https://doi.org/10.1186/s12915-023-01586-2

Mao, M., Yang, X., Poff, K., & Bennett, G. (2017). Comparative genomics of the dual-obligate symbionts from the treehopper, *Entylia carinata* (Hemiptera: Membracidae), provide insight into the origins and evolution of an ancient symbiosis. *Genome Biology and Evolution,* 9(6): 1803–1815. https://doi.org/10.1093/gbe/evx134

Moran, N. A. (2007). Symbiosis as an adaptive process and source of phenotypic complexity. *Proceedings of the National Academy of Sciences of the United States of America,* 104: 8627–8633. https://doi.org/10.1073/pnas.0611659104

Oliveira, N. C., Rodrigues, P. A. P, & Cônsoli, F. L. (2023) Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the western hemisphere. *Microbial Ecology*, 85: 1552–1563 https://doi.org/10.1007/s00248-022-02008-6

Oliver, K. M., Russell, J. A., Moran, N. A., & Hunter, M. S. (2003). Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. *Proceedings of the National Academy of Sciences of the United States of America.*, 100(4): 1803–1807. https://doi.org/73/pnas.0335320100

Russell, C. W., Bouvaine, S., Newell, P. D., & Douglasa, A. E. (2013). Shared metabolic pathways in a coevolved insect-bacterial symbiosis. *Applied and Environmental Microbiology,* 79(19): 6117–6123. https://doi.org/10.1128/AEM.01543-13

Salem, H., Kirsch, R., Pauchet, Y., Berasategui, A., Fukumori, K., Moriyama et al. (2020). Symbiont digestive range reflects host plant breadth in herbivorous beetles. *Current Biology*, 30(15): 2875-2886. https://doi.org/10.1016/j.cub.2020.05.043

Sudakaran, S., Kost, C., & Kaltenpoth, M. (2017). Symbiont acquisition and replacement as a source of ecological innovation. *Trends in Microbiology,* 25(5): 375–390. https://doi.org/10.1016/j.tim.2017.02.014

Voirol, L. R. P., Frago, E., Kaltenpoth, M., Hilker, M., & Fatouros, N. E. (2018). Bacterial symbionts in Lepidoptera: Their diversity, transmission, and impact on the host. *Frontiers in Microbiology*, 9: 556. https://doi.org/10.3389/fmicb.2018.00556

Wang, D., Lv, W., Yuan, Y., Zhang, T., Teng, H., Losey, J. E., & Chang, X. (2020). Mechanism of the different metabolome responses between *Plutella xylostella* and *Pieris rapae* treated with the diamide insecticides. *Ecotoxicology and Environmental Safety,* 203: 111033. https://doi.org/10.1016/j.ecoenv.2020.111033

Wang, Z., Liu, Y., Wang, H., Roy, A., Liu, H., Han, F., Zhang, X., & Lu, Q. (2023). Genome and transcriptome of *Ips nitidus* provide insights into high-altitude hypoxia adaptation and symbiosis. *IScience*, 26(10): 107793. https://doi.org/10.1016/j.isci.2023.107793

Wilson, A. C. C., & Duncan, R. P. (2015). Signatures of host/symbiont genome coevolution in insect nutritional endosymbioses. *Proceedings of the National Academy of Sciences of the United States of America*, 112(33): 10255–10261. https://doi.org/10.1073/pnas.1423305112

Wu, Y., Zheng, Y., Chen, Y., Wang, S., Chen, Y., Hu, F., & Zheng, H. (2020). Honeybee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. *Microbial Biotechnology,* 13(4): 1201–1212. https://doi.org/10.1111/1751-7915.13579

Xia, X., Sun, B., Gurr, G. M., Vasseur, L., Xue, M., & You, M. (2018). Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). *Frontiers in Microbiology*, 9: 25. https://doi.org/10.3389/fmicb.2018.00025

Zhang, X., Zhang, F., & Lu, X. (2022). Diversity and functional roles of the gut microbiota in lepidopteran insects. *Microorganisms,* 10(6): 1234. https://doi.org/10.3390/microorganisms10061234

Zilber-Rosenberg, I., & Rosenberg, E. (2008). Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. *FEMS Microbiology Reviews*, 32(5): 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

2. COMPARATIVE GENOMICS OF PESTICIDE-DEGRADING *Enterococcus* SYMBIONTS OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) LEADS TO THE IDENTIFICATION OF TWO NEW SPECIES AND THE REAPPRAISAL OF INSECT-ASSOCIATED *Enterococcus* species*

Abstract

Enterococcus species have been described as core members of the microbial community of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and have been previously reported as insecticide degrading agents. This study aimed to investigate the molecular composition of these microbial symbionts of *S. frugiperda* to better understand their association with the host and their potential for insecticide metabolization. Through comparative genomic analyses of several pesticide-degrading *Enterococcus* isolated from the gut of *S. frugiperda* larvae, we identified two new species: *Enterococcus entomosocium* n. sp. and *Enterococcus spodopteracolus* n. sp. Their identities as new species were confirmed by whole genome alignment, utilizing cut-offs of 95-96% for the average nucleotide identity (ANI) and 70% for the digital DNA: DNA hybridization (dDDH) values. The systematic positioning of these new species within the genus *Enterococcus* was resolved using genome-based analysis, revealing *Enterococcus casseliflavus* as a sister group of *E. entomosocium* n. sp., and *Enterococcus mundtii* as a sister group of *E. spodopteracolus* n. sp. Comparative genomic analyses of several isolates of *E. entomosocium* n. sp. and *E. spodopteracolus* n. sp. provided a better assessment of the interactions established in the symbiotic association with *S. frugiperda* and led to the discovery of misidentified new species of *Enterococcus* associated with insects. Our analyses indicated that the potential of *E. entomosocium* n. sp. and *E. spodopteracolus* n. sp. to metabolize different pesticides arises from molecular mechanisms that result in rapid evolution of new phenotypes in response to environmental stressors, in this case, the pesticides their host insect is exposed to.

Keywords: Comparative genomics; Fall armyworm; Genome-based taxonomy; Symbiont-mediated resistance; Pesticide-degrading bacteria

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2.1. Introduction

Research focused on the functional contribution of host-associated bacteria has revealed their involvement in defining host phenotypes, ranging from enhanced performance under stress conditions [1, 2] to changes in body coloration in aphid insects [3]. The diversity of microbial associations and contributions to insect hosts is as vast as the insect class itself. Insect-associated microbes directly influence the host´s response to various biotic and abiotic stressors and contribute to host fitness [4–6]. Microbes can affect insect adaptability through nutritional regulation, nutrient provisioning, digestion of challenging substrates, hormonal signaling, immune contribution, xenobiotic metabolism, and even act as pathogens [4,7, 8].

The megadiverse order Lepidoptera has a long coevolutionary history with plants and is almost entirely associated with angiosperms [9]. The divergence of the major ditrysian lineages during the Cretaceous period and the subsequent proliferation of lepidopterans approximately 90 million years ago resulted in the second largest group of phytophagous insects [9, 10]. Several lepidopteran species have significant implications for human interests, particularly as severe pests that threaten food security by infesting staple crops and other agricultural products [11].

Lepidopterans are known to host a wide array of microbes, with bacteria primarily colonizing their gut lumen [12, 13]. Early studies on gut microbial associations in lepidopterans initially disbelieved the existence of intimate microbial partnerships [14]. However, subsequent research on lepidopteran larvae has demonstrated that gut bacteria play crucial roles in food digestion facilitation [15], nutrient absorption enhancement [12], and direct contribution to host nutrition through production of essential nutrients (eg, L-tryptophan) [16]. Yet, gut bacteria of lepidopteran larvae can inhibit potentially harmful microorganisms by producing antimicrobial compounds [17] and reduce the toxicity of xenobiotics by the metabolization of host plant-derived defense molecules [18, 19] and pesticides [20–22].

Proteobacteria and *Firmicutes* are prevalent in the gut microbiota of insects, with their relative proportions reflecting host-insect groups [23, 24]. *Enterococcaceae,* a family within the *Firmicutes* phylum, is commonly found in the gut microbial communities of insects [24, 25]. The genus *Enterococcus,* within the *Enterococcaceae* family, has been reported to persist in several lepidopteran species, irrespective of their diet and metamorphic stage (reviewed by [12]). *Enterococcus* has also been associated with the metabolization of xenobiotics in lepidopterans [26, 27], including *Spodoptera* larvae [28, 29]. Its prevalence in the gut microbiota of *Spodoptera littoralis* and *Spodoptera frugiperda* larvae recognized *Enterococcus* as a core member of their microbial community [25, 30, 31]. However, there are instances where *Enterococcus* has been implicated in pathogenic interactions with lepidopteran larvae [32–34]. For example, *Enterococcus faecalis* has shown pathogenicity towards *Spodoptera exigua* [35], while *Enterococcus mundtii* [36] and *Enterococcus cloacae* [37] have been found to be pathogenic to *Spodoptera litura.*

Numerous *Enterococcus* colonies were isolated from the gut of laboratory-selected insecticide-resistant and susceptible strains of *S. frugiperda.* The isolates obtained from insecticide-resistant strains of *S. frugiperda* could grow on selective media containing the specific insecticide to which the host insect had evolved resistance, unlike the isolates obtained from susceptible larvae [28]. These isolates were putatively identified as *E. mundtii* and

Enterococcus casseliflavus by heuristic comparisons and phylogenetic analysis based on almost complete sequences of the 16S rRNA gene [28].

Given the diversity of interactions established by *Enterococcus*, including *E. mundtii* and *E. casseliflavus*, with their host insects [38], as well as the considerable variation in the potential for insecticide degradation exhibited by the putative *E. mundtii* and *E. casseliflavus* isolates obtained from insecticide-resistant strains of *S. frugiperda*, we applied comparative genomic analyses to explore the molecular diversity and the nature of the interaction between these *Enterococcus* species and *S. frugiperda* larvae. This investigation sheds light on their potential to metabolize insecticides and uncovers key features that they carry as symbionts.

2.2. Conclusions

Comparative genomics analysis allowed us to better characterize the enterococci symbionts of *S. frugiperda* with the recognition of two new species of enterococci that were named *Enterococcus entomosocium* n. sp. and *Enterococcus spodopteracolus* n. sp. The genomic features of these species indicate their potential role in host detoxification and provide support for the hypothesis that gut bacteria, like their insect hosts, are subject to selective pressure from insecticides.

References

1. Houtz JE, Taff CC, Vitousek MN (2022) Gut microbiome as mediator of stress resilience a reactive scope model framework. Integr Comp Biol 62:41–57. https://doi.org/10.1093/icb/icac030

2. Wang X, Eguchi A, Fujita Y, et al (2023) Abnormal compositions of gut microbiota and metabolites are associated with susceptibility versus resilience in rats to inescapable electric stress. J Affect Disord 331:369–379. https://doi.org/10.1016/j.jad.2023.03.073

3. Tsuchida T, Koga R, Horikawa M, et al (2010) Symbiotic bacterium modifies aphid body color. Science 330:1102–1104. https://doi.org/10.1126/science.1195463

4. Schmidt K, Engel P (2021) Mechanisms underlying gut microbiota-host interactions in insects. J Exp Biol 224(2): jeb207696. https://doi: 10.1242/jeb.207696.

5. Wang G, Berdy BM, Velasquez O, et al (2020) Changes in microbiome confer multigenerational host resistance after sub-toxic pesticide exposure. Cell Host Microbe 27:213–224. https://doi.org/10.1016/j.chom.2020.01.009

6. Zheng H, Perreau J, Powell JE, et al (2019) Division of labor in honey bee gut microbiota for plant polysaccharide digestion. Proc Natl Acad Sci U.S.A 116 (51):25909–25916. https://doi.org/10.1073/pnas.1916224116

7. Engel P, Moran NA (2013) The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 37:699–735. https://doi.org/10.1111/1574-6976.12025

8. Kucuk RA (2020) Gut bacteria in the holometabola: A review of obligate and facultative symbionts. J Pest Sci 20:1–12. https://doi.org/10.1093/jisesa/ieaa084

9. Scoble MJ (1992) The Lepidoptera: Form, function and diversity. OxfordUK: Oxford University Press

10. Wahlberg N, Wheat CW, Peña C (2013) Timing and patterns in the taxonomic diversification of Lepidoptera (butterflies and moths). PLoS One 8 (11):e80875. https://doi.org/10.1371/journal.pone.0080875

11. Holloway JD, Bradley JD, Carter DJ (1987) CIE Guides to insects of importance to man. I. Lepidoptera. CAB International

12. Voirol LRP, Frago E, Kaltenpoth M, et al (2018) Bacterial symbionts in Lepidoptera: Their diversity, transmission, and impact on the host. Front Microbiol 9:556. https://doi.org/10.3389/fmicb.2018.00556

13. Zhang X, Zhang F, Lu X (2022) Diversity and functional roles of the gut microbiota in Lepidopteran insects. Microorganisms 10 (6):1234. https://doi: 10.3390/microorganisms10061234

14. Hammer TJ, Janzen DH, Hallwachs W, et al (2017) Caterpillars lack a resident gut microbiome. Proc Natl Acad Sci U.S.A 114:9641–9646. https://doi.org/10.1073/pnas.1707186114

15. Visôtto LE, Oliveira MGA, Guedes RNC, et al (2009) Contribution of gut bacteria to digestion and development of the velvetbean caterpillar, *Anticarsia gemmatalis*. J Insect Physiol 55:185–191. https://doi.org/10.1016/j.jinsphys.2008.10.017

16. Liang X, He J, Zhang N, et al (2022) Probiotic potentials of the silkworm gut symbiont *Enterococcus casseliflavus* ECB140, a promising L-tryptophan producer living inside the host. J Appl Microbiol 133:1620–1635. https://doi.org/10.1111/jam.15675

17. Shao Y, Chen B, Sun C, et al (2017) Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. Cell Chem Biol 24:66–75. https://doi.org/10.1016/j.chembiol.2016.11.015

18. Chen B, Mason CJ, Peiffer M, et al (2022) Enterococcal symbionts of caterpillars facilitate the utilization of a suboptimal diet. J Insect Physiol 138:104369. https://doi.org/10.1016/j.jinsphys.2022.104369

19. Mazumdar T, Hänniger S, Shukla SP, et al (2023) 8-HQA adjusts the number and diversity of bacteria in the gut microbiome of *Spodoptera littoralis*. Front Microbiol 14:1075557. https://doi.org/10.3389/fmicb.2023.1075557

20. Gadad H, Vastrad AS (2016) Gut bacteria mediated insecticide resistance in *Spodoptera litura*. J Exp Zool India 19:1099–1102

21. Gomes AFF, Omoto C, Cônsoli FL (2020) Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. J Pest Sci 93:833–851. https://doi.org/10.1007/s10340-020-01202-0

22. Li D, Zhang Y, Li W, et al (2019) Fitness and evolution of insecticide resistance associated with gut symbionts in metaflumizone-resistant *Plutella xylostella*. Crop Prot 124:104869. https://doi.org/10.1016/j.cropro.2019.104869

23. Li D-D, Li J-Y, Hu Z-Q, et al (2022) Fall armyworm gut bacterial diversity associated with different developmental stages, environmental habitats, and diets. Insects 13:762. https://doi.org/10.3390/insects13090762

24. Shao Y, Arias-Cordero E, Guo H, et al (2014) In vivo Pyro-SIP assessing active gut microbiota of the cotton leafworm, *Spodoptera littoralis*. PLoS One 9(1):e85948. https://doi.org/10.1371/journal.pone.0085948

25. Tang X, Freitak D, Vogel H, et al (2012) Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. PLoS One 7(7):e36978. https://doi.org/10.1371/journal.pone.0036978

26. Xia X, Sun B, Gurr GM, et al (2018) Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). Front Microbiol 9:25. https://doi.org/10.3389/fmicb.2018.00025

27. Vilanova C, Baixeras J, Latorre A, Porcar M (2016) The generalist inside the specialist: Gut bacterial communities of two insect species feeding on toxic plants are dominated by *Enterococcus* sp. Front Microbiol 7:1005. https://doi.org/10.3389/fmicb.2016.01005

28. Almeida LG, de Moraes LAB, Trigo JR, et al (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. PLoS One 12(3):e0174754. https://doi.org/10.1371/journal.pone.0174754

