

METAGENOMIC ANALYSIS OF THE GUT BACTERIOME OF USHERHOPPER, *POEKILO CERUS BUFONIUS* (KLUG) FROM HADDA, SAUDI ARABIA

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Abstract. Microbial communities that colonize insect guts contribute positively to the absorption of nutrients, immunity, and the overall health of the host. Recent studies have been tapered towards only economically important arthropods, particularly honeybees. On the other hand, arthropods such as grasshoppers are considered pests because they create havoc leading to economic losses. Grasshoppers are considered phytophagous pests that have a large appetite for plant fibers, and digestion depends largely on the bacterial communities in their guts. This study characterizes the gut microbiome in Usherhopper, *Poekilocerus bufonius* using metagenomics methods through next generation sequencing (NGS). A total of 59,072,222 bacterial reads were recorded which were classified at the phylum and genus levels. *Proteobacteria* were the most common at the phylum level whereas *Wolbachia* was the most dominant genus based on the total reads. The host-microbiome interactions and their perceived influence on the ecosystem are yet to be fully explained, therefore a detailed study is pivotal in order to enforce effective conservation and pest management.

Keywords: gut microbiome, NGS, 16s rRNA, bacteria, archaea

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; OUT, operational taxonomic unit

Introduction

Insects are considered as the largest class of the Arthropoda phylum, which is the largest in the animal phyla in diversity, ecological adaptations and biomass (Muratore et al., 2020). Their diversity and evolutionary success are partly attributed to their interaction with the beneficial bacteria that colonize their digestive tracts (Engel and Moran, 2013; Jing et al., 2020). They assist host digestion and protect them from parasites and pathogenic bacteria by producing antimicrobial peptides (Chen et al., 2016) and influence host behaviors like aggregation into large groups (Dillon and Charnley, 2002). Interactions between insect hosts and their microbiomes show

comprehensive effects on the host, and by extension, the ecosystem (Lü et al., 2019). The contribution of gut bacteria to insects' function is highly important from a medicinal, agricultural and ecological perspective. In the order Orthoptera, which includes grasshoppers, katydids, and crickets although relatively abundant in the ecosystem, their microbiomes are yet to be extensively characterized unlike other more charismatic insect species such as butterflies, moths, caterpillars, and Hymenopterans like bees (Muratore et al., 2020; Ojha and Zhang, 2019).

Grasshoppers are an important herbivore in grassland ecosystems that provide crucial ecosystem services like nutrient cycling. In contrast, they are considered as pests that require effective management and control strategies (Wang et al., 2020). Studies have generalized that the gut microbiome of most insect herbivores is comprised predominantly of *Proteobacteria*, *Firmicutes*, and *Actinobacteria* (Wielkopolan and Obrepalska-Stepłowska, 2016). Whether this generalization holds true across all insect herbivores has not been fully investigated. Compared with termites and cockroaches, grasshoppers have a very sparse microbiome (Dillon and Dillon, 2004).

Research had shown that any alteration in the gut microflora constitution influences the survival rate of grasshoppers (Tan et al., 2020). The polyphagous Usherhopper *Poekilocerus bufonius* (Orthoptera: *Pyrgomorphidae*) has been typically recorded in different regions in Saudi Arabia with a particular higher presence in the western region (Alghamdi et al., 2017; Noor et al., 2020; Sayyed and Patel, 2011). This is where the *Rhazya stricta* plant is widely distributed in the rangeland of Hadda, Saudi Arabia. This plant is well known for its allelopathic effects and has been explored in traditional medicine (Baeshen et al., 2014; Noor et al., 2020). Thus, the present study aimed to determine the microbiota bacterial communities of *P. bufonius* that habituates the western region of Saudi Arabia and harvest *R. stricta* plant with all its life cycle forms, which inspired us to investigate the microbiome of this insect that lives on this toxic shrub which is avoided by many herbivores (Baeshen et al., 2015).

