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Corresponding Author
A. Mary Agnes,
Research Department of Zoology, Auxilium College (Autonomous), Vellore-6 Affiliated to Thiruvalluvar University Serkkadu, TN, India

Author Designation
[1]Research Scholar, PG
[2]Associate Professor, PG

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Diversity of Bacterial Community in the Gut of Spider – *Peucetia viridana*

[1]Rebecca Vinolia and [2]A. Mary Agnes
[1,2]Research Department of Zoology, Auxilium College, (Autonomous), Vellore-6 Affiliated to Thiruvalluvar University Serkkadu, TN, India

ABSTRACT
It is well recognised that insects' gut microbiomes greatly influence physiological aspects of their survival, such as diet, behaviour and health. Spiders are one of the primary insect predators in nature, yet much is known about their gut microbiomes. To learn more about the host-bacterial connection, it is essential to examine the gut microbiomes of spiders that inhabit the wild. Here, we examine the composition and diversity of the gut bacterial, based on 16S rRNA gene sequencing, we examined the composition and diversity of gut bacteria in *Peucetia viridana*. The abundance of bacterial microbiota was demonstrated by the results ($p<0.05$). Our findings suggest that a significant role in determining the gut bacterial community in *Peucetia viridana*spiders, although the noticeable changes in bacterial composition are mostly caused by their various dietary and physiological needs. The 40 bacterial classes, 156 family members and 251 genera were identified by analysis as being present in the stomach. *Acinetobacter, Pseudomonas, Clostridium, Acetobacter, Lactobacillus, Exiguobacterium, Lactococcus, Massilia, Flavobacterium* and *Stenotrophomonas* are among the genus-level organisms. The family Oxyopidae has significant richness and variety, according to a comparison between and within subfamilies based on diversity indices (alpha and beta diversity).

INTRODUCTION

Arthropods with healthy gut communities of bacteria can perform crucial and advantageous tasks for their hosts, including nutrient production, digestion, energy metabolism and immune system regulation[1-5]. Numerous groups, including ants[6], Drosophila[7-8] and isopterans[9], have explored the relationship between the phylogeny of the host species and the phylogeny of the gut bacteria.Generalist predators include spiders, which belong to the Order Araneae[10]. Few species are used as biological controlling agents on agricultural pests[11], such as for the control of cotton pests in China[12], apple orchard pests in Israel and Europe[13-15]. All species are not useful against a specific pest. Additionally, they serve as ecological indicators for environmental monitoring[16-18]. The endosymbionts in the stomach of spiders, like those of other arthropods, are in charge of altering the host's diet and sex ratio[19-22]. Extra oral digestion (EOD) is a technique used by spiders to immobilise their prey by injecting venom and regurgitating digestive fluid onto (or into) their prey before sucking the resultant liquefied tissue back up[23,24]. Spiders are an interesting model to investigate the make-up and function of their gut microbial communities due to this kind of feeding habit. However, little is known about this most diverse group's gut bacterial diversity.

Arthropods various metabolic processes are greatly influenced by their gut microbiome, which also provides insight into how they have evolved and helped them become more diverse as well as adapt to new environments[25]. The diversity of bacterial communities that were involved in many metabolic processes including diet-nutrient improvement, endosymbiosis, digestive assistance, mating and reproductive systems are part of what makes insects so abundant[3,26]. Researchers have investigated the gut bacterial communities of honeybees[27], mosquitos[28] and moths[29], as well as their potential roles in body metabolism and evolutionary patterns. These investigations have been made possible by advanced sequencing techniques like next-generation sequencing (NGS). The composition of the bacterial populations in spider guts and associated putative metabolic roles, however, are mostly unknown. The most varied member of the Arachnida class, spiders are found all over the world and play a significant part in preserving the ecological balance through a prey-predator interaction[30-31].

India and Myanmar are home to the spider species *Peucetia viridana*. A green lynxspider with brown dots on its cephalothorax. There are a few spines in the head area and in the middle, there is a deep, green fovea. Two black lines that start at the base of the middle anterior eyeballs go along the length of the clypeus. Heart-shaped, pointed behind and covered in hair and trees, the sternum is. The legs are powerful and lengthy, with prominent black markings and sharp, black spines covering them. Long and narrowing behind, the abdomen is covered with fine hair. The core of the abdomen is divided into lateral branches by a longitudinal deep brown line . The males are around 8-10 mm in size and the females are roughly 10-12 mm[32].