29. Shao Y, Spiteller D, Tang X, et al (2011) Crystallization of α- and β-carotene in the foregut of *Spodoptera* larvae feeding on a toxic food plant. Insect Biochem Mol Biol 41:273–281. https://doi.org/10.1016/j.ibmb.2011.01.004

30. Higuita Palacio MF, Montoya OI, Saldamando CI, et al (2021) Dry and rainy seasons significantly alter the gut microbiome composition and reveal a key *Enterococcus* sp. (Lactobacillales: Enterococcaceae) core component in *Spodoptera frugiperda* (Lepidoptera: Noctuidae) corn strain from northwestern Colombia. J Pest Sci 21(6):10. https://doi.org/10.1093/jisesa/ieab076

31. Oliveira NC, Rodrigues PAP, Cônsoli FL (2023) Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the western hemisphere. Microb Ecol 85(4):1552–1563. https://doi.org/10.1007/s00248-022-02008-6

32. Cappellozza S, Saviane A, Tettamanti G, et al (2011) Identification of *Enterococcus mundtii* as a pathogenic agent involved in the “flacherie” disease in *Bombyx mori* L. larvae reared on artificial diet. J Invertebr Pathol 106:386–393. https://doi.org/10.1016/j.jip.2010.12.007

33. Mason KL, Stepien TA, Blum JE, et al (2011) From commensal to pathogen: Translocation of *Enterococcus faecalis* from the midgut to the hemocoel of *Manduca sexta.* mBio 2(3):e00065-11. https://doi.org/10.1128/mBio.00065-11

34. Sun Y, Li X, Wang G, et al (2016) Genome sequence of *Enterococcus pernyi*, a pathogenic bacterium for the chinese oak silkworm, *Antheraea pernyi*. Genome Announc 4(3):e01764-15. https://doi.org/10.1128/genomeA.01764-15

35. Youngjin P, Kim K, Kim Y (2002) A pathogenic bacterium, *Enterococcus faecalis*, to the beet armyworm, *Spodoptera exigua*. J Asia Pac Entomol 5:221–225. https://doi.org/10.1016/S1226-8615(08)60156-9

36. Devi S, Saini HS, Kaur S (2022) Assessing the pathogenicity of gut bacteria associated with tobacco caterpillar *Spodoptera litura* (Fab.). Sci Rep 12(1):8257. https://doi.org/10.1038/s41598-022-12319-w

37. Thakur A, Dhammi P, Saini HS, Kaur S (2015) Pathogenicity of bacteria isolated from gut of *Spodoptera litura* (Lepidoptera: Noctuidae) and fitness costs of insect associated with consumption of bacteria. J Invertebr Pathol 127:38–46. https://doi.org/10.1016/j.jip.2015.02.007

38. Rodrigues PAP, Oliveira NC, Omoto C, Girke T, Cônsoli FL (2023) Host plant affects the larval gut microbial communities of the generalist herbivores *Helicoverpa armigera* and *Spodoptera frugiperda*. BioRxiv 2023.03.08.531690. https://doi.org/10.1101/2023.03.08.531690

39. Andrews S. (2010) FastQC: A quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc.

40. Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170

41. Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J. Comput. Biol. 19:455–477. https://doi.org/10.1089/cmb.2012.0021

42. Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes de novo assembler. Curr Protoc Bioinformatics 70(1):e102. https://doi.org/10.1002/cpbi.102

43. Brettin T, Davis JJ, Disz T, et al (2015) RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5(1):8365. https://doi.org/10.1038/srep08365

44. Alcock BP, Raphenya AR, Lau TTY, et al (2020) CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48:D517–D525. https://doi.org/10.1093/nar/gkz935

45. Wishart DS, Knox C, Guo AC, et al (2006) DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res 34:D668-D672. https://doi.org/10.1093/nar/gkj067

46. Saier MH, Reddy VS, Moreno-Hagelsieb G, et al (2021) The transporter classification database (TCDB): 2021 update. Nucleic Acids Res 49:D461–D467. https://doi.org/10.1093/nar/gkaa1004

47. Chen L, Yang J, Yu J, et al (2005) VFDB: A reference database for bacterial virulence factors. Nucleic Acids Res 33:D325-D328. https://doi.org/10.1093/nar/gki008

48. Sayers S, Li L, Ong E, et al (2019) Victors: A web-based knowledge base of virulence factors in human and animal pathogens. Nucleic Acids Res 47:693–700. https://doi.org/10.1093/nar/gky999

49. Medema MH, Blin K, Cimermancic P, et al (2011) AntiSMASH: Rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Nucleic Acids Res 39:W339-W346. https://doi.org/10.1093/nar/gkr466

50. Kautsar SA, Blin K, Shaw S, et al (2020) MIBiG 2.0: A repository for biosynthetic gene clusters of known function. Nucleic Acids Res 48: D454–D458. https://doi.org/10.1093/nar/gkz882

51. Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10(1):2182. https://doi.org/10.1038/s41467-019-10210-3

52. Goris J, Konstantinidis KT, Klappenbach JA, et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0

53. Rosselló-Móra R, Amann R (2015) Past and future species definitions for Bacteria and Archaea. Syst Appl Microbiol 38:209–216. https://doi.org/10.1016/j.syapm.2015.02.001

54. Meier-Kolthoff JP, Hahnke RL, Petersen J, et al (2014) Complete genome sequence of DSM 30083T, the type strain (U5/41T) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci 9:2. https://doi.org/10.1186/1944-3277-9-2

55. Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol 38:3022–3027. https://doi.org/10.1093/molbev/msab120

56. Lefort V, Desper R, Gascuel O (2015) FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. https://doi.org/10.1093/molbev/msv150

57. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797. https://doi.org/10.1093/nar/gkh340

58. Cock PJA, Antao T, Chang JT, et al (2009) Biopython: Freely available Python tools for computational molecular biology and bioinformatics. Bioinformatics 25:1422–1423. https://doi.org/10.1093/bioinformatics/btp163

59. Le SQ, Gascuel O (2008) An improved general amino acid replacement matrix. Mol Biol Evol 25:1307–1320. https://doi.org/10.1093/molbev/msn067

60. Gomes, AFF., De Almeida, LG., Cônsoli, FL (2023) Comparative genomics of pesticide-degrading *Enterococcus* symbionts of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) leads to the identification of two new

species and the reappraisal of insect-associated *Enterococcus* species. Microb Ecol 86:2583–2605. https://doi.org/10.1007/s00248-023-02264-0

61. Sánchez-Romero MA, Casadesús J (2020) The bacterial epigenome. Nat Rev Microbiol 18:7–20. https://doi.org/10.1038/s41579-019-0286-2

62. Seong HJ, Han SW, Sul WJ (2021) Prokaryotic DNA methylation and its functional roles. J Microbiol 59:242–248. https://doi: 10.1007/s12275-021-0674-y

63. Stephens C, Reisenauer A, Wright R, et al (1996) A cell cycle-regulated bacterial DNA methyltransferase is essential for viability. Natl Acad Sci U S A 93(3):1210-1214. https://doi: 10.1073/pnas.93.3.1210

64. Kahramanoglou C, Prieto AI, Khedkar S, et al (2012) Genomics of DNA cytosine methylation in *Escherichia coli* reveals its role in stationary phase transcription. Nat Commun 3(1):886. https://doi.org/10.1038/ncomms1878

65. Militello KT, Mandarano AH, Varechtchouk O, Simon RD (2014) Cytosine DNA methylation influences drug resistance in *Escherichia coli* through increased *sugE* expression. FEMS Microbiol Lett 350:100–106. https://doi: 10.1111/1574-6968.12299

66. Manso AS, Chai MH, Atack JM, et al (2014) A random six-phase switch regulates pneumococcal virulence via global epigenetic changes. Nat Commun 5(1):5055. https://doi.org/10.1038/ncomms6055

67. Li J, Li JW, Feng Z, et al (2016) Epigenetic switch driven by DNA inversions dictates phase variation in *Streptococcus pneumoniae*. PLoS Pathog 12(7):e1005762. https://doi.org/10.1371/journal.ppat.1005762

68. Oliver MB, Basu Roy A, Kumar R, et al (2017) *Streptococcus pneumoniae* TIGR4 phase-locked opacity variants differ in virulence phenotypes. mSphere 2(6):e00386-17. https://doi.org/10.1128/msphere.00386-17

69. Kumar S, Karmakar BC, Nagarajan D, et al (2018) N4-cytosine DNA methylation regulates transcription and pathogenesis in *Helicobacter pylori.* Nucleic Acids Res 46:3429–3445. https://doi.org/10.1093/NAR/GKY126

70. Estibariz I, Overmann A, Ailloud F, et al (2019) The core genome m5C methyltransferase JHP1050 (M.Hpy99III) plays an important role in orchestrating gene expression in *Helicobacter pylori.* Nucleic Acids Res 47:2336–2348. https://doi.org/10.1093/nar/gky1307

71. Mishra S, Lin Z, Pang S, et al (2021) Recent advanced technologies for the characterization of xenobiotic-degrading microorganisms and microbial communities. Front Bioeng Biotechnol 9:632095. https://doi.org/10.3389/fbioe.2021.632059

72. Russell RJ, Scott C, Jackson CJ, et al (2011) The evolution of new enzyme function: Lessons from xenobiotic metabolizing bacteria versus insecticide-resistant insects. Evol Appl 4:225–248. https://doi.org/10.1111/j.1752-4571.2010.00175.x

73. Bhatti S, Satyanarayana GNV, Patel DK, Satish A (2019) Bioaccumulation, biotransformation and toxic effect of fipronil in *Escherichia coli.* Chemosphere 231:207–215. https://doi.org/10.1016/j.chemosphere.2019.05.124

74. Gangola S, Bhatt P, Kumar AJ, et al (2022) Biotechnological tools to elucidate the mechanism of pesticide degradation in the environment. Chemosphere 296:133916. https://doi.org/10.1016/j.chemosphere.2022.133916

75. Sutherland TD, Horne I, Weir KM, et al (2004) Enzymatic bioremediation: From enzyme discovery to applications. Clin Exp Pharmacol Physiol 31(11):817-21. https://doi: 10.1111/j.1440-1681.2004.04088.x

76. Itoh H, Tago K, Hayatsu M, Kikuchi Y (2018) Detoxifying symbiosis: Microbe-mediated detoxification of phytotoxins and pesticides in insects. Nat Prod Rep 35(5):434-454. https://doi.org/10.1039/c7np00051k

77. Jaffar S, Ahmad S, Lu Y (2022) Contribution of insect gut microbiota and their associated enzymes in insect physiology and biodegradation of pesticides. Front Microbiol 13:979383. https://doi.org/10.3389/fmicb.2022.979383

78. Kikuchi Y, Hayatsu M, Hosokawa T, et al (2012) Symbiont-mediated insecticide resistance. Proc Natl Acad Sci U S A 109:8618–8622. https://doi.org/10.1073/pnas.1200231109

79. Cheng D, Guo Z, Riegler M, et al (2017) Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome 5:1-12. https://doi.org/10.1186/s40168-017-0236-z

80. Bhandari S, Poudel DK, Marahatha R, et al (2021) Microbial enzymes used in bioremediation. J Chem 2021:1-17. https://doi.org/10.1155/2021/8849512

81. Bose S, Kumar PS, Vo DVN (2021) A review on the microbial degradation of chlorpyrifos and its metabolite TCP. Chemosphere 283:131447. https://doi.org/10.1016/j.chemosphere.2021.131447

82. Sun R, Liu C, Zhang H, Wang Q (2015) Benzoylurea chitin synthesis inhibitors. J Agric Food Chem 63:6847–6865. https://doi.org/10.1021/acs.jafc.5b02460

83. Garrido-Sanz D, Manzano J, Martín M, et al (2018) Metagenomic analysis of a biphenyl-degrading soil bacterial consortium reveals the metabolic roles of specific populations. Front Microbiol 9:232. https://doi.org/10.3389/fmicb.2018.00232

84. Yu Y, Yin H, Peng H, et al (2020) Proteomic mechanism of decabromodiphenyl ether (BDE-209) biodegradation by *Microbacterium* Y2 and its potential in remediation of BDE-209 contaminated water-sediment system. J Hazard Mater 387:121708. https://doi.org/10.1016/j.jhazmat.2019.121708

85. Zhan H, Huang Y, Lin Z, et al (2020) New insights into the microbial degradation and catalytic mechanism of synthetic pyrethroids. Environ Res 182:109138. https://doi: 10.1016/j.envres.2020.109138

86. Zhu N, Li R, Zhang J, et al (2021) Photo-degradation behavior of seven benzoylurea pesticides with C3N4 nanofilm and its aquatic impacts on *Scendesmus obliquus*. Sci Total Environ 799:149470. https://doi.org/10.1016/j.scitotenv.2021.149470

87. Cleveland CB, Bormett GA, Saunders DG, et al (2002) Environmental fate of spinosad. 1. Dissipation and degradation in aqueous systems. J Agric Food Chem 50:3244–3256. https://doi.org/10.1021/jf011663i

88. Finkelstein ZI, Baskunov BP, Rietjens IMCM, et al (2001) Transformation of the insecticide teflubenzuron by microorganisms. J Environ Sci Health B 36:559–567. https://doi.org/10.1081/PFC-100106185

89. Lee Y, Jeong SE, Hur M, et al (2018) Construction and evaluation of a korean native microbial consortium for the bioremediation of diesel fuel-contaminated soil in Korea. Front Microbiol 9:2594. https://doi.org/10.3389/fmicb.2018.02594

90. Koga R, Moriyama M, Onodera-Tanifuji N, et al (2022) Single mutation makes *Escherichia coli* an insect mutualist. Nat Microbiol 7:1141–1150. https://doi.org/10.1038/s41564-022-01179-9

91. Simonsen AK (2022) Environmental stress leads to genome streamlining in a widely distributed species of soil bacteria. ISME J 16:423–434. https://doi.org/10.1038/s41396-021-01082-x

92. Antonelli P, Duval P, Luis P, et al (2022) Reciprocal interactions between anthropogenic stressors and insect microbiota. Environ Sci Pollut Res 29(43):64469-64488. https://doi.org/10.1007/s11356-022-21857-9

93. Hufnagel M, Koch S, Creti R, et al (2004) A putative sugar-binding transcriptional regulator in a novel gene locus in *Enterococcus faecalis* contributes to production of biofilm and prolonged bacteremia in mice. J Infect Dis 189(3):420-30. https://doi.org/10.1086/381150

94. Creti R, Koch S, Fabretti F, et al (2006) Enterococcal colonization of the gastro-intestinal tract: Role of biofilm and environmental oligosaccharides. BMC Microbiol 6(1):1-8. https://doi.org/10.1186/1471-2180-6-60

95. Klünemann M, Andrejev S, Blasche S, et al (2021) Bioaccumulation of therapeutic drugs by human gut bacteria. Nature 597:533–538. https://doi.org/10.1038/s41586-021-03891-8

96. Jindal S, Yang L, Day PJ, Kell DB (2019) Involvement of multiple influx and efflux transporters in the accumulation of cationic fluorescent dyes by *Escherichia coli*. BMC Microbiol 19(1):1-16. https://doi.org/10.1186/s12866-019-1561-0

97. Ahmed M, Lyass L, Markham PN, et al (1995) Two highly similar multidrug transporters of *Bacillus subtilis* whose expression is differentially regulated. J Bacteriol 177(14):3904-10. https://doi.org/10.1128/jb.177.14.3904-3910.1995

98. Gill MJ, Brenwald NP, Wise R (1999) Identification of an efflux pump gene, *pmrA*, associated with fluoroquinolone resistance in *Streptococcus pneumoniae.* Antimicrob Agents Chemother 43(1):187-9. https://doi.org/10.1128/aac.43.1.187

99. Schindler BD, Kaatz GW (2016) Multidrug efflux pumps of Gram-positive bacteria. Drug Resist Updat 27:1–13. https://doi.org/10.1016/j.drup.2016.04.003

100. Scharf ME, Wolfe ZM, Raje KR, et al (2022) Transcriptome responses to defined insecticide selection pressures in the German cockroach (*Blattella germanica* L.). Front Physiol 12:2570. https://doi.org/10.3389/fphys.2021.816675