Metagenomics based molecular techniques based on 16S rRNA sequencing have proven efficient in the characterization of insect's gut microbiome (Ahn et al., 2012; Prabhakar et al., 2013). The characterization of grasshopper gut microbiome communities in conjunction with information on host-associated variation in bacteria composition is obligatory for a total perception of insect ecology and the improvement of pest management action plan.

The aim of the study is to point out the bacterial and archaeal communities living inside the gut of the usherhopper that consumes toxic plants and lives in harsh environmental conditions.

Materials and methods

Sample collection

Usherhoppers were collected from the leaves of *R. stricta* plants, from Hadda (Coordinates: Lat: 21.444271 – Lon: 39.5316938), Saudi Arabia, in August 2014 at 11:00 am while the temperature was 40 °C and transported to the laboratory according to standard protocol (Mancini et al., 2018; Muratore et al., 2020).

Sample treatment

Usherhoppers were dissected in aseptic conditions. The entire intestinal tract was removed. Gut parts were separated, stored at -20 °C till further use.

Extraction of genomic DNA, metagenomics and bioinformatics analysis

The stored gut parts were crushed and powdered in liquid nitrogen. Total genomic DNA was extracted using the Wizard® Genomic DNA Purification kit (www.promega.com) and shipped to Beijing Genomics Institute, China for next generation sequencing.

Based on the PCR results, a high-quality original library was prepared by Illumina kit by removing short fragments. Illumina (USA) HiSeq/MiSeq 2000 encoded 16S rRNA gene amplicons were used to observe the gut microbiome diversity (Moussa et al., 2017). The quality of the raw data sequences was checked using *FastQC* v0.11.9. Filtration of the raw data was done to obtain clean reads, and tags were clustered at 97% sequence identity to an operational taxonomic unit (OTU), which assigned its taxonomy.

Results

Sequence analysis

Sequence analysis was performed by EDGE bioinformatics (<https://edgebioinformatics.org/>). A total of 6,000,838 paired-end reads were obtained by sequencing and 5,907,222 clean tags were generated after splicing and filtering the paired-end reads with a covered percentage of 98.44%, and the unpaired reads were 83,418 with a percentage of 1.41% (Table 1).

Table 1. Assembly's result of the *P. bufonius* sample

Sample name	Before trimming		After trimming		Unpaired reads	Paired reads	GC (%)
	Total bases	Read count	Total bases	Read count			
<i>Poekilocerus bufonius</i> gut bacteriome	600083800	6000838	582766134 (97.11%)	5907222 (98.44%)	83418 (1.41%)	5823804 (98.59%)	58.93%

The results of the bacterial communities in the samples at phylum-level taxonomic distribution show that they belong to eight phyla based on the total reads. *Proteobacteria* were the most shared at the phylum-level and followed by; *Actinobacteria*, *Firmicutes*, *Nitrospirae*, *Bacteroidetes*, *Tenericutes*, *Cyanobacteria*, and *Acidobacteria* (Fig. 1).

At the genus-level, the taxonomic distribution showed that they belong to eighty-nine (89) genera. *Wolbachia* were the most abundants at the genus-level and followed by, *Acinetobacter*, *Pseudomonas*, *Azospira*, *Enterobacter*, *Shewanella*, *Vibrio*, *Photobacterium*, *Serratia*, *Acidovorax*, *Sphingobium*, *Nitrospira*, *Sphingomonas*, *Rhodoferrax*, *Stenotrophomonas*, and *Bacillus* (Fig. 2).

At the species-level, the taxonomic distribution showed that they belong to one hundred eighty-two (182) species. *Wolbachia* sp. wRi, *Wolbachia* endosymbiont of *Drosophila simulans*, *Acinetobacter oleivorans*, *Acinetobacter pittii*, *Azospira oryzae*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Enterobacter lignolyticus*, *Pseudomonas* sp. UW4, *Pseudomonas stutzeri*, *Pseudomonas brassicacearum*, *Pseudomonas savastanoi*, *Pseudomonas syringae*, *Photobacterium profundum* were the most abundant based on the total reads (Fig. 3). A heatmap was generated at the genus

and species level to explain the diversity in the composition of the taxonomy based on all the total reads (Fig. 4) while the bacterial communities that are present in the sample are attached (see Appendix).

