Metagenomic Analysis of the Gut Bacteriome of Usherhopper, *Poekilocerus bufonius* (Klug) from Hadda, Saudi Arabia

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Abstract

Background: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 as pests because they create havoc leading to economic losses. Grasshoppers are considered phytophagous pests that have a large appetite for plant fibers, whose digestion depend largely on the bacterial communities in their guts.

This study characterises the gut microbiome in Usherhopper, *Poekilocerus bufonius* using the metagenomics methods through the next generation sequencing (NGS).

Results:A total of 59,072,222 bacterial reads were recorded which were classified into phylum and genus levels. *Proteobacteria* were the most shared at the phylum-level whereas *Wolbachia* were the most dominant genera based on the total reads.

Conclusions: 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.

Introduction

Insects are considered as the largest class of the phylum arthropods, which is the largest in the animal phyla in diversity, ecological adaptations and biomass (1). Their diversity and evolutionary success are partly attributed to their interaction with the beneficial bacteria that colonize their digestive tracts(2), (3). They assist host digestion and protect them from parasites and pathogenic bacteria by producing antimicrobial peptides (4) and influence host behaviours like aggregation into large groups(5). Interactions between insect hosts and their microbiomes show comprehensive effects on the host, and by extension, the ecosystem (6). 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, and caterpillars, and Hymenopterans like bees (1),(7)

Grasshoppers are an important herbivore in grassland ecosystems that provide important ecosystem services like nutrient cycling. In contrast, they are considered as pests that require effective management and control strategies (8). Studies have generalised that the gut microbiome of most insect herbivores is comprised predominantly of *Proteobacteria, Firmicutes, and Actinobacteria* (9). 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 (10). Research had shown that any alteration in the gut microflora constitution influences the survival rate of grasshoppers (11). 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 (12, 13), (14). 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(15),(13)

Thus, the present study aimed to determine the microbiota bacterial communities of *P. bufonius* that habituates the western region of Saudi Arabia which feeds on the *R. stricta* plant. Metagenomics based molecular techniques based on 16S rRNA sequencing have proven efficient in the characterization of insect's gut microbiome (16), (17). The characterization of grasshopper gut microbiome communities in conjunction with information on host-associated variation in bacteria composition is obligatory for a total perception on insect ecology and the improvement of pest management action plan.

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^{o}C and transported to the laboratory according to standard protocol (1), (18).

Sample Treatment

Usherhoppers were dissected in aseptic conditions. The entire intestinal tract was removed. Midgut and hindgut parts were separated, stored at -20^{o}C till further use.

Extraction Of Metagenomic Dna 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 (19). 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 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			
Poekilocerus bufonius 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 belongs 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* (Figure. 1).

At the genus-level, the taxonomic distribution showed that they belong to eighty-nine (89) genera. *Wolbachia* were the most abounds at the genus-level and followed by, *Acinetobacter, Pseudomonas, Azospira, Enterobacter, Shewanella, Vibrio, Photobacterium, Serratia, Acidovorax, Sphingobium, Nitrospira, Sphingomonas, Rhodoferax, Stenotrophomonas*, and *Bacillus* (Figure. 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 (Figure. 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 (Figure. 4) while the bacterial communities that are present in the sample are attached (See supplementary file 1).

Discussion

The microbiomes present in the guts of insects serve as major contributors to nutrient absorption, enhancing immunity and ecological fitness (1), (20). The evolutionary powers of *P. bufonius* in terms of their high tolerance to varying environmental conditions is evidenced by the bacterial community presented in their guts (21). 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-characterised microbiota of honeybees(22),(23), (24). On other hand, Cyanobacteria were appeared remarkably in the sample because it is part of the diet of some insects such as mosquito *Larvae* (25), In other studies, (26) high abundance of cyanobacteria family was found present in dessert soils of Israel, while (27) 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 (94) genera. This diversity is attributed to a combination of factors such as nutrition, environmental conditions, and the gut environment (28), (8), (29). *Wolbachia* were the most abounds at the genus-level and followed by, *Acinetobacter, Pseudomonas, Azospira, Enterobacter, Shewanella, Vibrio, Photobacterium, Serratia, Acidovorax, Sphingobium, Nitrospira, Sphingomonas, Rhodoferax, Stenotrophomonas*, and *Bacillus* based on the total reads. Many studies demonstrated that *Wolbachia* have a role in controlling the reproductive characteristics of host insects (30), (31). In addition, grasshopper gut bacteria, such as Acinetobacter, Pseudomonas and Klebsiella, have the ability to produce siderophore (32). 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(33), (34), (35). 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 (8). The study of the gut bacteria in *P. bufonius* carried out by (14) was 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 the metagenomic methods. Metagenomic methods are powerful techniques and future features that can open the space to understand the diversities and the functions of the bacterial community in their environment (36) .