101. Yu L, Yang H, Cheng F, et al (2021) Honey bee *Apis mellifera* larvae gut microbial and immune, detoxication responses towards flumethrin stress. Environ Pollut 290:118107. https://doi.org/10.1016/j.envpol.2021.118107

102. Dethlefsen L, McFall-Ngai M, Relman DA (2007) An ecological and evolutionary perspective on humang-microbe mutualism and disease. Nature 449:811–818. https://doi.org/10.1038/nature06245

103. Stoy KS, Gibson AK, Gerardo NM, Morran LT (2020) A need to consider the evolutionary genetics of host–symbiont mutualisms. J Evol Biol 33:1656–1668. https://doi.org/10.1111/jeb.13715

104. Mason CJ, Peiffer M, Chen B, et al (2022) Opposing growth responses of lepidopteran larvae to the establishment of gut microbiota. Microbiol Spectr 10(4):e01941-22. https://doi.org/10.1128/spectrum.01941-22

105. Verneuil N, Rincé A, Sanguinetti M, et al (2005) Contribution of a PerR-like regulator to the oxidative-stress response and virulence of *Enterococcus faecalis*. Microbiology 151(12):3997-4004. https://doi.org/10.1099/mic.0.28325-0

106. Michel A, Agerer F, Hauck CR, et al (2006) Global regulatory impact of ClpP protease of *Staphylococcus aureus* on regulons involved in virulence, oxidative stress response, autolysis, and DNA repair. J Bacteriol 188:5783–5796. https://doi.org/10.1128/JB.00074-06

107. de Diego-Diaz B, Treu L, Campanaro S, et al (2018) Genome sequence of *Enterococcus mundtii* EM01, isolated from *Bombyx mori* midgut and responsible for flacherie disease in silkworms reared on an artificial diet. Genome Announc 6(3):e01495-17. https://doi.org/10.1128/genomeA.01495-17

108. Audisio MC, Terzolo HR, Apella MC (2005) Bacteriocin from honey bee beebread *Enterococcus avium*, active against *Listeria monocytogenes*. Appl Environ Microbiol 71:3373–3375. https://doi.org/10.1128/AEM.71.6.3373-3375.2005

109. Kawamoto S, Shima J, Sato R, et al (2002) Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. Appl Environ Microbiol 68:3830–3840. https://doi.org/10.1128/AEM.68.8.3830-3840.2002

110. Liang X, Sun C, Chen B, et al (2018) Insect symbionts as valuable grist for the biotechnological mill: an alkaliphilic silkworm gut bacterium for efficient lactic acid production. Appl Microbiol Biotechnol 102:4951–4962. https://doi.org/10.1007/s00253-018-8953-1

111. Douglas AE (2017) The B vitamin nutrition of insects: the contributions of diet, microbiome and horizontally acquired genes. Curr Opin Insect Sci 23:65–69. https://doi.org/10.1016/j.cois.2017.07.012

112. Michalkova V, Benoit JB, Weiss BL, et al (2014) Vitamin B6 generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. Appl Environ Microbiol 80:5844–5853. https://doi.org/10.1128/AEM.01150-14

113. Srinivasan R, Karaoz U, Volegova M, et al (2015) Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One 10(2):e117617. https://doi.org/10.1371/journal.pone.0117617

114. Fox, GE, Wisotzkey, JD, Jurtshuk, P (1992). How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int J Syst Bacteriol 42(1):166–170. https://doi.org/10.1099/00207713-42-1-166

115. Vandamme P, Pot B, Gillis M, et al (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60(2):407-38. https://doi: 10.1128/mr.60.2.407-438

116. Vandamme P, Peeters C (2014) Time to revisit polyphasic taxonomy. Antonie van Leeuwenhoek 106:57–65. https://doi: 10.1007/s10482-014-0148-x

117. Chun J, Oren A, Ventosa A, et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.002516

3. COMPARATIVE GENOMICS AND CHARACTERIZATION OF MULTIPLE INSECTICIDE-METABOLIZING *Acinetobacter* AND *Pseudomonas* ASSOCIATED WITH *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) LARVAE

Abstract

Exploring the microbial communities within insects provides valuable insights into their functional roles and ecological significance. This study focuses on the phenotypic and genomic characterization of *Acinetobacter* and *Pseudomonas* isolates from the gut of field collected *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae. Previous screening using insecticide-selective media revealed their distinct abilities to metabolize several insecticides used in *S. frugiperda* control. Comparative genomics revealed distinct characteristics of these isolates. Specific resistance patterns and metabolic potentials were identified by antibiotic susceptibility and carbohydrate metabolism analyses. Genome assembly identified diverse coding sequences associated with metabolism, energy production, and xenobiotic degradation, while the analysis of shared and unique sequences provided insights into common traits and individual differences among the isolates. Whole genome alignment and taxonomy confirmed their taxonomic positions. Remarkably, IILSFSC100 was proposed as *Pseudomonas fraudulenta* n. sp. while the remaining isolates of *Pseudomonas* were classified in two new subspecies clades of the recently described *Pseudomonas bharatica*. All isolates of *Acinetobacter* investigated were like *Acinetobacter soli*. *Acinetobacter* and *Pseudomonas* have been identified as core members of the gut microbial community of *S. frugiperda*, but their low abundance in the gut microbiota of field-collected larvae and their failure to colonize the gut of laboratory-reared larvae suggest that their association with *S. frugiperda* occurs only under insecticide selection pressure. This comparative genomics study uncovered intricate genetic aspects and revealed the functional diversity, ecological relevance, and host-environment interactions of these isolates.

Keywords: Insect gut microbiota; Phenotypic diversity; Genomic analysis; Sustainable pest management; Bacterial metabolism

3.1. Introduction

Bacterial symbionts play a crucial role in the survival and development of insects. One of the most common and well-known contribution of bacterial symbionts to host insects is the provision of essential nutrients (Engel and Moran 2013). Associations with nutrient-provisioning symbionts often result from a long coevolutionary history between hosts and bacteria, leading to a significant reduction in the genome size of symbiotic bacteria (Moran et al. 2003; McCutcheon and Moran 2012). However, even bacteria with shorter histories of association with their hosts have been shown to contribute to various insect fitness traits (Oliver et al. 2003; Engel and Moran 2013; Gurung et al. 2019). Nutrient-provisioning bacteria are more often represented by endocytobionts, while extracellular endosymbionts offer more diverse contributions to hosts (Sudakaran et al. 2017; Ganesan et al. 2022).

The involvement of free-living endosymbionts and endocytobionts in pesticide degradation has been observed in several species of chewing and sap-sucking insects (Itoh et al. 2018a; Miksanek and Tuda 2023). In many of these cases, the hosts are economically important agricultural insect pests, and symbiont-mediated metabolization of insecticides has been implicated in the insect resistance to these chemicals (Cheng et al. 2017; Chen et al. 2020; Sato et al. 2021). The active contribution of insect-associated symbionts to insecticide resistance evolution adds complexity to the implementation of insecticide resistance management strategies and sustainable agricultural practices (Itoh et al. 2018b; Bras et al. 2022). Insecticide-degrading symbionts can be acquired from the environment (e.g., soil) and/or evolve this ability under the exposure to insecticide selection pressure (Cheng et al. 2017; Itoh et al. 2018b; Gomes et al. 2020). Furthermore, some of these symbionts are vertically transmitted (Guo et al. 2017).

Several lepidopteran species are among the most destructive pests in agriculture, particularly due to the voracious feeding and polyphagous behavior of their larval stage (Cunningham et al. 1999; Mitra et al. 2021). The observed geographic range expansion of several lepidopteran pests, coupled with potential failures of chemical control tactics, increases the threat they pose to global food security (Suckling et al. 2017). The fall armyworm (FAW) *Spodoptera frugiperda* (JE Smith, 1797) (Lepidoptera: Noctuidae) is an incredible destructive lepidopteran pest that attacks numerous host plants, including major staple crops like maize, millet, rice, and sorghum (Montezano et al. 2018; Wan et al. 2021). *Spodoptera frugiperda* has recently expanded its geographical range from the Americas to Africa, Asia, and Oceania (Goergen et al. 2016; Maino et al. 2021). Reports of FAW control failures are common in the literature, as this species can evolve resistance to various organic chemical compounds (reviewed by Van Den Berg and Du Plessis 2022) and Bt-based technologies (Storer et al. 2010; Omoto et al. 2016).

Analysis of the gut microbiota of several insecticide-resistant strains of FAW selected under laboratory conditions revealed a diverse range of bacterial species capable of degrading different types of organic chemistries used in FAW control (Almeida et al. 2017; Gomes et al. 2020). A comprehensive analysis of bacterial diversity associated with field-collected larvae from different populations of major maize-producing areas in Brazil identified a much higher diversity of insecticide-degrading bacteria in the gut microbiota of the FAW larvae (Gomes et al. 2020). Field-collected FAW larvae not only harbored a greater richness of bacteria capable of degrading pesticides, but also many bacteria with the ability to metabolize a wider range of chemical structures (Gomes et al. 2020). Two bacterial species, putatively identified through heuristic comparisons of nearly complete 16S rRNA gene sequences, *Acinetobacter soli* and *Pseudomonas japonica*, were consistently found in all studied populations and showed the ability to metabolize multiple insecticides (Gomes et al. 2020).

Both *Acinetobacter* and *Pseudomonas* belong to the phylum *Proteobacteria*. They exhibit considerable genetic diversity and are widely distributed in various environments, including soil, water, plants, and animals (Silby et al. 2011; Minard et al. 2013). Both genera are commonly found in the gut microbial communities of insects (Guégan et al. 2018; Teoh et al. 2021), where they can act as opportunistic pathogens (Tao et al. 2011; Flury et al. 2017; McKenna et al. 2022) or mutualists by providing nutrients to hosts (Guégan et al. 2018; Fabryová et al. 2018; Weglarz et al. 2018) or protection against predators (Kador et al. 2011), microbial infections (Saati-Santamaría et al. 2018), and plant-produced defense molecules (Boone et al. 2013; Xu et al. 2016; Zhang et al. 2020).

Acinetobacter and *Pseudomonas* species are metabolic versatile and can utilize a wide range of carbon sources to adapt to different environmental conditions (Viggor et al. 2020; Li et al. 2023). These bacteria can acquire genetic material from the environment through a process called bacterial natural transformation (Seitz and Blokesch 2013). Natural transformation involves the uptake and incorporation of extracellularly available DNA into the bacterial genome, allowing for the expression of newly acquired genes (Johnston et al. 2014; Blokesch 2016). Their ability to undergo natural transformation, along with mechanisms such as transduction and conjugation, plays a crucial role in their genetic plasticity and adaptation to changing environments (Johnston et al. 2014; Averhoff et al. 2021). It enables them to acquire new genetic traits, including antibiotic resistance genes, metabolic capabilities, and virulence factors through horizontal gene transfer (Wiedenbeck and Cohan 2011; Arnold et al. 2022).

The metabolic versatility of *Acinetobacter* and *Pseudomonas* species allows them to thrive in different environmental niches and utilize different organic compounds as sources of energy and nutrients, such as sugars, organic acids, lipids, and aromatic compounds (Frimmersdorf et al. 2010; Jung and Park 2015). They are well-known for their ability to degrade complex compounds and detoxify toxic substances such as petroleum pollutants (Liu et al. 2013; Xia et al. 2014; Sui et al. 2023), phenolic compounds (Krastanov et al. 2013; Liu et al. 2020), heavy metals (Kang et al. 2020; Vélez et al. 2021), and pesticides (Singh et al. 2004; Gong et al. 2018; Aswathi et al. 2019; Gur Ozdal and Algur 2022). Consequently, they are often the focus of studies related to bioremediation (Bhandari et al. 2021). *Acinetobacter* and *Pseudomonas* strains have been reported to metabolize organophosphates, carbamates, triazines, organochlorines, and pyrethroids (Singh et al. 2004; Xie et al. 2009; Abraham et al. 2014; Gong et al. 2018; Gomes et al. 2020).

Thus, given the importance of gut associated microbes in the metabolization of pesticides and their contribution to host resistance to pesticides, and the consequent threat to sustainable food production and food security, we sequenced and performed comparative genomic analyses of several multiple insecticide-degrading isolates of *A. soli* and *P. japonica* that were obtained from field-collected larvae of *S. frugiperda* in five different agricultural regions in Brazil (Gomes et al. 2020). Our aims are to provide a better systematic characterization of the isolates obtained and to identify and understand the genetic and metabolic traits they carry for metabolizing a diversified set of insecticidal chemistries. The data obtained from this study are expected to provide valuable information for understanding the potential of these bacteria in xenobiotic metabolism, adaptability, and host detoxification in the context of agricultural conditions.

3.2. Conclusions

Our study of insect-associated *Acinetobacter* and *Pseudomonas* isolates through comparative genomics and phenotypic assays has provided valuable insights into their ability to metabolize xenobiotics and interact with the host *Spodoptera frugiperda*. We have identified genetic adaptations and metabolic pathways involved in insecticide degradation and highlighted the need to re-evaluate taxonomy based solely on 16S sequencing. Based on our data, we hypothesized that the gut colonization of *S. frugiperda* larvae by *A. soli* and *P. bharatica* occurs only when the host insect is under insecticide selection pressure, and the association with these bacteria would benefit the host as we identified that the abundance and prevalence of both species in the gut of the host are negatively correlated with other core species of the gut microbial community of *S. frugiperda*. This integrated approach improves our understanding of bacterial metabolism, insect-bacteria interactions, and has implications for the development of sustainable pest management strategies in agricultural and environmental contexts.

References

Abraham J, Silambarasan S, Logeswari P (2014) Simultaneous degradation of organophosphorus and organochlorine pesticides by bacterial consortium. J Taiwan Inst Chem Eng 45:2590–2596. https://doi.org/10.1016/j.jtice.2014.06.014

Adewoyin MA, Okoh AI (2018) The natural environment as a reservoir of pathogenic and non-pathogenic *Acinetobacter* species. Rev Environ Health 33:265–272. https://doi.org/10.1515/reveh-2017-0034

Almeida LG, De Moraes LAB, Trigo JR, et al (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. PLoS One 12(3):e0174754. https://doi.org/10.1371/journal.pone.0174754

Andrews S. (2010) FastQC: A quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Arnold BJ, Huang IT, Hanage WP (2022) Horizontal gene transfer and adaptive evolution in bacteria. Nat Rev Microbiol 20:206–218. https://doi.org/10.1038/s41579-021-00650-4

Aswathi A, Pandey A, Sukumaran RK (2019) Rapid degradation of the organophosphate pesticide – chlorpyrifos by a novel strain of *Pseudomonas nitroreducens* AR-3. Bioresour Technol 292:122025. https://doi.org/10.1016/j.biortech.2019.122025

Averhoff B, Kirchner L, Pfefferle K, Yaman D (2021) Natural transformation in Gram-negative bacteria thriving in extreme environments: From genes and genomes to proteins, structures and regulation. Extremophiles 25:425–436. https://doi.org/10.1007%2Fs00792-021-01242-z

Banerjee S, Kamila B, Barman S, et al (2019) Interlining Cr (VI) remediation mechanism by a novel bacterium *Pseudomonas brenneri* isolated from coalmine wastewater. J Environ Manage 233:271–282. https://doi.org/10.1016/j.jenvman.2018.12.048

Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 4:493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

Bhandari S, Poudel DK, Marahatha R, et al (2021) Microbial enzymes used in bioremediation. J Chem 2021:8849512. https://doi.org/10.1155/2021/8849512

Bilal M, Rasheed T, Iqbal HMN, Yan Y (2018) Peroxidases-assisted removal of environmentally related hazardous pollutants with reference to the reaction mechanisms of industrial dyes. Sci Total Environ 644:1–13. https://doi.org/10.1016/j.scitotenv.2018.06.274

Blokesch M (2016) Natural competence for transformation. Curr Biol 26(21):1126–1130. https://doi.org/10.1016/j.cub.2016.08.058

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Boone CK, Keefover-Ring K, Mapes AC, et al (2013) Bacteria associated with a tree-killing insect reduce concentrations of plant defense compounds. J Chem Ecol 39:1003–1006. https://doi.org/10.1007/s10886-013-0313-0