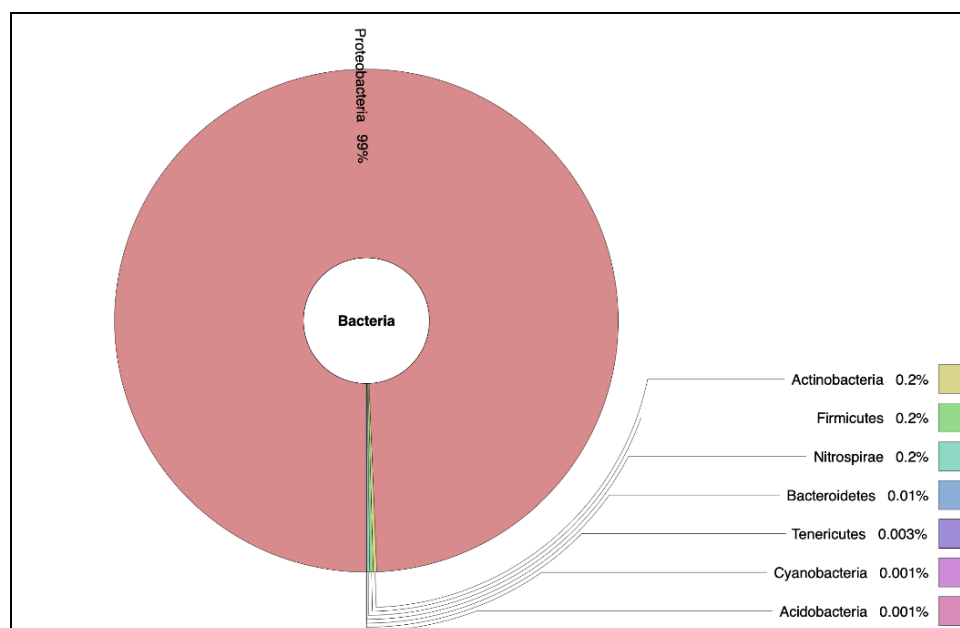


Figure 1. The bacterial communities at the phylum-level

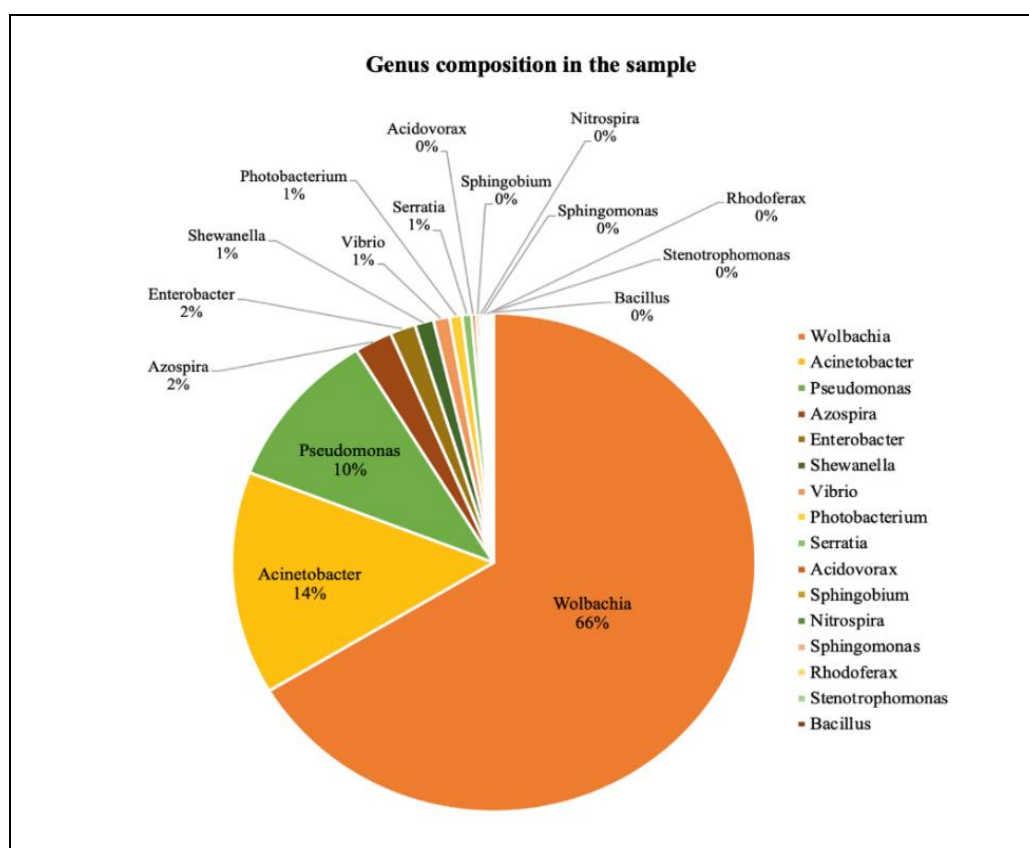