MATERIALS AND METHODS

Spider and sample collection: From January 2021 to December 2021, adult *P. viridana* specimens were randomly collected using the pipe buckle method handpicked and stored in sterile containersin the Auxilium College campus in Katpadi, Vellore, Tamil Nadu, India. Each plastic tube was 30 mm in diameter and 110 mm long and cotton that had been moistened was placed at the bottom to preserve air humidity. All spiders were brought to the lab and maintained individually in these tubes.

Each spider was sterilised by 75% ethanol for 5 min before to dissection and impurities on its body surface were removed by washing the spider three times in sterile water both before and after sterilisation. The remaining water on the spider's surface was then sucked up by sterilised filter paper. The gut was dissected under a microscope using a sterilised scissor and sterile phosphate-buffered saline (PBS) solution. To get rid of the non-native microbes in the stomach, spiders were fasted for 10 days before being dissected[5]. The aseptic table was sterilised with 75% ethanol three times and exposed to an ultraviolet light for 60 min to guarantee sterility during dissection. It was then cleaned with sterile water, put into a 1.5 mL microcentrifuge tube and temporarily kept in a refrigerator (Haier BCD-252WBCS, Qingdao, China) at -20°C. On ice, the dissection procedure was completed. AUCMA DW86L500, Qingdao, China) -80°C freezer until DNA extraction. Ten guts were put to a tube as one sample and immediately quick-frozen in liquid nitrogen.

DNA extraction and 16S rRNA gene amplicon sequencing: Each pooled sample's total DNA was extracted according to the manufacturer's instructions using the Fast DNA Spin Kit for Soil (MP Biomedicals, USA). The concentrations and purities of the DNA samples were examined using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) and their quality and integrity using 1% agarose gel electrophoresis. The 16S rRNA gene

V3-V4 (Product size: ~459 bp) region of the 16S RNA gene was used to amp up the DNA using certain primers. Re-amplifying the PCR-amplified product using particular V3-F and V4-R primers and an overhang adaptor is known as PCR.

V3-F =16S Amplicon PCR Forward Primer
TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG
V4-R =16S Amplicon PCR Reverse Primer
GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC.

The following ingredients are used in the Polymerase Chain Reaction (PCR) amplification: 4 µL of 5 buffer, 2 µL of dNTPs (2.5 mM), 0.8 µL each of forward and reverse primers (5 mM), 0.4 µL of DNA polymerase, 10 ng of template DNA and lastly 20 µL of ddH_2O. Denaturation at 95 degrees Celsius for three minutes is followed by 29 cycles of annealing at 53° Celsius for 30 sec, extension at 72° Celsius for 45 seconds and final extension at 72 degrees Celsius for ten minutes in the PCR process. According to the manufacturer's instructions, the PCR product was extracted from a 2% agarose gel, purified using an AxyPrep DNA Gel Extraction Kit from Axygen Biosciences in Union City, California and quantified using a QuantusTM Fluorometer from Promega in the United States. An Illumina MiSeq platform was used for the sequencing at Majorbio BioPharm Technology Co., Ltd. in Shanghai, China[33].

Bioinformatics, sequence analysis and statistical analysis: After the assignment of reads to species and strain level, DESeq2 differential abundance testing for sequencing data, was performed. padj values lower than 0.05 and log2 fold-change greater than 1.5 were considered to determine statistical differences in taxonomic and gene data according to the intervention (WD or FMD) or the stability of the microbiota (robust or non-robust). Statistically significant clades of the different species of the spider were analysed through hierarchical all-against-all association testing (HAIIA) (huttenhower.sph.harvard.edu/halla last accessed on 19 November 2020)[34]. Pairwise Pearson coefficients were calculated considering q-values and a Bonferroni false discovery rate of 0.05. Moreover, multi-omic data integration using the DIABLO biomarker discovery pipeline, was used to determine the microbial population profiles. The population profile was further evaluated for alpha diversity using the QIIME/ MOTHUR/KRAKEN/BRACKEN workflows. The alpha diversity profile was interpreted in the form of pie charts for the purpose of graphical representation. This workflow enables highly accurate investigations at genus level. The databases used are SILVA/ GREENGENES/NCBI. Each read is classified based on % coverage and identity. The 16S workflow will be useful in identifying pathogens in a mixed sample or understanding the composition of a microbial community. All statistical tests were computed on R v3.5.0.