Bouteiller M, Dupont C, Bourigault Y, et al (2021) *Pseudomonas* flagella: Generalities and specificities. Int J Mol Sci 22(7):3337. https://doi.org/10.3390/ijms22073337

Bras A, Roy A, Heckel DG, et al (2022) Pesticide resistance in arthropods: Ecology matters too. Ecol Lett 25:1746–1759. https://doi.org/10.1111/ele.14030

Brettin T, Davis JJ, Disz T, et al (2015) RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365

Ceja-Navarro JA, Vega FE, Karaoz U, et al (2015) Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee. Nat Commun 6:7618. https://doi.org/10.1038/ncomms8618

Choi AHK, Slamti L, Avci FY, et al (2009) The *pgaABCD* locus of *Acinetobacter baumannii* encodes the production of poly-β-1-6-N-acetylglucosamine, which is critical for biofilm formation. J Bacteriol 191:5953–5963. https://doi.org/10.1128/JB.00647-09

Chen B, Zhang N, Xie S, et al (2020) Gut bacteria of the silkworm *Bombyx mori* facilitate host resistance against the toxic effects of organophosphate insecticides. Environ Int 143:105886. https://doi.org/10.1016/j.envint.2020.105886

Cheng D, Guo Z, Riegler M, et al (2017) Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome 5:13. https://doi.org/10.1186/s40168-017-0236-z

Chun J, Oren A, Ventosa A, et al (2018) Proposed minimal standards for the use of genome data for the taxonomy of prokaryotes. Int J Syst Evol Microbiol 68:461–466. https://doi.org/10.1099/ijsem.0.002516

Collier LS, Nichols NN, Neidle EL (1997) *Benk* encodes a hydrophobic permease-like protein involved in benzoate degradation by *Acinetobacte*r sp. strain ADP1. J Bacteriol 79:5943-6. https://doi.org/10.1128/jb.179.18.5943-5946.1997

Cornelis P (2010) Iron uptake and metabolism in pseudomonads. Appl Microbiol Biotechnol 86:1637–1645. https://doi.org/10.1007/s00253-010-2550-2

Cunningham JP, Zalucki MP, West SA (1999) Learning in *Helicoverpa armigera* (Lepidoptera: Noctuidae): A new look at the behaviour and control of a polyphagous pest. Bull Entomol Res 89:201–207. https://doi.org/10.1017/S0007485399000310

Deng W, Li C, Xie J (2013) The underling mechanism of bacterial TetR/AcrR family transcriptional repressors. Cell Signal 25:1608–1613. https://doi.org/10.1016/j.cellsig.2013.04.003

Dirix G, Monsieurs P, Dombrecht B, et al (2004) Peptide signal molecules and bacteriocins in Gram-negative bacteria: A genome-wide in silico screening for peptides containing a double-glycine leader sequence and their cognate transporters. Peptides 25:1425–1440. https://doi.org/10.1016/j.peptides.2003.10.028

Douglas AE (2017) The B vitamin nutrition of insects: The contributions of diet, microbiome and horizontally acquired genes. Curr Opin Insect Sci 23:65–69. https://doi.org/10.1016/j.cois.2017.07.012

Duc HD, Thuy NTD, Thanh LU, et al (2022) Degradation of diuron by a bacterial mixture and shifts in the bacterial community during bioremediation of contaminated soil. Curr Microbiol 79:11. https://doi.org/10.1007/s00284-021-02685-5

Engel P, Moran NA (2013) The gut microbiota of insects – diversity in structure and function. FEMS Microbiol Rev 37:699–735. https://doi.org/10.1111/1574-6976.12025

Fabryová A, Kostovčík M, Díez-Méndez A, et al (2018) On the bright side of a forest pest-the metabolic potential of bark beetles' bacterial associates. Sci Total Environ 619–620:9–17. https://doi.org/10.1016/j.scitotenv.2017.11.074

Flury P, Vesga P, Péchy-Tarr M, et al (2017) Antimicrobial and insecticidal: cyclic lipopeptides and hydrogen cyanide produced by plant-beneficial *Pseudomonas* strains CHA0, CMR12a, and PCL1391 contribute to insect killing. Front Microbiol 8. https://doi.org/10.3389/fmicb.2017.00100

Fournier PE, Raoult D (2009) Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann N Y Acad Sci 1166:1–11. https://doi.org/10.1111/j.1749-6632.2009.04528.x

Fox GE, Wisotzkey JD, Jurtshuk P (1992) How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int J Syst Bacteriol 42(1):166–170. https://doi.org/10.1099/00207713-42-1-166

Frimmersdorf E, Horatzek S, Pelnikevich A, et al (2010) How *Pseudomonas aeruginosa* adapts to various environments: A metabolomic approach. Environ Microbiol 12:1734–1747. https://doi.org/10.1111/j.1462-2920.2010.02253.x

Ganesan R, Wierz JC, Kaltenpoth M, Flórez L V. (2022) How it all begins: Bacterial factors mediating the colonization of invertebrate hosts by beneficial symbionts. Microbiol Mol Biol Rev 86(4):e00126-21. https://doi.org/10.1128/mmbr.00126-21

Gangola S, Bhatt P, Kumar AJ, et al (2022) Biotechnological tools to elucidate the mechanism of pesticide degradation in the environment. Chemosphere 296:133916. https://doi.org/10.1016/j.chemosphere.2022.133916

Gilbert MTP, Moore W, Melchior L, Worebey M (2007) DNA extraction from dry museum beetles without conferring external morphological damage. PLoS One 2(3):e272. https://doi.org/10.1371/journal.pone.0000272

Giltner CL, Nguyen Y, Burrows LL (2012) Type IV pilin proteins: Versatile molecular modules. Microbiol Mol Biol Rev 76(4):740–772. https://doi.org/10.1128/mmbr.00035-12

Goergen G, Kumar PL, Sankung SB, et al (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11(10):e0165632. https://doi.org/10.1371/journal.pone.0165632

Gomes AFF, Omoto C, Cônsoli FL (2020) Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. J Pest Sci 93:833–851. https://doi.org/10.1007/s10340-020-01202-0

Gong T, Xu X, Dang Y, et al (2018) An engineered *Pseudomonas putida* can simultaneously degrade organophosphates, pyrethroids and carbamates. Sci Total Environ 628–629:1258–1265. https://doi.org/10.1016/j.scitotenv.2018.02.143

Goris J, Konstantinidis KT, Klappenbach JA, et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0

Granada GA, Sequeira L (1983) Survival of *Pseudomonas solanacearum* in soil, rhizosphere, and plant roots. Can J Microbiol 29:433–440. https://doi.org/https://doi.org/10.1139/m83-070

Graupner S, Frey V, Hashemi R, et al (2000) Type IV pilus genes *pilA* and *pilC* of *Pseudomonas stutzeri* are required for natural genetic transformation, and *pilA* can be replaced by corresponding genes from nontransformable species. J Bacteriol 182:2184–2190. https://doi.org/10.1128/JB.182.8.2184-2190.2000

Guégan M, Zouache K, Démichel C, et al (2018) The mosquito holobiont: Fresh insight into mosquito-microbiota interactions. Microbiome 6:49. https://doi.org/10.1186/s40168-018-0435-2

Guo Z, Lu Y, Yang F, et al (2017) Transmission modes of a pesticide-degrading symbiont of the oriental fruit fly *Bactrocera dorsalis* (Hendel). Appl Microbiol Biotechnol 101:8543–8556. https://doi.org/10.1007/s00253-017-8551-7

Gur Ozdal O, Algur OF (2022) Biodegradation α-endosulfan and α-cypermethrin by *Acinetobacter schindleri* B7 isolated from the microflora of grasshopper (*Poecilimon tauricola*). Arch Microbiol 204:159. https://doi.org/10.1007/s00203-022-02765-5

Gurung K, Wertheim B, Falcao Salles J (2019) The microbiome of pest insects: It is not just bacteria. Entomol Exp Appl 167:156–170. https://doi.org/10.1111/eea.12768

Haggblom MM (1992) Microbial breakdown of halogenated aromatic pesticides and related compounds. FEMS Microbiol Lett 103:29–72. https://doi.org/10.1111/j.1574-6968.1992.tb05823.x

Han L, Yuan Z, Shao X, et al (2023) Synthesis, insecticidal activity, and stability study of novel nitromethylene neonicotinoids with five-membered aromatic heterocycles. Chin Chem Lett 34(6):107868. https://doi.org/10.1016/j.cclet.2022.107868

Harding CM, Tracy EN, Carruthers MD, et al (2013) *Acinetobacter baumannii* strain M2 produces type IV pili which play a role in natural transformation and twitching motility but not surface-associated motility. mBio 4(4). https://doi.org/10.1128/mBio.00360-13

Heilbronner S, Krismer B, Brötz-Oesterhelt H, Peschel A (2021) The microbiome-shaping roles of bacteriocins. Nat Rev Microbiol 19:726–739. https://doi.org/10.1038/s41579-021-00569-w

Hernandez RE, Gallegos-Monterrosa R, Coulthurst SJ (2020) Type VI secretion system effector proteins: Effective weapons for bacterial competitiveness. Cell Microbiol 22(9):e13241. https://doi.org/10.1111/cmi.13241

Itoh H, Tago K, Hayatsu M, Kikuchi Y (2018a) Detoxifying symbiosis: Microbe-mediated detoxification of phytotoxins and pesticides in insects. Nat Prod Rep 35:434–454. https://doi.org/10.1039/c7np00051k

Itoh H, Hori T, Sato Y, et al (2018b) Infection dynamics of insecticide-degrading symbionts from soil to insects in response to insecticide spraying. ISME Journal 12:909–920. https://doi.org/10.1038/s41396-017-0021-9

Johnston C, Martin B, Fichant G, et al (2014) Bacterial transformation: distribution, shared mechanisms and divergent control. Nat Rev Microbiol 12:181–196. https://doi.org/10.1038/nrmicro3199

Jung J, Park W (2015) *Acinetobacter* species as model microorganisms in environmental microbiology: Current state and perspectives. Appl Microbiol Biotechnol 99:2533–2548. https://doi.org/10.1007/s00253-015-6439-y

Kador M, Horn MA, Dettner K (2011) Novel oligonucleotide probes for in situ detection of pederin-producing endosymbionts of *Paederus riparius* rove beetles (Coleoptera: Staphylinidae). FEMS Microbiol Lett 319:73–81. https://doi.org/10.1111/j.1574-6968.2011.02270.x

Kang W, Zheng J, Bao J, et al (2020) Characterization of the copper resistance mechanism and bioremediation potential of an *Acinetobacter calcoaceticus* strain isolated from copper mine sludge. Environ Sci Pollut Res 27:7922–7933. https://doi.org/10.1007/s11356-019-07303-3

Kasten JP, Precetti AACM, Parra JRP (1978) Dados biológicos comparativos de *Spodoptera frugiperda* (J. E. Smith, 1797) em duas dietas artificiais e substrato natural. Rev Agri 53:68–78.

Kawamoto S, Shima J, Sato R, et al (2002) Biochemical and genetic characterization of mundticin KS, an antilisterial peptide produced by *Enterococcus mundtii* NFRI 7393. Appl Environ Microbiol 68:3830–3840. https://doi.org/10.1128/AEM.68.8.3830-3840.2002

Kramer J, Özkaya Ö, Kümmerli R (2020) Bacterial siderophores in community and host interactions. Nat Rev Microbiol 18:152–163. https://doi.org/10.1038/s41579-019-0284-4

Krastanov A, Alexieva Z, Yemendzhiev H (2013) Microbial degradation of phenol and phenolic derivatives. Eng Life Sci 13:76–87. https://doi.org/10.1002/elsc.201100227

Labroussaa F, Ionescu M, Zeilinger AR, et al (2017) A chitinase is required for *Xylella fastidiosa* colonization of its insect and plant hosts. Microbiology 163:502–509. https://doi.org/10.1099/mic.0.000438

Lefort V, Desper R, Gascuel O (2015) FastME 2.0: A comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. https://doi.org/10.1093/molbev/msv150

Li T, Luo D, Ning N, et al (2023) *Acinetobacter baumannii* adaptation to the host pH microenvironment is mediated by allelic variation in a single residue of BauA protein. PNAS Nexus 2(4):pgad079. https://doi.org/10.1093/pnasnexus/pgad079

Liu Y, Wang W, Shah SB, et al (2020) Phenol biodegradation by *Acinetobacter radioresistens* APH1 and its application in soil bioremediation. Appl Microbiol Biotechnol 104:427–437. https://doi.org/10.1007/s00253-019-10271-w

Liu YS, Han HJ, Fang F (2013) Degradation of long-chain n-alkanes by *Acinetobacter* sp. Adv Mat Res 726:2151–2155. https://doi.org/10.4028/www.scientific.net/AMR.726-731.2151

Lossi NS, Dajani R, Freemont P, Filloux A (2011) Structure-function analysis of HsiF, a gp25-like component of the type VI secretion system, in *Pseudomonas aeruginosa*. Microbiology 157:3292–3305. https://doi.org/10.1099/mic.0.051987-0

Lu Y, Zhou G, Ewald J, et al (2023) MicrobiomeAnalyst 2.0: Comprehensive statistical, functional, and integrative analysis of microbiome data. Nucleic Acids Res 51(1):310–318. https://doi.org/10.1093/nar/gkad407

Maino JL, Schouten R, Overton K, et al (2021) Regional and seasonal activity predictions for fall armyworm in Australia. Curr Res Insect Sci 1:100010. https://doi.org/10.1016/j.cris.2021.100010

Matsumura E, Ooi S, Murakami S, et al (2004) Constitutive synthesis, purification, and characterization of catechol 1,2-dioxygenase from the aniline-assimilating bacterium *Rhodococcus* sp. AN-22. J Biosci Bioeng 98:71-76. https://doi.org/10.1016/s1389-1723(04)70245-5

McCutcheon JP, Moran NA (2012) Extreme genome reduction in symbiotic bacteria. Nat Rev Microbiol 10:13–26. https://doi.org/10.1038/nrmicro2670

McKenna CH, Asgari D, Crippen TL, et al (2022) Gene expression in *Lucilia sericata* (Diptera: Calliphoridae) larvae exposed to *Pseudomonas aeruginosa* and *Acinetobacter baumannii* identifies shared and microbe-specific induction of immune genes. Insect Mol Biol 31:85–100. https://doi.org/10.1111/imb.12740

Mea HJ, Yong PVC, Wong EH (2021) An overview of *Acinetobacter baumannii* pathogenesis: Motility, adherence, and biofilm formation. Microbiol Res 247:126722. https://doi.org/10.1016/j.micres.2021.126722

Meier-Kolthoff JP, Auch AF, Klenk H-P, Oker MG (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3

Micalella C, Martignon S, Bruno S, et al (2011) X-ray crystallography, mass spectrometry and single crystal microspectrophotometry: A multidisciplinary characterization of catechol 1,2 dioxygenase. Biochim Biophys Acta Proteins Proteom 1814:817–823. https://doi.org/10.1016/j.bbapap.2010.09.008

Miksanek JR, Tuda M (2023) Endosymbiont-mediated resistance to entomotoxic nanoparticles and sex-specific responses in a seed beetle. J Pest Sci 96:1257–1270. https://doi.org/10.1007/s10340-023-01596-7

Minard G, Tran FH, Raharimalala FN, et al (2013) Prevalence, genomic and metabolic profiles of *Acinetobacter* and *Asaia* associated with field caught *Aedes albopictus* from Madagascar. FEMS Microbiol Ecol 83:63–73. https://doi.org/10.1111/j.1574-6941.2012.01455.x

Mitra S, Firake DM, Umesh KP, et al (2021) Polyphagous caterpillars of *Spodoptera litura* switch from a trap crop to the main crop, improve fitness, and shorten generation time. J Pest Sci 94:1091–1103. https://doi.org/10.1007/s10340-021-01351-w

Mohapatra B, Nain S, Sharma R, Phale PS (2022) Functional genome mining and taxono-genomics reveal eco-physiological traits and species distinctiveness of aromatic-degrading *Pseudomonas bharatica* sp. nov. Environ Microbiol Rep 14:464–474. https://doi.org/10.1111/1758-2229.13066

Montezano DG, Specht A, Sosa-Gómez DR, et al (2018) Host Plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. African Entomology 26:286–300. https://doi.org/10.4001/003.026.0286