Figure 2. The bacterial communities at the genus level

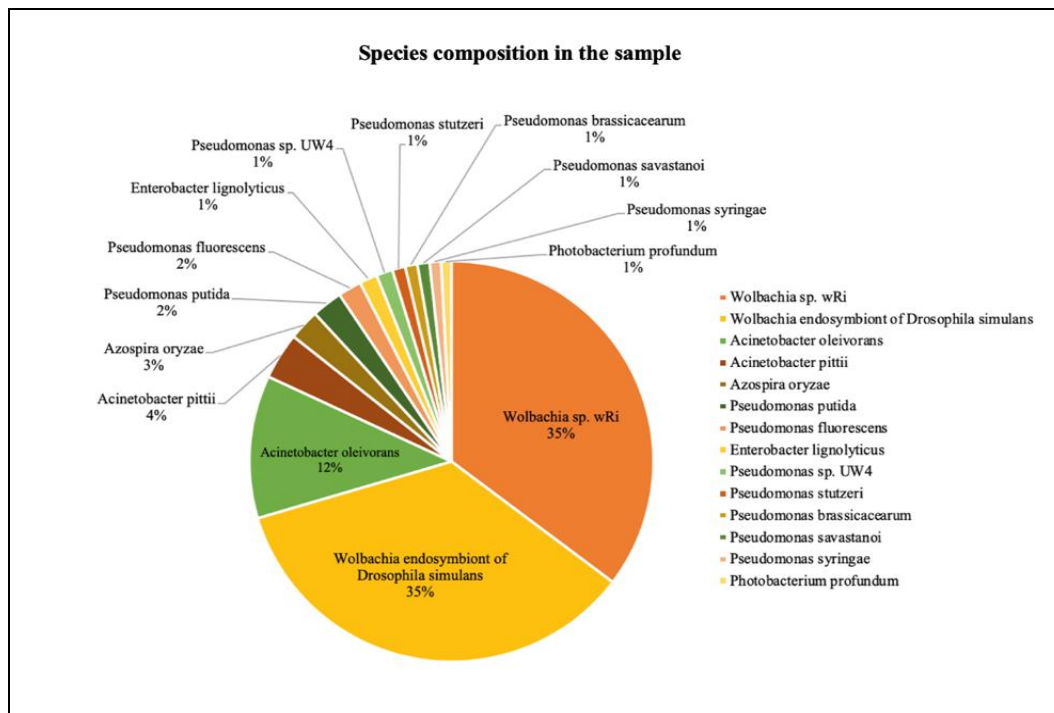


Figure 3. The bacterial communities at the species level