Conclusion

In this study, we demonstrated the conservation of one dominant phyla, *Proteobacteria* present in the *P. bufonius*, grasshoppers. Differences in the bacterial community compositions is 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 is yet to be fully established, therefore a detailed study is needed to ensure effective conservation and pest management.

Abbreviations

RNA
Ribonucleic acid
DNA

Deoxyribonucleic acid
OTU
Operational Taxonomic Unit

Declarations

Acknowledgements:

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Conflict of interest

Authors declare no conflicts of interest or ethical problems.

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Figures

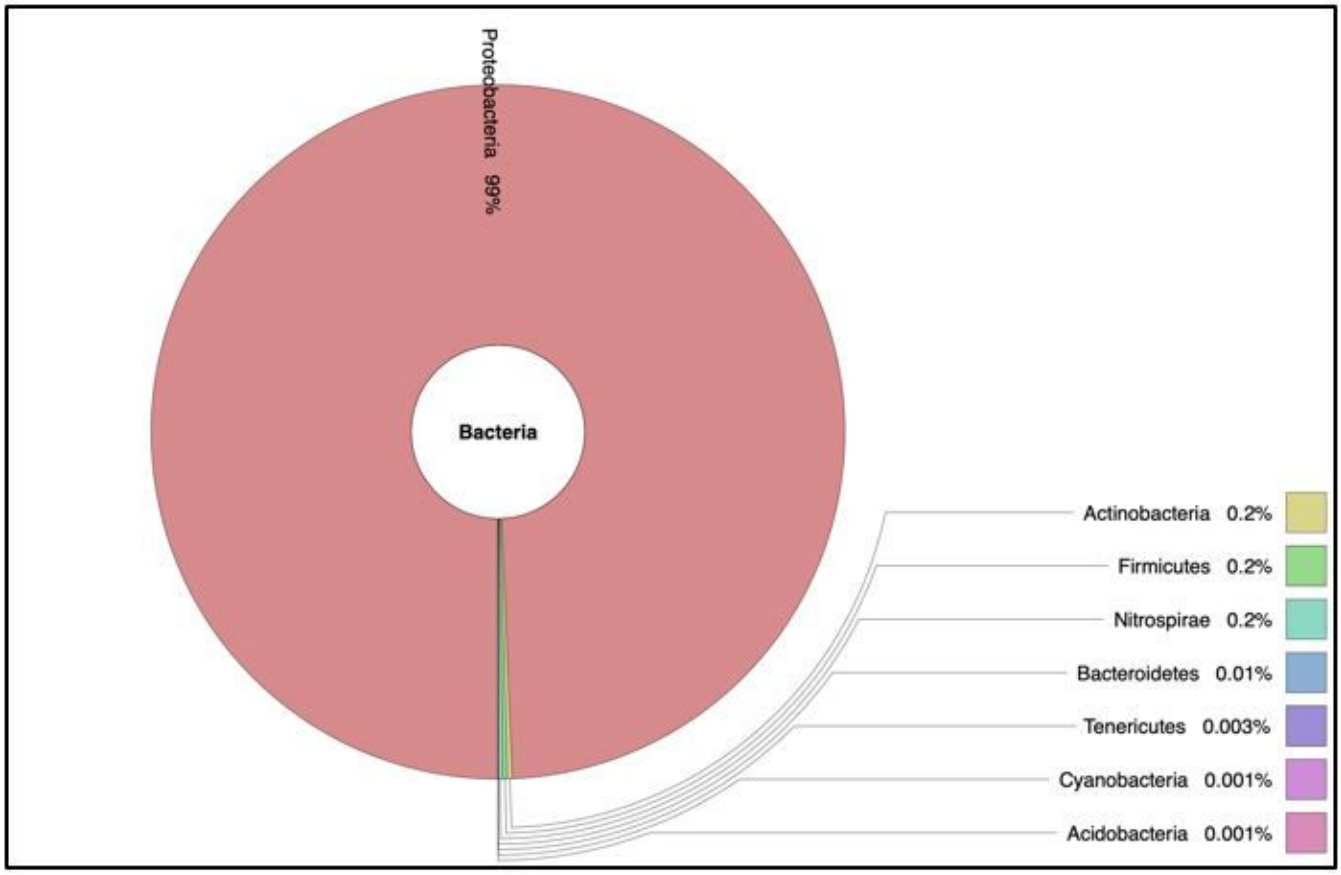

Figure 1

The bacterial communities at the phylum-level

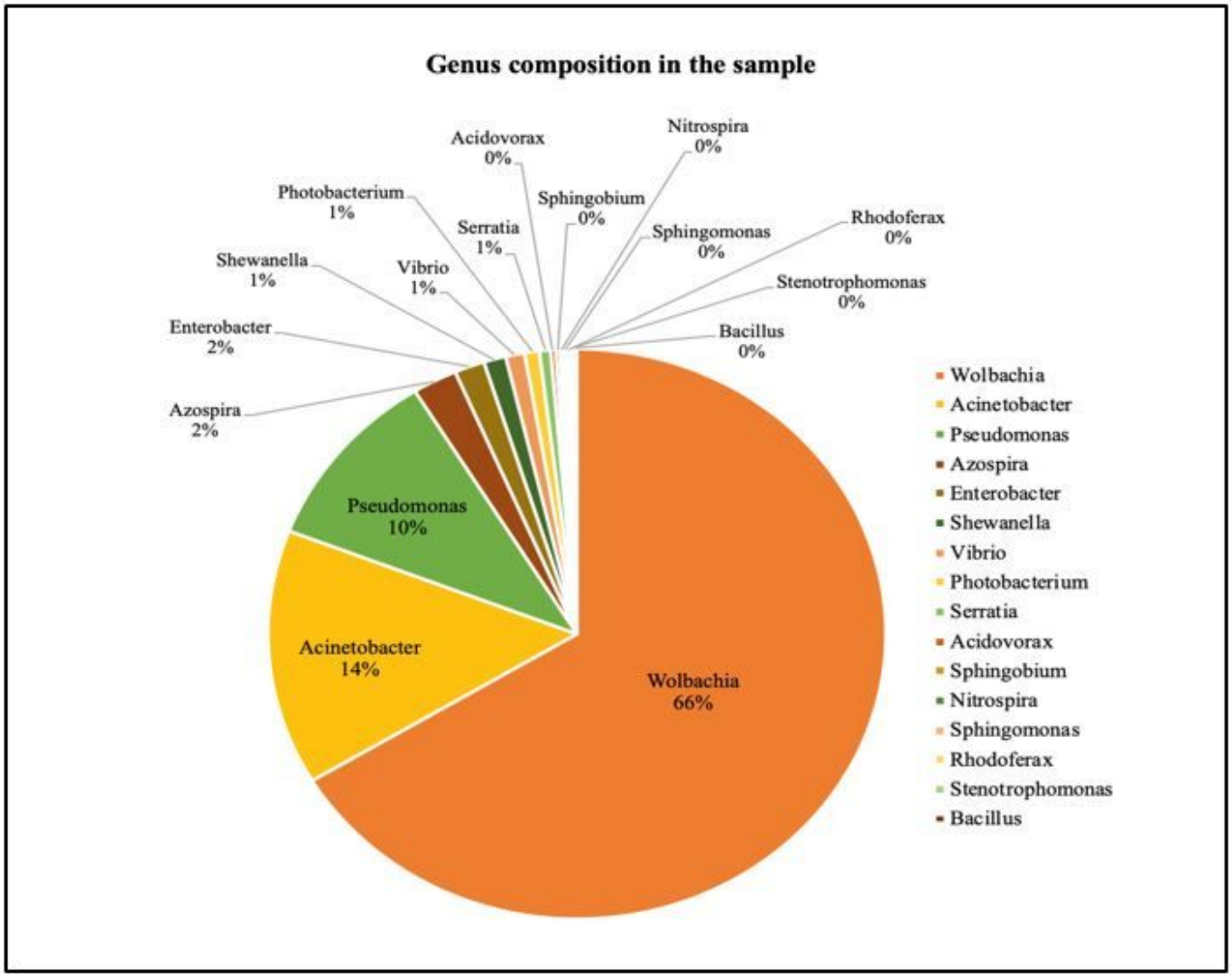

Figure 2

The bacterial communities at the genus -level.