Moran NA, Plague GR, Sandströ JP, Wilcox JL (2003) A genomic perspective on nutrient provisioning by bacterial symbionts of insects. Proc Natl Acad Sci U S A 100:14543–14548. https://doi.org/https://doi.org/10.1073/pnas.2135345100

Nanra JS, Buitrago SM, Crawford S, et al (2013) Capsular polysaccharides are an important immune evasion mechanism for *Staphylococcus aureus*. Hum Vaccin Immunother 9(3):480–487. https://doi.org/10.4161/hv.23223

Oliveira NC, Rodrigues PAP, Cônsoli FL (2023) Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the western hemisphere. Microb Ecol 85(4):1552–1563 https://doi.org/10.1007/s00248-022-02008-6

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci U S A 100(4):1803–1807. https://doi.org/10.1073/pnas.0335320100

Omoto C, Bernardi O, Salmeron E, et al (2016) Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag Sci 72:1727–1736. https://doi.org/10.1002/ps.4201

Pang R, Xing K, Yuan L, et al (2021) Peroxiredoxin alleviates the fitness costs of imidacloprid resistance in an insect pest of rice. PLoS Biol 19(4):e3001190. https://doi.org/10.1371/journal.pbio.3001190

Parks AR, Peters JE (2009) Tn7 elements: Engendering diversity from chromosomes to episomes. Plasmid 61(1):1–14. https://doi.org/10.1016/j.plasmid.2008.09.008

Parks AR, Peters JE (2007) Transposon Tn7 is widespread in diverse bacteria and forms genomic islands. J Bacteriol 189:2170–2173. https://doi.org/10.1128/JB.01536-06

Pavlidi N, Vontas J, Van Leeuwen T (2018) The role of glutathione S-transferases (GSTs) in insecticide resistance in crop pests and disease vectors. Curr Opin Insect Sci 27:97–102. https://doi.org/10.1016/j.cois.2018.04.007

Peters JE, Fricker AD, Kapili BJ, Petassi MT (2014) Heteromeric transposase elements: Generators of genomic islands across diverse bacteria. Mol Microbiol 93:1084–1092. https://doi.org/10.1111/mmi.12740

Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. Appl Microbiol Biotechnol 67:170–191. https://doi.org/10.1007/s00253-004-1810-4

Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes De Novo assembler. Curr Protoc Bioinformatics 70(1):e102. https://doi.org/10.1002/cpbi.102

Quast C, Pruesse E, Yilmaz P, et al (2013) The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41(1):590–596. https://doi.org/10.1093/nar/gks1219

Repizo GD, Gagné S, Foucault-Grunenwald ML, et al (2015) Differential role of the T6SS in *Acinetobacter baumannii* virulence. PLoS One 10(9):e0138265. https://doi.org/10.1371/journal.pone.0138265

Rodríguez-Salazar J, Almeida-Juarez AG, Ornelas-Ocampo K, et al (2020) Characterization of a novel functional trimeric catechol 1,2-Dioxygenase from a *Pseudomonas stutzeri* isolated from the Gulf of Mexico. Front Microbiol 11:1100. https://doi.org/10.3389/fmicb.2020.01100

Rohmer L, Guttman DS, Dangl JL (2004) Diverse evolutionary mechanisms shape the type III effector virulence factor repertoire in the plant pathogen *Pseudomonas syringae*. Genetics 167:1341–1360. https://doi.org/10.1534/genetics.103.019638

Roskova Z, Skarohlid R, McGachy L (2022) Siderophores: An alternative bioremediation strategy? Sci Total Environ 819:153144. https://doi.org/10.1016/j.scitotenv.2022.153144

Rosselló-Móra R, Amann R (2015) Past and future species definitions for bacteria and archaea. Syst Appl Microbiol 38:209–216. https://doi.org/10.1016/j.syapm.2015.02.001

Saati-Santamaría Z, López-Mondéjar R, Jiménez-Gómez A, et al (2018) Discovery of phloeophagus beetles as a source of *Pseudomonas* strains that produce potentially new bioactive substances and description of *Pseudomonas bohemica* sp. nov. Front Microbiol 9:913. https://doi.org/10.3389/fmicb.2018.00913

Sato Y, Jang S, Takeshita K, et al (2021) Insecticide resistance by a host-symbiont reciprocal detoxification. Nat Commun 12:6432. https://doi.org/10.1038/s41467-021-26649-2

Seitz P, Blokesch M (2013) Cues and regulatory pathways involved in natural competence and transformation in pathogenic and environmental Gram-negative bacteria. FEMS Microbiol Rev 37(3):336–363. https://doi.org/10.1111/j.1574-6976.2012.00353.x

Shao Y, Arias-Cordero E, Guo H, et al (2014) In vivo Pyro-SIP assessing active gut microbiota of the cotton leafworm, *Spodoptera littoralis*. PLoS One 9(1):e85948. https://doi.org/10.1371/journal.pone.0085948

Shao Y, Chen B, Sun C, et al (2017) Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. Cell Chem Biol 24:66–75. https://doi.org/10.1016/j.chembiol.2016.11.015

Silby MW, Winstanley C, Godfrey SAC, et al (2011) *Pseudomonas* genomes: Diverse and adaptable. FEMS Microbiol Rev 35(4):652–680. https://doi.org/10.1111/j.1574-6976.2011.00269.x

Singh P, Suri CR, Cameotra SS (2004) Isolation of a member of *Acinetobacter* species involved in atrazine degradation. Biochem Biophys Res Commun 317:697–702. https://doi.org/10.1016/j.bbrc.2004.03.112

Sirithanakorn C, Cronan JE (2021) Biotin, a universal and essential cofactor: Synthesis, ligation, and regulation. FEMS Microbiol Rev 45:1–18. https://doi.org/10.1093/femsre/fuab003

Srinivasan R, Karaoz U, Volegova M, et al (2015) Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One 10(2):e0117617. https://doi.org/10.1371/journal.pone.0117617

Storer NP, Babcock JM, Schlenz M, et al (2010) Discovery and characterization of field resistance to Bt Maize: *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in Puerto Rico. J Econ Entomol 103(4):1031–1038. https://doi.org/10.1603/EC10040

Strepis N, Naranjo HD, Meier-Kolthoff J, et al (2020) Genome-guided analysis allows the identification of novel physiological traits in *Trichococcus* species. BMC Genomics 21:24. https://doi.org/10.1186/s12864-019-6410-x

Subhadra B, Kim J, Kim DH, et al (2018) Local repressor AcrR regulates AcrAB efflux pump required for biofilm formation and virulence in *Acinetobacter nosocomialis*. Front Cell Infect Microbiol 8:270. https://doi.org/10.3389/fcimb.2018.00270

Suckling DM, Conlong DE, Carpenter JE, et al (2017) Global range expansion of pest Lepidoptera requires socially acceptable solutions. Biol Invasions 19:1107–1119. https://doi.org/10.1007/s10530-016-1325-9

Sudakaran S, Kost C, Kaltenpoth M (2017) Symbiont acquisition and replacement as a source of ecological innovation. Trends Microbiol 25(5):375–390. https://doi.org/10.1016/j.tim.2017.02.014

Sui X, Li Y, Wang X, et al (2023) Genomics and degradation law of *Acinetobacter junii* in response to petroleum pollution. Process Biochemistry 126:41–50. https://doi.org/10.1016/j.procbio.2022.12.021

Tang T, Zhang Y, Cai T, et al (2021) Antibiotics increased host insecticide susceptibility via collapsed bacterial symbionts reducing detoxification metabolism in the brown planthopper, *Nilaparvata lugens.* J Pest Sci 94:757–767. https://doi.org/10.1007/s10340-020-01294-8

Tao A, Wang T, Pang F, et al (2022) Characterization of a novel chitinolytic *Serratia marcescens* strain TC-1 with broad insecticidal spectrum. AMB Express 12:100. https://doi.org/10.1186/s13568-022-01442-6

Tao HP, Shen ZY, Zhu F, et al (2011) Isolation and identification of a pathogen of silkworm *Bombyx mori.* Curr Microbiol 62:876–883. https://doi.org/10.1007/s00284-010-9796-x

Teoh MC, Furusawa G, Veera Singham G (2021) Multifaceted interactions between the pseudomonads and insects: Mechanisms and prospects. Arch Microbiol 203:1891–1915. https://doi.org/10.1007/s00203-021-02230-9

Teufel R, Mascaraque V, Ismail W, et al (2010) Bacterial phenylalanine and phenylacetate catabolic pathway revealed. Proc Natl Acad Sci U S A 107:14390–14395. https://doi.org/10.1073/pnas.1005399107

Uniyal S, Paliwal R, Verma M, et al (2016) Isolation and characterization of fipronil degrading *Acinetobacter calcoaceticus* and *Acinetobacter oleivorans* from rhizospheric zone of *Zea mays*. Bull Environ Contam Toxicol 96:833–838. https://doi.org/10.1007/s00128-016-1795-6

Vandamme P, Peeters C (2014) Time to revisit polyphasic taxonomy. Antonie van Leeuwenhoek 106(1):57–65. https://doi.org/10.1007/s10482-014-0148-x

Vandamme P, Pot B, Gillis M, et al (1996) Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol Rev 60: 407–38. https://doi.org/10.1128/mr.60.2.407-438.1996

Van Den Berg J, Du Plessis H (2022) Chemical control and insecticide resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Econ Entomol 115:1761–1771. https://doi.org/10.1093/jee/toac108

Vélez JMB, Martínez JG, Ospina JT, Agudelo SO (2021) Bioremediation potential of *Pseudomonas* genus isolates from residual water, capable of tolerating lead through mechanisms of exopolysaccharide production and biosorption. Biotechnol Rep 32:e00685. https://doi.org/10.1016/j.btre.2021.e00685

Vianney A, Jubelin G, Renault S, et al (2005) *Escherichia coli tol* and *rcs* genes participate in the complex network affecting curli synthesis. Microbiology 151:2487–2497. https://doi.org/10.1099/mic.0.27913-0

Viggor S, Jõesaar M, Soares-Castro P, et al (2020) Microbial metabolic potential of phenol degradation in wastewater treatment plant of crude oil refinery: Analysis of metagenomes and characterization of isolates. Microorganisms 8(5):652. https://doi.org/10.3390/microorganisms8050652

Wan J, Huang C, Li C you, et al (2021) Biology, invasion, and management of the agricultural invader: fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Integr Agric 20:646–663. https://doi.org/10.1016/S2095-3119(20)63367-6

Weber BS, Kinsella RL, Harding CM, Feldman MF (2017) The secrets of *Acinetobacter* secretion. Trends Microbiol 25:532–545. https://doi.org/10.1016%2Fj.tim.2017.01.005

Weglarz KM, Havill NP, Burke GR, Von Dohlen CD (2018) Partnering with a pest: Genomes of hemlock woolly adelgid symbionts reveal atypical nutritional provisioning patterns in dual-obligate bacteria. Genome Biol Evol 10:1607–1621. https://doi.org/10.1093/gbe/evy114

Wiedenbeck J, Cohan FM (2011) Origins of bacterial diversity through horizontal genetic transfer and adaptation to new ecological niches. FEMS Microbiol Rev 35:957–976. https://doi.org/10.1111/j.1574-6976.2011.00292.x

Xia W, Du Z, Cui Q, et al (2014) Biosurfactant produced by novel *Pseudomonas* sp. WJ6 with biodegradation of n-alkanes and polycyclic aromatic hydrocarbons. J Hazard Mater 276:489–498. https://doi.org/10.1016/j.jhazmat.2014.05.062

Xie S, Liu J, Li L, Qiao C (2009) Biodegradation of malathion by *Acinetobacter johnsonii* MA19 and optimization of cometabolism substrates. J Environ Sci 21:76–82. https://doi.org/10.1016/S1001-0742(09)60014-0

Xu LT, Lu M, Sun JH (2016) Invasive bark beetle-associated microbes degrade a host defensive monoterpene. Insect Sci 23:183–190. https://doi.org/10.1111/1744-7917.12255

Yao X-H, Min H, Lv Z-M (2006) Response of superoxide dismutase, catalase, and ATPase activity in bacteria exposed to acetamiprid. Biomed Environ Sci 19(4):309-314. PMID: 17044650

Zhang S, Shu J, Xue H, et al (2020) The gut microbiota in camellia weevils are influenced by plant secondary metabolites and contribute to saponin degradation. mSystems 5(2):10-1128. https://doi.org/10.1128/msystems.00692-19

Zhao K, Tang H, Zhang B, et al (2022) Microbial production of vitamin B5: Current status and prospects. Crit Rev Biotechnol 43(8):1172-1192. https://doi.org/10.1080/07388551.2022.2104690

4. METABOLOMICS OF SUSCEPTIBLE AND INSECTICIDE-RESISTANT STRAINS OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE) AND METATRANSCRIPTOMICS OF THEIR MIDGUT-ASSOCIATED MICROBIOTA

Abstract

The gut microbiota of insects can influence host physiology and, like the host, can be affected by stressors. In this study, we investigated the effects of insecticide exposure on the metabolome and gut microbial metatranscriptome of insecticide resistant and susceptible *Spodoptera frugiperda* larvae. Metabolomics analysis revealed distinct metabolic profiles influenced by genotype and treatment with three insecticides: flubendiamide, spinosad and teflubenzuron. Amino acids, monosaccharides and fatty acids were prominent among the differentially abundant metabolites. Genotype emerges as an important factor influencing the metabolic profile of *S. frugiperda* in response to insecticide exposure. For certain metabolites, this influence is observed constitutively, independent of treatment. This study highlights the role of members of the gut microbiota contributing with metabolites involved in host detoxification. In addition, it demonstrates that insecticide exposure and resistance evolution may alter host immunity and its interaction with the gut microbial community, potentially affecting the host's interaction with associated viruses. Our findings help to clarify the complex interplay between insecticide exposure, gut microbiota, and host response, and provide insights into the dynamics of the gut microbiota of *S. frugiperda* in response to insecticides.

Keywords: Gut microbiota; Metabolomics; Metatranscriptomics; Insecticide exposure; Holobiont

4.1. Introduction

The gut microbiota of insects plays an increasingly recognized role in shaping host physiology, including the response to stressors like xenobiotics (Jing et al. 2020; Antonelli et al. 2022). The complex interplay between insect hosts, their gut microbiota, and toxic compounds, such as insecticides, has attracted considerable scientific interest not only for its ecological implications, but also for its potential applications in pest management strategies (Pan et al. 2020; Rupawate et al. 2023).

The Fall armyworm (FAW) *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a key pest that endangers food security. The FAW is notorious for its polyphagy, remarkable adaptability to different environments, and rapid evolution of resistance to a wide range of insecticide and biotechnology-based pest control strategies (Carvalho et al. 2013; Omoto et al. 2016; Van Den Berg and Du Plessis 2022). Insecticide-resistant strains of *Spodoptera* species often possess a range of well-established and widely recognized mechanisms underlying their resistance to chemical insecticides (Yu et al. 2003; Dawkar et al. 2013; Boaventura et al. 2020;

Nascimento et al. 2022). These mechanisms can include target site mutations (Boaventura et al. 2020), alterations in activity of the detoxication machinery, like an elevated expression of cytochrome P450s, glutathione S-transferases, and esterases genes (Yu et al. 2003; Wang et al. 2006; Nascimento et al. 2022), and behavioral changes that minimize insecticide exposure or penetration of toxins (Dawkar et al. 2013; Zalucki and Furlong 2017).

The contribution of the gut microbiota to the enzymatic repertoire and adaptation of insects to crop rotation systems (Chu et al. 2013) and similar enzymatic contribution of the gut microbiota to metabolization of natural and organic xenobiotics has been reported for several insects (Genta et al. 2006; Shukla and Beran 2020; Jaffar et al. 2022; Zhou et al. 2023). In some cases, the contribution of the gut microbiota to the degradation of organic xenobiotics has been associated with insecticide resistance (Kikuchi et al. 2012; Cheng et al. 2017; Wu et al. 2020; Sato et al. 2021). The association of insecticide-degrading bacteria with laboratory-selected strains of insecticide-resistant *S. frugiperda* suggests that gut microbes also undergo a process of selection to adapt to insecticide exposure, just as their host (Almeida et al. 2017), indicating that the host and associated microbiota respond as a unit, a holobiont (Zilber-Rosenberg and Rosenberg 2008), to such stressful conditions. The higher diversity of insecticide-degrading bacteria in the gut microbiota of field collected larvae of *S. frugiperda*, and moreover the higher capacity of isolates of these bacteria to metabolize different chemistries of insecticides add an additional layer to the intricate mechanisms that may be involved in the evolution of insecticide resistance in *S. frugiperda* (Gomes et al. 2020). This symbiotic relationship between insects and their associated bacteria highlights the need for a comprehensive understanding of both well-established and emerging mechanisms of resistance in our pursuit of effective pest management strategies.