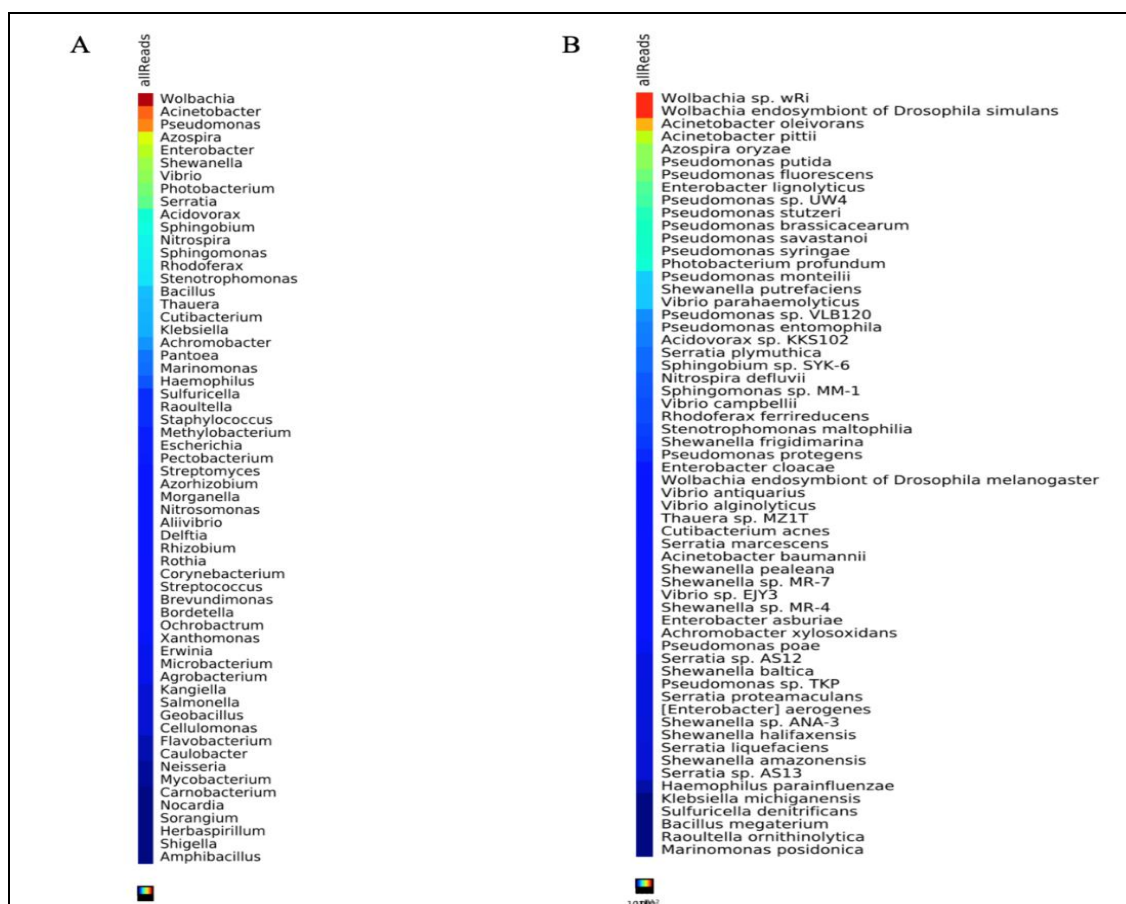


Figure 4. The heatmap of the bacterial community at; (A) genus level, (B) species level