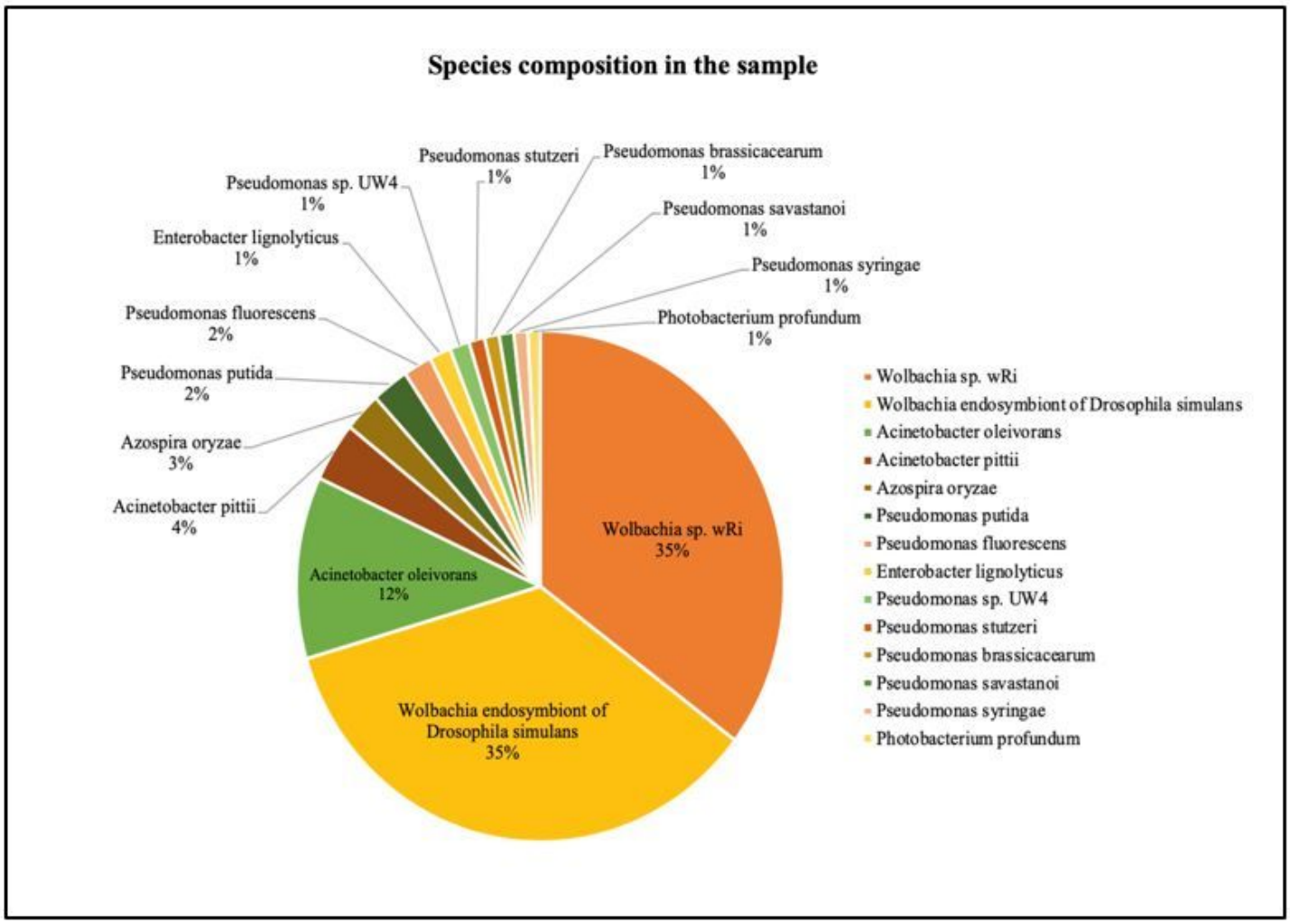

Figure 3

The bacterial communities at the species -level.