The objective of this study was to investigate the effects of insecticide exposure on the metabolome and gut microbial metatranscriptome of insecticide-resistant and susceptible *S. frugiperda* larvae. Our research hypothesis is that the gut microbiota of insecticide resistant *S. frugiperda* strains may have metabolic pathways and transcriptional profiles that enhance the insect's ability to tolerate or detoxify these chemicals. This study aims to investigate mechanisms by which the gut microbiota may enhance insecticide resistance, potentially opening new avenues for more sustainable pest management strategies.

4.2. Conclusions

The study revealed that the metabolome of both FAW larvae and its gut microbiota were influenced by host genotype, exposure to insecticides and/or by the interaction of these factors. The analyses revealed that the members of the gut microbiota may contribute with metabolites relevant to host detoxification, and demonstrated that insecticide exposure and resistance evolution may affect host immunity and its interaction with its gut microbial community, with apparent effects on the host's interactions with associated viruses. Genotype emerged as the major factor contributing to the differential metabolic profile of *S. frugiperda* in response to insecticide exposure. Resistant genotypes showed differential abundance of mainly amino acids, monosaccharides, and fatty acids, potentially related to stress response, energy metabolism and xenobiotic detoxification. Overall, this highlights the intricate nature of the relationship between FAW larvae and their gut microbial community, shedding light on the biological processes and potential metabolic responses associated with insecticide resistance.

References

Ahn, S., Jung, J., Jang, I. A., Madsen, E. L., Park, W. (2016). Role of glyoxylate shunt in oxidative stress response. J Biol Chem, 291(22): 11928–11938. https://doi.org/10.1074/jbc.M115.708149

Allocati, N., Federici, L., Masulli, M., Di Ilio, C. (2009). Glutathione transferases in bacteria. FEBS J, 276(1): 58–75. https://doi.org/10.1111/j.1742-4658.2008.06743.x

Almeida, L. G., De Moraes, L. A. B., Trigo, J. R., Omoto, C., Cônsoli, F. L. (2017). The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. PLoS ONE, 12(3): e0174754. https://doi.org/10.1371/journal.pone.0174754

Andrews (2010) FastQC: A quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Antonelli, P., Duval, P., Luis, P., Minard, G., Valiente Moro, C. (2022). Reciprocal interactions between anthropogenic stressors and insect microbiota. Environ Sci Pollut Res, 29(43): 64469-64488. https://doi.org/10.1007/s11356-022-21857-9

Araújo, R. A., Guedes, R. N. C., Oliveira, M. G. A., Ferreira, G. H. (2008). Enhanced activity of carbohydrate- and lipid-metabolizing enzymes in insecticide-resistant populations of the maize weevil, *Sitophilus zeamais*. Bull Entom Res, 98(4): 417–424. https://doi.org/10.1017/S0007485308005737

Arvas M., Pakula T., Smit B., et al (2011) Correlation of gene expression and protein production rate - a system wide study. BMC Genomics 12: 616. https://doi.org/10.1186/1471-2164-12-616

Aucoin R., Guillet G., Murray C., Philogène, B. J. R., Arnason, J. T. (1995) How do insect herbivores cope with the extreme oxidative stress of phototoxic host plants? Arch Insect Biochem Physiol, 29(2): 211-226. https://doi.org/10.1002/arch.940290210

Bayoumi, A. E., Pérez-Pertejo, Y., Ordóñez, C., et al (2000) Changes in the glutathione-redox balance induced by the pesticides heptachlor, chlordane, and toxaphene in CHO-K1 cells. Bull Environ Contam Toxicol, 65(6): 748–755. https://doi.org/10.1007/s0012800186

Boaventura, D., Bolzan, A., Padovez, F. E. O., Okuma, D. M., Omoto, C., Nauen, R. (2020). Detection of a ryanodine receptor target-site mutation in diamide insecticide resistant fall armyworm, *Spodoptera frugiperda*. Pest Manag Sci, 76(1): 47–54. https://doi.org/10.1002/ps.5505

Bolger, A. M., Lohse, M., Usadel, B. (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics, 30(15): 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bolzan, A., Padovez, F. E. O., Nascimento, A. R. B., et al (2019) Selection and characterization of the inheritance of resistance of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to chlorantraniliprole and cross-resistance to other diamide insecticides. Pest Manag Sci, 75:2682–2689. https://doi.org/10.1002/ps.5376

Buchfink, B., Xie, C., Huson, D. H. (2015) Fast and sensitive protein alignment using DIAMOND. Nat Methods, 12: 59–60. https://doi.org/10.1038/nmeth.3176

Carvalho, R. A., Omoto, C., Field, L. M., Williamson, M. S., Bass, C. (2013). Investigating the molecular mechanisms of organophosphate and pyrethroid resistance in the fall armyworm *Spodoptera frugiperda*. PLoS ONE, 8(4): e62268. https://doi.org/10.1371/journal.pone.0062268

Champion, C. J., Xu, J. (2018) Redox state affects fecundity and insecticide susceptibility in *Anopheles gambiae*. Sci Rep, 8: 13054. https://doi.org/10.1038/s41598-018-31360-2

Chen, Y-P., Li, Y-H., Sun, Z-X., et al (2022) Effects of host plants on bacterial community structure in larvae midgut of *Spodoptera frugiperda*. Insects, 13(4): 373. https://doi.org/10.3390/insects13040373

Cheng, D., Guo, Z., Riegler, M., Xi, Z., Liang, G., Xu, Y. (2017). Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome, 5: 13. https://doi.org/10.1186/s40168-017-0236-z

Chetverin, A. B., Spirin, A. S. (1982) Bioenergetics and protein synthesis. Biochim Biophys Acta, 683(2): 153–179. https://doi.org/10.1016/0304-4173(82)90009-x

Chew, S. Y., Than, L. T. L. (2021) Glucose metabolism and use of alternative carbon sources in medically-important fungi. Encyclopedia of Mycology, 1: 220-229. http://dx.doi.org/10.1016/B978-0-12-819990-9.00068-8

Chio, E. H., Li, Q. X. (2022). Pesticide research and development: General discussion and spinosad case. J Agric Food Chem, 70(29): 8913–8919. https://doi.org/10.1021/acs.jafc.2c03821

Chu, C. C., Spencer, J. L., Curzi, M. J., Zavala, J. A., Seufferheld, M. J. (2013). Gut bacteria facilitate adaptation to crop rotation in the western corn rootworm. Proc Natl Acad Sci U S A, 110(29):11917-11922. https://doi.org/10.1073/pnas.1301886110

Conesa, A., Götz, S., García-Gómez, J. M., et al (2005) Blast2GO: A universal tool for annotation, visualization and analysis in functional genomics research. Bioinformatics, 21(18): 3674–3676. https://doi.org/10.1093/bioinformatics/bti610

Dawkar, V. V., Chikate, Y. R., Lomate, P. R., Dholakia, B. B., Gupta, V. S., Giri, A. P. (2013). Molecular insights into resistance mechanisms of lepidopteran insect pests against toxicants. J Proteome Res, 12(11): 4727–4737. https://doi.org/10.1021/pr400642p

De Vadder, F., Kovatcheva-Datchary, P., Zitoun, C., et al (2016) Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. Cell Metab, 24(1): 151–157. https://doi.org/10.1016/j.cmet.2016.06.013

Di Prisco, G., Cavaliere, V., Annoscia, D., et al (2013) Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. Proc Natl Acad Sci U S A, 110: 18466–18471. https://doi.org/10.1073/pnas.1314923110

Dolan, S. K., Welch, M. (2018) The glyoxylate shunt, 60 years on. Annu Rev Microbiol., 72: 309–330. https://doi.org/10.1146/annurev-micro-090817-062257

Dunn, M. F., Ramírez-Trujillo, J. A., Hernández-Lucas, I. (2009). Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis. Microbiology, 155(10): 3166–3175. https://doi.org/10.1099/mic.0.030858-0

Erdos, Z., Chandler, D., Bass, C., Raymond, B. (2021). Controlling insecticide resistant clones of the aphid, *Myzus persicae*, using the entomopathogenic fungus *Akanthomyces muscarius*: Fitness cost of resistance under pathogen challenge. Pest Manag Sci, 77(11): 5286–5293. https://doi.org/10.1002/ps.6571

Farhad, H. A. (2021). An overview of stress in cellular and molecular levels and the importance of studying responses to stresses in biology. Res J Biotechnol, 16(4): 258–282.

Fulda, S., Gorman, A. M., Hori, O., Samali, A. (2010). Cellular stress responses: Cell survival and cell death. Int J Cell Biol, 1–23. https://doi.org/10.1155/2010/214074

Gamarra, Y., Santiago, F. C., Molina-López, J., Castaño, J., Herrera-Quintana, L., Domínguez, Á., Planells, E. (2019). Pyroglutamic acidosis by glutathione regeneration blockage in critical patients with septic shock. Crit Care, 23: 162. https://doi.org/10.1186/s13054-019-2450-5

Gao B, Chi L, Tu P., et al (2019a) The carbamate aldicarb altered the gut microbiome, metabolome, and lipidome of C57BL/6J mice. Chem Res Toxicol, 32(1): 67–79. https://doi.org/10.1021/acs.chemrestox.8b00179

Gao, X., Li, W., Luo, J., et al (2019b) Biodiversity of the microbiota in *Spodoptera exigua* (Lepidoptera: Noctuidae). J Appl Microbiol, 126: 1199–1208. https://doi.org/10.1111/jam.14190

Gassmann, A. J., Carrière, Y., Tabashnik, B. E. (2009). Fitness costs of insect resistance to *Bacillus thuringiensis.* Annu Rev Entomol, 54: 147–163. https://doi.org/10.1146/annurev.ento.54.110807.090518

Ge, S. X, Son, E. W., Yao, R. (2018) iDEP: An integrated web application for differential expression and pathway analysis of RNA-Seq data. BMC Bioinformatics 19: 534. https://doi.org/10.1186/s12859-018-2486-6

Genta, F. A., Dillon, R. J., Terra, W. R., Ferreira, C. (2006). Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae. J Insect Physiol, 52(6): 593-601. https://doi.org/10.1016/j.jinsphys.2006.02.007

Giambò, F., Teodoro, M., Costa, C., Fenga, C. (2021). Toxicology and microbiota: How do pesticides influence gut microbiota? J. Environ. Res. Public Health, 18(11): 5510. https://doi.org/10.3390/ijerph18115510

Gomes, A. F. F., Omoto, C., Cônsoli, F. L. (2020). Gut bacteria of field collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory selected resistant strains. J Pest Sci, 93(2): 833–851. https://doi.org/10.1007/s10340-020-01202-0

Haas, B. J., Papanicolaou, A., Yassour, M., et al (2013) *De novo* transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. Nat Protoc, 8: 1494–1512. https://doi.org/10.1038/nprot.2013.084

Hannig, G. T., Ziegler, M., Paula, G. M. (2009). Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. Pest Manag Sci, 65(9): 969–974. https://doi.org/10.1002/ps.1781

Hiler, S., Charnetzky, W. T. (1981). Glyoxylate bypass enzymes in *Yersinia* species and multiple forms of isocitrate lyase in *Yersinia pestis.* J Bacteriol, 145(1): 452–458. https://doi.org/10.1128/jb.145.1.452-458.1981

Hill, B. G., Shiva, S., Ballinger, S., et al (2019) Bioenergetics and translational metabolism: Implications for genetics, physiology and precision medicine. Biol Chem, 401(1): 3-29. https://doi.org/10.1515/hsz-2019-0268

Hou, J., Yu, J., Qin, Z., et al (2021) Guadipyr, a new insecticide, induces microbiota dysbiosis and immune disorders in the midgut of silkworms (*Bombyx mori*). Environ Pollut, 286: 117531. https://doi.org/10.1016/j.envpol.2021.117531

Huang, L., Li, J., Peng, L., Xie, R., Su, X., He, P., Xu, J., Jia, Z., Luo, X., Chen, X. G., Li, H. (2021). The differential metabolic profiles between deltamethrin-resistant and -susceptible strains of *Aedes albopictus* (Diptera: Culicidae) by 1H-NMR. J Med Entomol, 58(3): 1256–1263. https://doi.org/10.1093/jme/tjaa273

Huynh, M. P., Hibbard, B. E., Ho, K. Van, Shelby, K. S. (2022). Toxicometabolomic profiling of resistant and susceptible western corn rootworm larvae feeding on Bt maize seedlings. Sci Rep, 12: 11639. https://doi.org/10.1038/s41598-022-15895-z

Hyung, Ko J., Gyu Kim, B., Joong-Hoon, A. (2006) Glycosylation of flavonoids with a glycosyltransferase from *Bacillus cereus*. FEMS Microbiol Lett, 258(2): 263–268. https://doi.org/10.1111/j.1574-6968.2006.00226.x

Jaffar, S., Ahmad, S., Lu, Y. (2022) Contribution of insect gut microbiota and their associated enzymes in insect physiology and biodegradation of pesticides. Front Microbiol, 13: 979383. https://doi.org/10.3389/fmicb.2022.979383

James, R. R., Xu, J. (2012) Mechanisms by which pesticides affect insect immunity. J Invertebr Pathol, 109: 175–182. https://doi.org/10.1016/j.jip.2011.12.005

Jing, T. Z., Qi, F. H., Wang, Z. Y. (2020) Most dominant roles of insect gut bacteria: Digestion, detoxification, or essential nutrient provision? Microbiome, 8: 38. https://doi.org/10.1186/s40168-020-00823-y

Jung, J., Noh, J., Park, W. (2011). Physiological and metabolic responses for hexadecane degradation in *Acinetobacter oleivorans* DR1. J Microbiol, 49(2): 208–215. https://doi.org/10.1007/s12275-011-0395-8

Jung, J., Park, W. (2013). Comparative genomic and transcriptomic analyses reveal habitat differentiation and different transcriptional responses during pectin metabolism in *Alishewanella* species. Appl Environ Microbiol, 79(20): 6351–6361. https://doi.org/10.1128/AEM.02350-13

Kasten, J. P., Precetti, A. A. C. M., Parra, J. R. P. (1978). Dados biológicos comparativos de *Spodoptera frugiperda* (J. E. Smith, 1797) em duas dietas artificiais e substrato natural. Rev Agri, 53: 68–78.