Discussion

The microbiomes present in the guts of insects serve as major contributors to nutrient absorption, enhancing immunity and ecological fitness (Morimoto et al., 2019; Muratore et al., 2020). The evolutionary powers of *P. bufonius* in terms of their high tolerance to varying environmental conditions are evidenced by the bacterial community presented in their guts (Griffith et al., 2018). Metagenomic analysis used to study the bacterial diversity in the gut of *P. bufonius* resulted in 59072222 sequences which were classified into phylum and genus levels. A total of 182 bacterial taxa at the species level were present with *Wolbachia* sp. were having high relative abundance. Meanwhile, results of sequencing showed that the taxonomic distribution of the bacterial communities at the phylum-level indicated eight phyla, indicating that *Proteobacteria* were the most shared at the phylum-level and followed by: *Actinobacteria*, *Firmicutes*, *Nitrospirae*, *Bacteroidetes*, *Tenericutes*, *Cyanobacteria*, and *Acidobacteria*. Several studies have shown that *Proteobacteria*, *Firmicutes*, and *Actinobacteria* have been found in the intestinal bacteria of locusts, orthopterans and the well-characterized microbiota of honeybees (Anjum et al., 2018; Engel et al., 2012; Stoops et al., 2016).

On the other hand, *Cyanobacteria* appeared remarkably in the sample because it is part of the diet of some insects such as mosquito *Larvae* (Berry, 2014). In other studies, (Meng et al., 2019) high abundance of cyanobacteria family was found present in desert soils of Israel, while (Wynn-Williams, 2000) observed that *Cyanobacteria* constitute a large population of microalgae in hot desert rocks as a result of their ability to withstand high temperatures.

At the genus-level, the taxonomic distribution showed eighty-nine (89) genera. This diversity is attributed to a combination of factors such as nutrition, environmental conditions, and the gut environment (Brune and Dietrich, 2015; Wang et al., 2020; Yun et al., 2014). *Wolbachia* were the most abundants at the genus-level and followed by, *Acinetobacter*, *Pseudomonas*, *Azospira*, *Enterobacter*, *Shewanella*, *Vibrio*, *Photobacterium*, *Serratia*, *Acidovorax*, *Sphingobium*, *Nitrospira*, *Sphingomonas*, *Rhodospirillum rubrum*, *Stenotrophomonas*, and *Bacillus* based on the total reads. Many studies demonstrated that *Wolbachia* has a role in controlling the reproductive characteristics of host insects (Hancock et al., 2011; Saridaki and Bourtzis, 2010). In addition, grasshopper gut bacteria, such as *Acinetobacter*, *Pseudomonas* and *Klebsiella*, have the ability to produce siderophore (Sonawane et al., 2018). The bacteria that produce siderophore have an effective role in promoting plant growth and are considered effective in insecticides, which are environmentally friendly and can be a valuable alternative solution to chemical insecticides (Kramer et al., 2020; Rungin et al., 2012; Sepehri and Khatabi, 2021). Microorganisms colonizing the gut of *P. bufonius* are capable of degrading cellulose, which is the functional role of *Bacillus* sp and *Pseudomonas* sp in the gut (Wang et al., 2020). The study of the gut bacteria in *P. bufonius* carried out by (Alghamdi et al., 2017) was done through traditional methods by isolation. Their work showed four bacteria: *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella* sp. and *Streptococcus* sp. However, the results of this study revealed ninety-two species that describe the whole bacterial community using metagenomic methods. Metagenomics is considered as a powerful technique and future feature that can open the space to understand the diversities and the functions of the bacterial community in their environment (Baeshen et al., 2020).

Conclusion

In this study, we demonstrated the conservation of one dominant phylum, *Proteobacteria* present in *P. bufonius*. Differences in the bacterial community compositions are likely influenced by their dietary preferences including *R. stricta* plant as compared to other studies on grasshoppers. The host-microbiome interactions and their subsequent impact on the ecosystem are yet to be fully established, therefore a detailed study is needed to ensure effective conservation, pest management, and other biotechnological applications in industry and environment.

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ELECTRONIC APPENDIX

This manuscript has an electronic appendix.