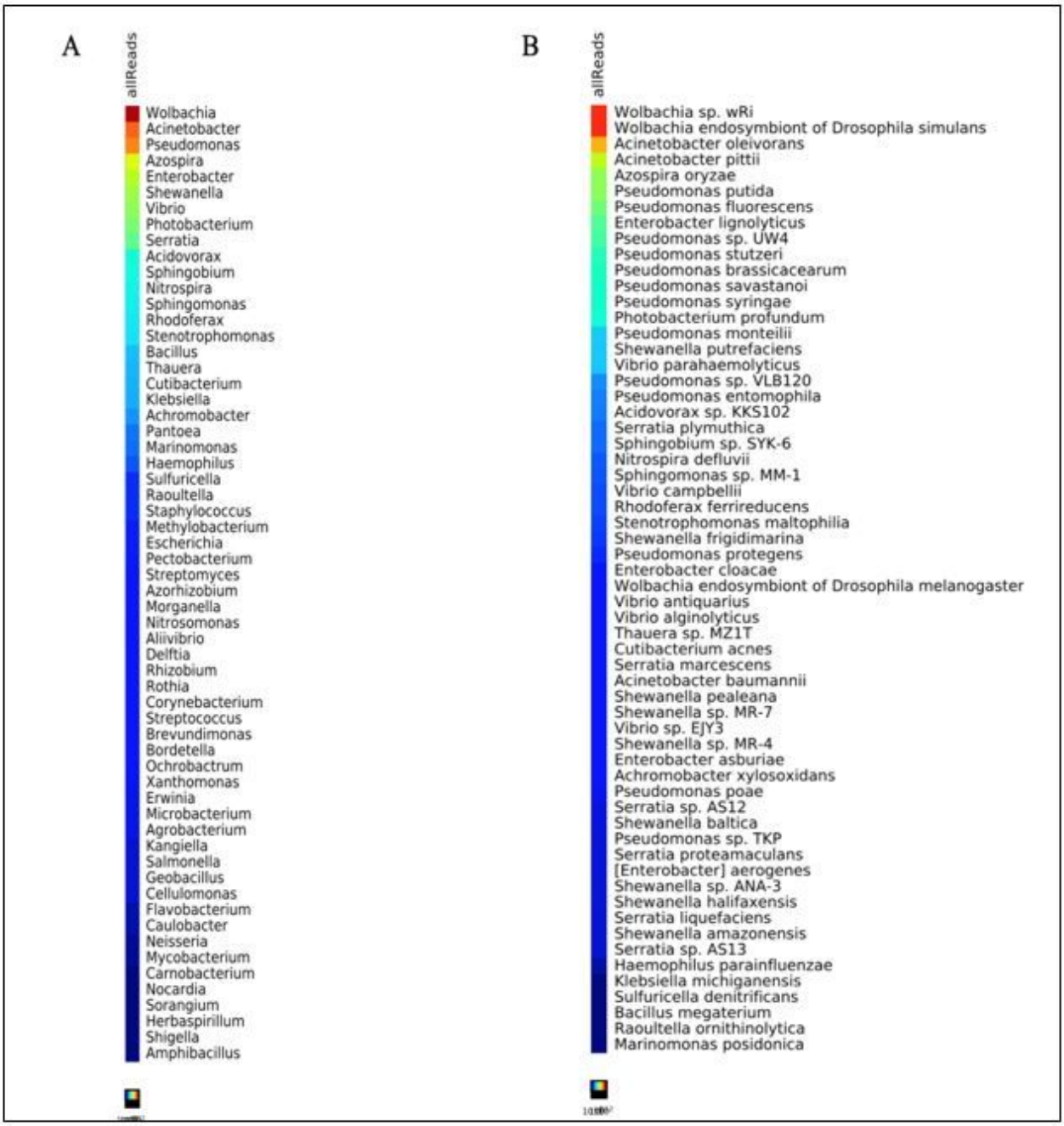

Figure 4

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

Supplementary Files

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- Sup1.Thediversityofbacteriathatisolatedfromthesamplefromphylumtogenuslevel..docx