Kikuchi, Y., Hayatsu, M., Hosokawa, T., Nagayama, A., Tago, K., Fukatsu, T. (2012). Symbiont-mediated insecticide resistance. Proc Natl Acad Sci U S A, 109(22): 8618–8622. https://doi.org/10.1073/pnas.1200231109

Kolawole, A. O., Kolawole, A. N. (2020) Insecticides and bio-insecticides modulate the glutathione-related antioxidant defense system of cowpea storage bruchid (*Callosobruchus maculatus*). Int J Insect Sci, 6(1): IJIS-S18029. https://doi.org/10.1177/IJIS.S18029

Kondrashov, F. A., Koonin, E. V., Morgunov, I. G., Finogenova, T. V., Kondrashova, M. N. (2006). Evolution of glyoxylate cycle enzymes in Metazoa: Evidence of multiple horizontal transfer events and pseudogene formation. Biol Direct, 1: 31. https://doi.org/10.1186/1745-6150-1-31

Krüger, A., Grüning, N. M., Wamelink, M. M. C., Kerick, M., Kirpy, A., Parkhomchuk, D., Bluemlein, K., Schweiger, M. R., Soldatov, A., Lehrach, H., Jakobs, C., Ralser, M. (2011). The pentose phosphate pathway is a metabolic redox sensor and regulates transcription during the antioxidant response. Antioxid Redox Signal, 15(2): 311–324. https://doi.org/10.1089/ars.2010.3797

Li, B., Dewey, C.N. (2011) RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 12:323. https://doi.org/10.1186/1471-2105-12-323

Li, Y., Li, Y., Wang, G., Li, J., Zhang, M., Wu, J., Liang, C., Zhou, H., Tang, J., Zhu, G. (2022). Differential metabolome responses to deltamethrin between resistant and susceptible *Anopheles sinensis*. Ecotoxicol Environ Saf, 237(1): 113553. https://doi.org/10.1016/j.ecoenv.2022.113553

Lindsey, T. L., Hagins, J. M., Sokol, P. A., Silo-Suh, L. A. (2008) Virulence determinants from a cystic fibrosis isolate of *Pseudomonas aeruginosa* include isocitrate lyase. Microbiology, 154: 1616–1627. https://doi.org/10.1099/mic.0.2007/014506-0

Liu, Y., Shen, Z., Yu, J., Li, Z., Liu, X., Xu, H. (2020). Comparison of gut bacterial communities and their associations with host diets in four fruit borers. Pest Manag Sci, 76(4): 1353–1362. https://doi.org/10.1002/ps.5646

Lorenz, M. C., Fink, G. R. (2002) Life and death in a macrophage: Role of the glyoxylate cycle in virulence. Eukaryot Cell, 1(5): 657–662. https://doi.org/10.1128%2FEC.1.5.657-662.2002

Love, M. I., Huber, W., Anders, S. (2014) Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol, 15: 550. https://doi.org/10.1186/s13059-014-0550-8

Mcfadden, B. A., Purohit, S. (1977). Itaconate, an isocitrate lyase-directed inhibitor in *Pseudomonas indigofera*. J Bacteriol, 131(1):136–144. https://doi.org/10.1128/jb.131.1.136-144.1977

McVey, A. C., Medarametla, P., Chee, X., et al (2017) Structural and functional characterization of malate synthase g from opportunistic pathogen *Pseudomonas aeruginosa.* Biochemistry, 56: 5539–5549. https://doi.org/10.1021/acs.biochem.7b00852

Merzendorfer, H. (2013). Chitin synthesis inhibitors: Old molecules and new developments. Insect Sci, 20(2): 121–138. https://doi.org/10.1111/j.1744-7917.2012.01535.x

Moine, L., Rivoira, M., de Barboza, G.D., et al (2018) Glutathione depleting drugs, antioxidants and intestinal calcium absorption. World J Gastroenterol, 24: 4979–4988. https://doi.org/10.3748%2Fwjg.v24.i44.4979

Nagare, M., Ayachit, M., Agnihotri, A., et al (2021) Glycosyltransferases: The multifaceted enzymatic regulator in insects. Insect Mol Biol, 30(2): 123–137. https://doi.org/10.1111/imb.12686

Nascimento, A. R. B., Pavinato, V. A. C., Rodrigues, J. G., Silva-Brandão, K. L., Consoli, F. L., Michel, A., Omoto, C. (2022). There is more than chitin synthase in insect resistance to benzoylureas: Molecular markers associated with teflubenzuron resistance in *Spodoptera frugiperda*. J Pest Sci, 95(1): 129-144. https://doi.org/10.1007/s10340-021-01373-4

Nascimento A. R. B., Fresia, P., Cônsoli, F.L., Omoto, C. (2015) Comparative transcriptome analysis of lufenuron-resistant and susceptible strains of *Spodoptera frugiperda* (Lepidoptera: Noctuidae). BMC Genomics, 16: 985. https://doi.org/10.1186/s12864-015-2183-z

Nguyen, T. Van, Alfaro, A. C., Young, T., Green, S., Zarate, E., Merien, F. (2019). Itaconic acid inhibits growth of a pathogenic marine *Vibrio* strain: A metabolomics approach. Sci Rep, 9: 5937. https://doi.org/10.1038/s41598-019-42315-6

Okuma, D. M., Bernardi, D., Horikoshi, R.J., et al (2018) Inheritance and fitness costs of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) resistance to spinosad in Brazil. Pest Manag Sci, 74: 1441–1448. https://doi.org/10.1002/ps.4829

Oliveira, N. C., Rodrigues, P. A. P., Cônsoli, F. L. (2023) Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the western hemisphere. Microb Ecol, 85(4): 1552–1563. https://doi.org/10.1007/s00248-022-02008-6

Omoto, C., Bernardi, O., Salmeron, E., Sorgatto, R. J., Dourado, P. M., Crivellari, A., Carvalho, R. A., Willse, A., Martinelli, S., Head, G. P. (2016). Field-evolved resistance to Cry1Ab maize by *Spodoptera frugiperda* in Brazil. Pest Manag Sci, 72(9): 1727–1736. https://doi.org/10.1002/ps.4201

Pan, X., Wang, X., Zhang, F. (2020). New insights into cockroach control: Using functional diversity of *Blattella germanica* symbionts. Insects, 11(10): 1–17. https://doi.org/10.3390/insects11100696

Panevska, A., Skočaj, M., Križaj, I., Maček, P., Sepčić, K. (2019). Ceramide phosphoethanolamine, an enigmatic cellular membrane sphingolipid. Biochim Biophys Acta Biomembr, 1861(7): 1284–1292. https://doi.org/10.1016/j.bbamem.2019.05.001

Panini M, Manicardi GC, Moores GD, Mazzoni E (2016) An overview of the main pathways of metabolic resistance in insects. Invertebrate Surviv J, 13: 326–335.

Pastore, A., Federici, G., Bertini, E., Piemonte, F. (2003). Analysis of glutathione: Implication in redox and detoxification. Clin Chim Acta, 333(1): 19–39. https://doi.org/10.1016/S0009-8981(03)00200-6

Pirog, T. P., Kuz’minskaya, Y. V. (2003) Some characteristics of central metabolism in *Acinetobacter* sp. grown on ethanol. Microbiology, 72: 408-413. https://doi.org/10.1023/A:1025080320168

Pozdniakov, M. A., Zhuk, I. V., Lyapunova, M. V., et al (2019) Glyoxylic acid: synthesis, isolation, and crystallization. Russ Chem Bull, 68: 472-279. https://doi.org/10.1007/s11172-019-2442-2

Rauf, A., Wilkins, R. M. (2022). Malathion-resistant *Tribolium castaneum* has enhanced response to oxidative stress, immunity, and fitness. Pestic Biochem Phys, 184: 105128. https://doi.org/10.1016/j.pestbp.2022.105128

Rechner, A. R., Kuhnle, G., Hu, H., Roedig-Penman, A., Van Den Braak, M. H., Moore, K. P., Rice-Evans, C. A. (2002). The metabolism of dietary polyphenols and the relevance to circulating levels of conjugated metabolites. Free Radic Res, 36(11): 1229–1241. https://doi.org/10.1080/246-1071576021000016472

Ren, L., Zhang, H., Zhou, J., Wu, Y., Liu, B., Wang, S., Liu, X., Hao, X., Zhao, L. (2023). Unique and generic crossed metabolism in response to four sub-lethal environmental stresses in the oriental fruit fly, *Bactrocera dorsalis* Hendel. Ecotoxicol Environ Saf, 264: 115434. https://doi.org/10.1016/j.ecoenv.2023.115434

Rupawate, P. S., Roylawar, P., Khandagale, K., Gawande, S., Ade, A. B., Jaiswal, D. K., Borgave, S. (2023). Role of gut symbionts of insect pests: A novel target for insect-pest control. Front Microbiol, 14: 1146390. https://doi.org/10.3389/fmicb.2023.1146390

Sano, M., Tanaka, T., Ohara, H., Aso, Y. (2020). Itaconic acid derivatives: structure, function, biosynthesis, and perspectives. Appl Microbiol Biotechnol, 104(21): 9041–9051. https://doi.org/10.1007/s00253-020-10908-1

Sato, Y., Jang, S., Takeshita, K., Itoh, H., Koike, H., Tago, K., Hayatsu, M., Hori, T., Kikuchi, Y. (2021). Insecticide resistance by a host-symbiont reciprocal detoxification. Nat Commun, 12: 6432. https://doi.org/10.1038/s41467-021-26649-2

Scherz-Shouval, R., Shvets, E., Fass, E., Shorer, H., Gil, L., Elazar, Z. (2007). Reactive oxygen species are essential for autophagy and specifically regulate the activity of Atg4. EMBO J, 26(7): 1749–1760. https://doi.org/10.1038/sj.emboj.7601623

Seo, J. S., Keum, Y. S., Li, Q. X. (2009). Bacterial degradation of aromatic compounds. Int J Environ Res Public Health, 6(1): 278–309. https://doi.org/10.3390/ijerph6010278

Shukla, S. P., Beran, F. (2020). Gut microbiota degrades toxic isothiocyanates in a flea beetle pest. Mol Ecol, 29(23): 4692-4705. https://doi.org/10.1111/mec.15657

Skye, G. E., van Handel, E. (1974). Malate synthase in insects. Comp Biochem Physiol B Biochem, 49(1): 83–86. https://doi.org/10.1016/0305-0491(74)90225-9

Szumowski, S. C., Troemel, E. R. (2015). Microsporidia-host interactions. Curr Opin Microbiol, 26: 10–16. https://doi.org/10.1016/j.mib.2015.03.006

Teixeira, L. A., Andaloro, J. T. (2013). Diamide insecticides: Global efforts to address insect resistance stewardship challenges. Pestic Biochem Phys, 106(3): 76–78. https://doi.org/10.1016/j.pestbp.2013.01.010

Tohnishi, M., Nakao, H., Furuya, T., Seo, A., Kodama, H., Tsubata, K., Fujioka, S., Kodama, H., Hirooka, T., Nishimatsu, T. (2005). Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. J Pestic Sci, 30(4): 354–360. https://doi.org/10.1584/jpestics.30.354

Trzebny, A., Slodkowicz-Kowalska, A., Björkroth, J., Dabert, M. (2023). Microsporidian infection in mosquitoes (Culicidae) is associated with gut microbiome composition and predicted gut microbiome functional content. Microb Ecol, 85(1): 247–263. https://doi.org/10.1007/s00248-021-01944-z

Van Den Berg, J., Du Plessis, H. (2022). Chemical control and insecticide resistance in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Econ Entomol, 115(6): 1761–1771. https://doi.org/10.1093/jee/toac108

Wang, D., Lv, W., Yuan, Y., Zhang, T., Teng, H., Losey, J. E., Chang, X. (2020). Mechanism of the different metabolome responses between *Plutella xylostella* and *Pieris rapae* treated with the diamide insecticides. Ecotoxicol Environ Saf, 203: 111033. https://doi.org/10.1016/j.ecoenv.2020.111033

Wang, W., Mo, J., Cheng, J., Zhuang, P., Tang, Z. (2006). Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). Pestic Biochem Phys, 84(3): 180–187. https://doi.org/10.1016/j.pestbp.2005.07.002

Weiss, L. M., Becnel, J. J. (2014). Microsporidia: Pathogens of opportunity, First Edition. Edited Microsporidia in Insects Chapter 21.

Wu, Y., Zheng, Y., Chen, Y., Wang, S., Chen, Y., Hu, F., Zheng, H. (2020). Honey bee (*Apis mellifera*) gut microbiota promotes host endogenous detoxification capability via regulation of P450 gene expression in the digestive tract. Microb Biotechnol, 13(4): 1201–1212. https://doi.org/10.1111/1751-7915.13579

Yerra, A., Challa, S., Valluri, S. V., Mamillapalli, A. (2016). Spermidine alleviates oxidative stress in silk glands of *Bombyx mori*. J Asia-Pac Entomol, 19(4): 1197–1202. https://doi.org/10.1016/j.aspen.2016.10.014

Yu, S. J., Nguyen, S. N., Abo-Elghar, G. E. (2003). Biochemical characteristics of insecticide resistance in the fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Pestic Biochem Phys, 77(1): 1–11. https://doi.org/10.1016/S0048-3575(03)00079-8

Zalucki, M. P., Furlong, M. J. (2017). Behavior as a mechanism of insecticide resistance: Evaluation of the evidence. Curr Opin Insect Sci, 21: 19–25. Elsevier Inc. https://doi.org/10.1016/j.cois.2017.05.006

Zhang, Q., Wang, Q., Zhai, Y., et al (2022) Impacts of imidacloprid and flupyradifurone insecticides on the gut microbiota of *Bombus terrestris*. Agriculture, 12(3): 389. https://doi.org/10.3390/agriculture12030389

Zhou, F., Liang, Q., Zhao, X., Wu, X., Fan, S., Zhang, X. (2023). Comparative metaproteomics reveal co-contribution of onion maggot and its gut microbiota to phoxim resistance. Ecotoxicol Environ Saf, 267: 115649. https://doi.org/10.1016/j.ecoenv.2023.115649

Zilber-Rosenberg, I., Rosenberg, E. (2008) Role of microorganisms in the evolution of animals and plants: The hologenome theory of evolution. FEMS Microbiol Rev, 32: 723–735. https://doi.org/10.1111/j.1574-6976.2008.00123.x

5. DIVERSITY OF THE RECENTLY DESCRIBED GUT SYMBIONTS OF *Spodoptera frugiperda* (LEPIDOPTERA: NOCTUIDAE), *Enterococcus entomosocium* AND *Enterococcus spodopteracolus* IN NEOTROPICAL SPECIES OF *Spodoptera*

Abstract

Enterococcus is a core member of the larval gut microbiota of *Spodoptera* species, and it is proposed to provide the host with antibiotics to avoid the proliferation of pathogenic bacteria in the gut. Recent functional genomic analysis of *Enterococcus* isolates obtained from *S. frugiperda* led to the characterization of two new species, *E. entomosocium* and *E. spodopteracolous* that have been mistakenly identified as *E. mundtii* and *E. casseliflavus,* respectively because of the use of 16S rRNA as the only molecular marker. In *S. frugiperda, Enterococcus* is the most abundant member of the gut microbial community, and *E. entomosocium* and *E. spodopteracolous* were shown to evolve the ability to metabolize insecticides and possibly to contribute with the detoxification of hosts intoxicated with insecticides. *Spodoptera frugiperda* is one of the major agricultural pests in Brazil, and together with *S. albula, S. cosmioides,* and *S. eridania* they form what is called the *Spodoptera* complex, causing severe damage to major cash crops. The potential contribution of the *Enterococcus* species associated with *S. frugiperda* to insecticide detoxification and insecticide resistance evolution call for investigations on the occurrence, diversity, and host specificity of *Enterococcus* gut associates of *Spodoptera*. Bacterial isolates were obtained from the larval gut of *S. albula, S. cosmioides, S. eridania*, and *S. frugiperda*. The *Enterococcus* isolates obtained were subjected to genome sequencing and comparative functional genomic analysis, resulting in their classification as the recently described *E. entomosocium* or *E. spodopteracolus*. The genomic variation carried by isolates obtained from different species of *Spodoptera* is not large enough to suggest that the *Enterococcus* species identified coevolved with each of their host species. This study confirmed the need to re-evaluate taxonomic identifications based solely on 16S rRNA gene sequencing and provided insights into the functional characteristics of the *E. entomosocium* and *E. spodopteracolus* and the potential ways in which they interact and contribute to host success and performance in the field.

Keywords: *Spodoptera* complex ; Symbiosis; *Enterococcus*; Coevolution; Phylosymbiosis

5.1. Introduction

Insects play a key role in ecological systems and are of great importance in a wide variety of ecosystems (Yang and Gratton 2014). Among the diverse range of insects, larvae of the genus *Spodoptera* have received considerable attention due to their great diversity and the risk they represent to food production worldwide (Kergoat et al. 2012; Early et al. 2018). *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) has spread from the Americas to Africa, Asia, and Oceania (Goergen et al. 2016; Maino et al. 2021), expanding its threat to global food safety by damaging major cash and staple crops (maize, millet, rice, and sorghum) (Montezano et al. 2018; Wan et al. 2021). In South America, several species of *Spodoptera* can co-occur in certain crops being collectively called by *Spodoptera* complex (Saldamando and Marquez 2012). In Brazil, *Spodoptera albula* (Walker, 1857) (Lepidoptera: Noctuidae), *Spodoptera cosmioides* (Walker, 1858) (Lepidoptera: Noctuidae), *Spodoptera eridania (*Stoll, 1782) (Lepidoptera: Noctuidae), and *S. frugiperda* are the species represented in the *Spodoptera* complex, which are responsible for extensive damage in agricultural production areas (Horikoshi et al. 2021).

The pest potential of *Spodoptera* species can be related to their well-known polyphagous habits, high reproductive potential, short life cycle and high dispersal capacity (Montezano et al. 2013ab, 2014ab, 2019; Specht and Roque-Specht 2016, 2019). But their association with symbionts has only been discussed for some species of *Spodoptera*, such as *S. frugiperda*, *S. littoralis*, *S. litura*, and *S. exigua* (Tang et al. 2012; Chen et al. 2016; Gao et al. 2019; Oliveira et al. 2023; Xia et al. 2020). In most of them, *Enterococcus* is often among the most abundant and important players in the gut microbial community of *Spodoptera* (Tang et al. 2012; Chen et al. 2016; Gao et al. 2019; Oliveira et al. 2023).

The *Enterococcus* isolates obtained from *Spodoptera* species studied have been identified as *E. mundtii* and *E. casseliflavus* usually by using the 16S rRNA gene as a molecular marker (Tang et al. 2012; Chen et al. 2016; Gao et al. 2019; Oliveira et al. 2023). But recent genomic analysis of these *Enterococcus* species associated with *S. frugiperda* revealed that they represent new taxonomical entities named *Enterococcus entomosocium* and *Enterococcus spodopteracolus* (Gomes et al. 2023; Chapter 2 of this thesis). Besides several potential contributions these species can provide to their host based on the identification of genomic features, these symbionts were demonstrated to degrade insecticides and potentially contribute to host detoxification (Almeida et al. 2017; Gomes et al. 2023). At the genus level, *Enterococcus* species were also confirmed as the main representatives of the gut microbial community of laboratory strains of *S. albula*, *S. cosmioides*, and *S. eridania* (Gomes 2018).

The relationship with symbiotic partners has been suggested as a driving force behind the host' evolution and response to selection (Klepzig et al. 2009; Shapira 2016; Henry et al. 2021). In a close relationship with the host organism, the composition of the host microbiota may reflect the evolutionary history or phylogeny of the host - a pattern known as phylosymbiosis (Brucker and Bordenstein 2013; Brooks et al. 2016; Mallott and Amato 2021). In insects, examples of phylosymbiosis have been demonstrated for parasitoid wasp species (Brucker and Bordenstein 2013) and flies (Rudman et al. 2019), illustrating the complex ways in which host-microbe interactions can shape the evolutionary trajectory of these organisms.

However, little is known about the co-evolutionary aspects of lepidopteran pests and their gut microbiota.

Thus, the main objective of this chapter is to characterize the diversity of *Enterococcus* symbionts in the *Spodoptera* complex occurring in Brazil – *S. albula*, *S. cosmioides*, *S. eridania*, and *S. frugiperda* - and to verify whether they show enough differences to be distinguished according to their host. This will shed light on the extent of their specialization and coevolution with the host, providing valuable insights into the coevolutionary dynamics of these insects and their symbionts. We used a comprehensive analysis of the *Spodoptera* - *Enterococcus* association to gain a deeper understanding if the *Enterococcus* symbionts are behind the ecological and/or evolutionary forces that drive the success of this complex of agricultural pests.

5.2. Conclusions

The analysis of *Enterococcus* isolates from *S. albula*, *S. cosmioides*, *S. eridania*, and *S. frugiperda* larvae allowed a better understanding of the relationship between *Spodoptera* species and their *Enterococcus* symbionts, shedding light on the diversity of these associations. Their taxonomic recognition as representatives of the newly described species *E. entomosocium* and *E. spodopteracolus*, and their genomic assessment support the need to reevaluate taxonomic identifications based solely on 16S rRNA gene sequencing, providing insights into the functional attributes of these symbionts. The genomic differences among the isolates from the *Spodoptera* species complex from Brazil did not show sufficient genomic differences among them to provide any evidence of the existence of an old *Enterococcus* – *Spodoptera* association that would demonstrate their co-evolutionary history. The assessment of *Enterococcus* species associated with basal lineages of *Spodoptera* from the Old World would provide a much broader understanding on the evolutionary history of the *Enterococcus* – *Spodoptera* association.

References

Alcock BP, Raphenya AR, Lau TTY, et al (2020) CARD 2020: Antibiotic resistome surveillance with the comprehensive antibiotic resistance database. Nucleic Acids Res 48(1):517–525. https://doi.org/10.1093/nar/gkz935

Almeida LG, De Moraes LAB, Trigo JR, et al (2017) The gut microbiota of insecticide-resistant insects houses insecticide-degrading bacteria: A potential source for biotechnological exploitation. PLoS One 12(3):e0174754. https://doi.org/10.1371/journal.pone.0174754

Andrews S. (2010) FastQC: A quality control tool for high throughput sequence data. Available online at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc

Bankevich A, Nurk S, Antipov D, et al (2012) SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30(15):2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brettin T, Davis JJ, Disz T, et al (2015) RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365

Brooks AW, Kohl KD, Brucker RM, et al (2016) Phylosymbiosis: Relationships and functional effects of microbial communities across host evolutionary history. PLoS Biol 14(11):e2000225. https://doi.org/10.1371/journal.pbio.2000225

Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: Gut bacteria cause hybrid lethality in the genus *Nasonia*. Science 341:667–669. https://doi.org/10.1126/science.1240659

Chen B, Teh BS, Sun C, et al (2016) Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. Sci Rep 6:29505. https://doi.org/10.1038/srep29505

Chen L, Yang J, Yu J, et al (2005) VFDB: A reference database for bacterial virulence factors. Nucleic Acids Res 33(1):325–328. https://doi.org/10.1093/nar/gki008

Early R, González-Moreno P, Murphy ST, Day R (2018) Forecasting the global extent of invasion of the cereal pest *Spodoptera frugiperda*, the fall armyworm. NeoBiota 40:25–50. https://doi.org/10.3897/neobiota.40.28165

Fournier PE, Raoult D (2009) Current knowledge on phylogeny and taxonomy of *Rickettsia* spp. Ann N Y Acad Sci 1166:1–11. https://doi.org/10.1111/j.1749-6632.2009.04528.x

Fox GE, Wisotzkey JD, Jurtshuk P (1992) How close is close: 16S rRNA sequence identity may not be sufficient to guarantee species identity. Int J Syst Bacteriol 42(1):166–170. https://doi.org/10.1099/00207713-42-1-166

Fu J, Wang J, Huang X, et al (2023) Composition and diversity of gut microbiota across developmental stages of *Spodoptera frugiperda* and its effect on the reproduction. Front Microbiol 14:1237684. https://doi.org/10.3389/fmicb.2023.1237684

Gao X, Li W, Luo J, et al (2019) Biodiversity of the microbiota in *Spodoptera exigua* (Lepidoptera: Noctuidae). J Appl Microbiol 126(4):1199–1208. https://doi.org/10.1111/jam.14190

Goergen G, Kumar PL, Sankung SB, et al (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera, Noctuidae), a new alien invasive pest in West and Central Africa. PLoS One 11(10):e0165632. https://doi.org/10.1371/journal.pone.0165632

Gomes AFF (2018) Microbiota intestinal de espécies Americanas de *Spodoptera* Guenée (Lepidoptera: Noctuidae). In: Microbiota intestinal de *Spodoptera* Guenée (Lepidoptera: Noctuidae) associada aos agrossistemas do Novo Mundo: Diversidade e capacidade de utilização de inseticidas. Dissertação (Mestrado), Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Piracicaba, Brasil.

Gomes AFF, de Almeida LG, Cônsoli FL (2023) Comparative genomics of pesticide-degrading *Enterococcus* symbionts of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) leads to the identification of two new species and the reappraisal of insect-associated *Enterococcus* species. Microb Ecol 86:2583–2605. https://doi.org/10.1007/s00248-023-02264-0

Gomes AFF, Omoto C, Cônsoli FL (2020) Gut bacteria of field-collected larvae of *Spodoptera frugiperda* undergo selection and are more diverse and active in metabolizing multiple insecticides than laboratory-selected resistant strains. J Pest Sci 93:833–851. https://doi.org/10.1007/s10340-020-01202-0

Goris J, Konstantinidis KT, Klappenbach JA, et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91. https://doi.org/10.1099/ijs.0.64483-0

Henry LP, Bruijning M, Forsberg SKG, Ayroles JF (2021) The microbiome extends host evolutionary potential. Nat Commun 12(1):541. https://doi.org/10.1038/s41467-021-25315-x

Horikoshi RJ, Dourado PM, Berger GU, et al (2021) Large-scale assessment of lepidopteran soybean pests and efficacy of Cry1Ac soybean in Brazil. Sci Rep 11:15956. https://doi.org/10.1038/s41598-021-95483-9

Kergoat GJ, Prowell DP, Le Ru BP, et al (2012) Disentangling dispersal, vicariance and adaptive radiation patterns: A case study using armyworms in the pest genus *Spodoptera* (Lepidoptera: Noctuidae). Mol Phylogenet Evol 65(3):855–870. https://doi.org/10.1016/j.ympev.2012.08.006

Klepzig KD, Adams AS, Handelsman J, Raffa KF (2009) Symbioses: A key driver of insect physiological processes, ecological interactions, evolutionary diversification, and impacts on humans. Environ Entomol 38(1):67–77. https://doi.org/10.1603/022.038.0109

Maino JL, Schouten R, Overton K, et al (2021) Regional and seasonal activity predictions for fall armyworm in Australia. Curr Res Insect Sci 1:100010. https://doi.org/10.1016/j.cris.2021.100010

Mallott EK, Amato KR (2021) Host specificity of the gut microbiome. Nat Rev Microbiol 19:639–653. https://doi.org/10.1038/s41579-021-00562-3

Meier-Kolthoff JP, Auch AF, Klenk H-P, Oker MG (2013) Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinform 14:60. https://doi.org/10.1186/1471-2105-14-60

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182. https://doi.org/10.1038/s41467-019-10210-3

Montezano DG, Specht A, Bortolin TM, et al (2013a) Immature stages of *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae): Developmental parameters and host plants. An Acad Bras Cienc 85(1):271–284. https://doi.org/10.1590/S0001-37652013000100013

Montezano DG, Specht A, Sosa-Gómez DR, et al (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. Afr Entomol 26:286–300. https://doi.org/10.4001/003.026.0286

Montezano DG, Specht A, Sosa-Gómez DR, et al (2019) Biotic potential and reproductive parameters of *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae). J Agric Sci 11(13):240. https://doi.org/10.5539/jas.v11n13p240

Montezano DG, Specht A, Sosa-Gómez DR, et al (2014a) Biotic potential, fertility and life table of *Spodoptera albula* (Walker) (Lepidoptera: Noctuidae), under controlled conditions. An Acad Bras Cienc 86:723–732. https://doi.org/10.1590/0001-3765201402812

Montezano DG, Specht A, Sosa-Gómez DR, et al (2013b) Biotic potential and reproductive parameters of *Spodoptera eridania* (Stoll) (Lepidoptera, Noctuidae) in the laboratory. Rev Bras Entomol 57(3):340–346. https://doi.org/10.1590/S0085-56262013005000026

Montezano DG, Specht A, Sosa-Gómez DR, et al (2014b) Immature stages of *Spodoptera eridania* (Lepidoptera: Noctuidae): Developmental parameters and host plants. J Insect Sci 14(1):238. https://doi.org/10.1093/jisesa/ieu100

Moran NA (2003) Tracing the evolution of gene loss in obligate bacterial symbionts. Curr Opin Microbiol 6(5):512–518. https://doi.org/10.1016/j.mib.2003.08.001

Oliveira NC., Rodrigues PAP, Cônsoli FL (2023) Host-adapted strains of *Spodoptera frugiperda* hold and share a core microbial community across the western hemisphere. Microb Ecol 85:1552–1563 https://doi.org/10.1007/s00248-022-02008-6

Prjibelski A, Antipov D, Meleshko D, et al (2020) Using SPAdes *de novo* assembler. Curr Protoc Bioinformatics 70(1):e102. https://doi.org/10.1002/cpbi.102

Rosselló-Móra R, Amann R (2015) Past and future species definitions for bacteria and archaea. Syst Appl Microbiol 38:209–216. https://doi.org/10.1016/j.syapm.2015.02.001

Rudman SM, Greenblum S, Hughes RC, et al (2019) Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. Proc Natl Acad Sci U S A 116(40):20025–20032. https://doi.org/10.1073/pnas.1907787116

Saier MH, Reddy VS, Moreno-Hagelsieb G, et al (2021) The transporter classification database (TCDB): 2021 update. Nucleic Acids Res 49:461–467. https://doi.org/10.1093/nar/gkaa1004

Saldamando CI, Marquez, E.J (2012). Approach to *Spodoptera* (Lepidoptera: Noctuidae) phylogeny based on the sequence of the cytocrhome oxydase I (COI) mitochondrial gene. Rev Biol Trop 60(3): 1237-1248.

Salem H, Bauer E, Kirsch R, et al (2017) Drastic genome reduction in an herbivore's pectinolytic symbiont. Cell 171:1520-1531.e13. https://doi.org/10.1016/j.cell.2017.10.029

Sambrook J (2001). Molecular cloning : A laboratory manual. Cold Spring Harbor, N.Y. : Cold Spring Harbor Laboratory Press

Sayers S, Li L, Ong E, et al (2019) Victors: A web-based knowledge base of virulence factors in human and animal pathogens. Nucleic Acids Res 47:693–700. https://doi.org/10.1093/nar/gky999

Shao Y, Chen B, Sun C, et al (2017) Symbiont-derived antimicrobials contribute to the control of the lepidopteran gut microbiota. Cell Chem Biol 24:66–75. https://doi.org/10.1016/j.chembiol.2016.11.015

Shapira M (2016) Gut microbiotas and host evolution: scaling up symbiosis. Trends Ecol Evol 31(7):539–549. https://doi.org/10.1016/j.tree.2016.03.006

Sloan DB, Moran NA (2012) Genome reduction and co-evolution between the primary and secondary bacterial symbionts of psyllids. Mol Biol Evol 29(12):3781–3792. https://doi.org/10.1093/molbev/mss180

Specht A, Roque-Specht VF (2019) Biotic potential and reproductive parameters of *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) in the laboratory. Braz J Biol 79:488–494. https://doi.org/10.1590/1519-6984.184595

Specht A, Roque-Specht VF (2016) Immature stages of *Spodoptera cosmioides* (Lepidoptera: Noctuidae): Developmental parameters and host plants. Zoologia 33(4):e20160053. https://doi.org/10.1590/S1984-4689zool-20160053

Strepis N, Naranjo HD, Meier-Kolthoff J, et al (2020) Genome-guided analysis allows the identification of novel physiological traits in *Trichococcus species*. BMC Genomics 21:24. https://doi.org/10.1186/s12864-019-6410-x

Tang X, Freitak D, Vogel H, et al (2012) Complexity and variability of gut commensal microbiota in polyphagous lepidopteran larvae. PLoS One 7(7):e36978. https://doi.org/10.1371/journal.pone.0036978

Turner S, Pryer KM, Miao VPW, Palmer JD (1999) Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. J Eukaryot Microbiol 46:327–338. https://doi.org/10.1111/j.1550-7408.1999.tb04612.x

Wan J, Huang C, Li C-Y, et al (2021) Biology, invasion and management of the agricultural invader: Fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). J Integr Agric 20:646–663. https://doi.org/10.1016/S2095-3119(20)63367-6

Wishart DS, Knox C, Guo AC, et al (2006) DrugBank: A comprehensive resource for *in silico* drug discovery and exploration. Nucleic Acids Res 34:668–672. https://doi.org/10.1093/nar/gkj067

Xia X, Lan B, Tao X, et al (2020) Characterization of *Spodoptera litura* gut bacteria and their role in feeding and growth of the host. Front Microbiol 11:1492. https://doi.org/10.3389/fmicb.2020.01492

Yang LH, Gratton C (2014) Insects as drivers of ecosystem processes. Curr Opin Insect Sci 2:26–32. https://doi.org/10.1016/j.cois.2014.06.004