RESULTS AND DISCUSSION

The study on the diversity and structure of the bacterial community in the gut of the spider *Peucetia viridana* sheds light on the intriguing relationship between arachnids and their gut microbiota. The *Peucetia viridana* species produced a total of 23070 readings. All projected coverage values were over 99%, indicating that the variety of the sample of bacterial communities was well represented by the existing sequences. The number of reads (measured in millions) was 0.2M, while GC content was 53%. The QC details concentration is >30 ng µL. The amplicon size (bp) in g DNA gel is about 600 bp. To perform quality control checks on the raw sequence data that high throughput sequencing processes produce. FastQC data are combined by MultiQC into a single report. The current work is an exhaustive comparative analysis of the gut bacterial diversity of *Peucetia viridana* spider (Fig. 1).

Members of the phyla Proteobacteria, Firmicutes, Actinobacteria and Bacteroidetes make up the core microbiome, which is crucial for nutrition and energy metabolism[5]. The group of taxa that are found in a significant portion of the population over a certain

Fig. 1: gDNA Gel Image and 16s PCR GEL Image

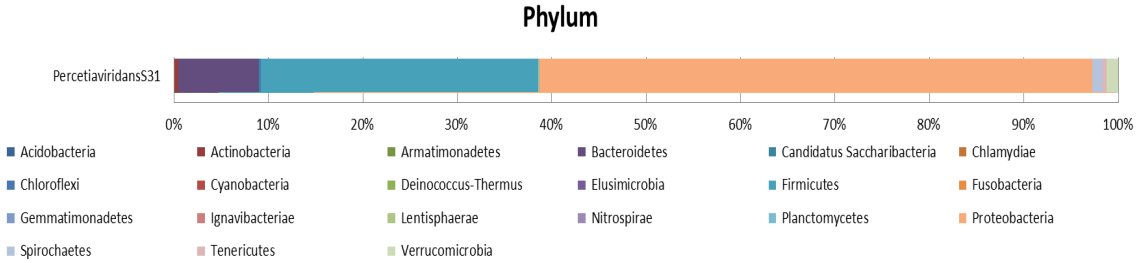

Fig. 2: Phylum of *Peucetia viridana* spider species

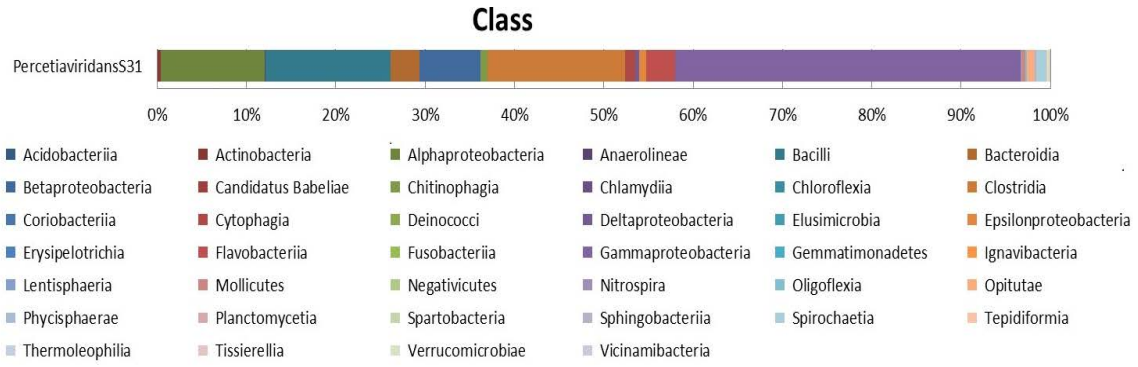

Fig. 3: Class of *Peucetia viridana* spider species

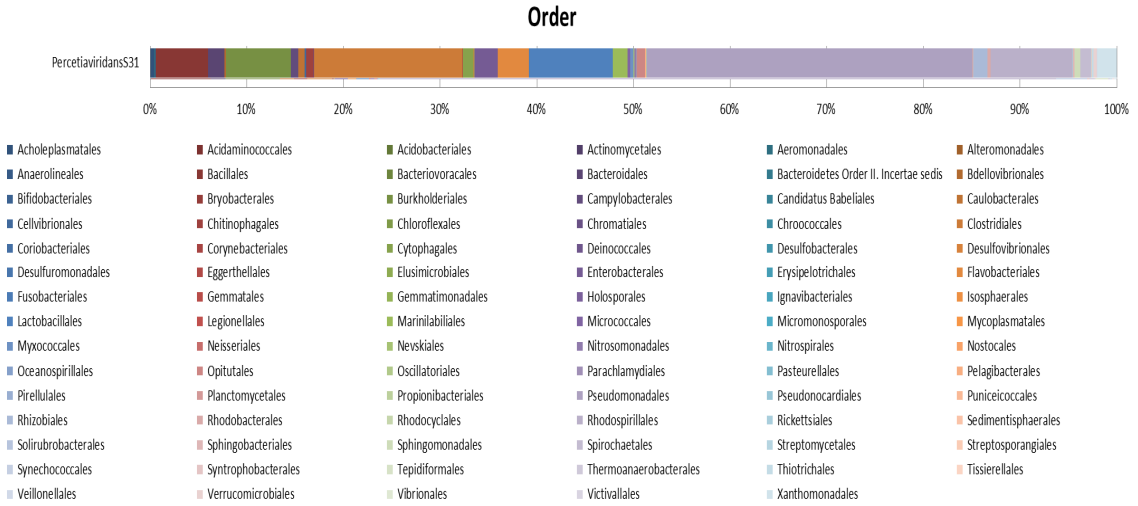

Fig. 4: Orderof *Peucetia viridana* spider species

Table 1: QC details

QC details lane	Sample name	Conc (ng μL^{-1})	Amplicon size (bp)	Library QC	QC
5	EGL/PON/21/00126/001	>30	600bp	Passed	Passed

abundance threshold is referred to as the core microbiome. In order to do this analysis, the count data is converted to compositional (relative) abundance. The data was shown with a relative abundance of 0.1% and a sample prevalence of 20%. The research reveals a rich and diverse community of bacteria residing in the gut of *P. viridana*. This diversity is consistent with emerging studies highlighting the importance of gut microbiota in various arthropods, including insects and spiders[2] (Table 1) (Fig. 2-6).

Our findings suggest that sexual variation plays a significant role in determining the gut bacterial community in *Peucetia viridana* spiders, although the noticeable changes in bacterial composition are mostly

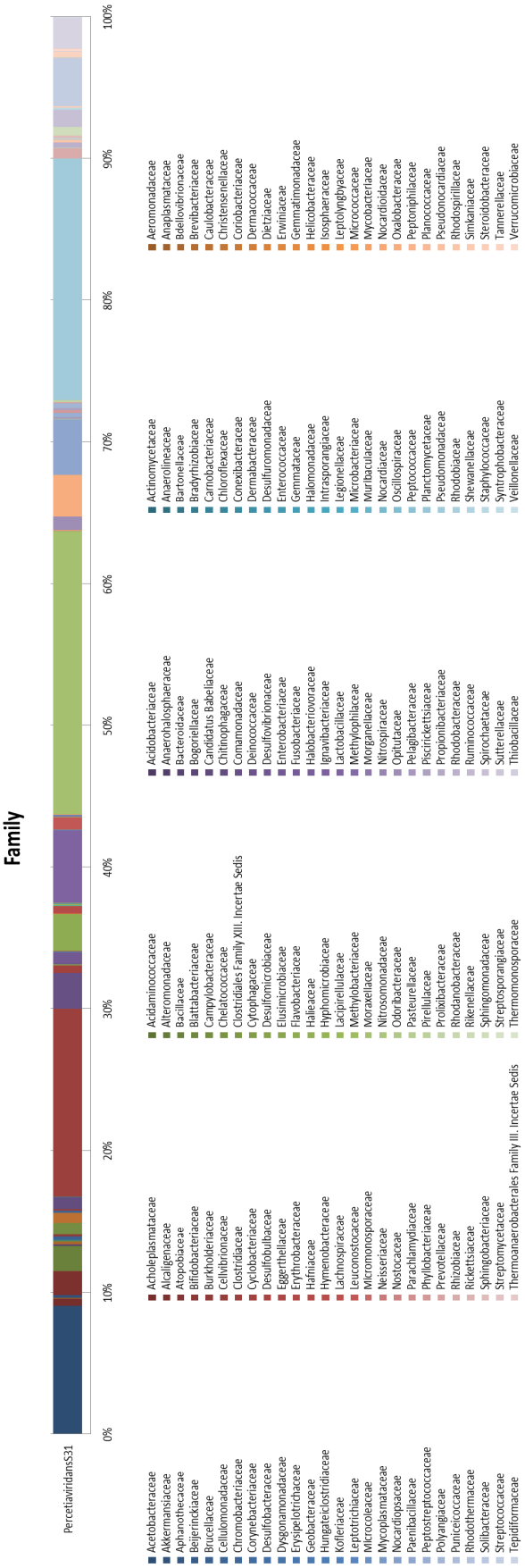

Fig. 5: Family of *Peucetia viridana* spider species

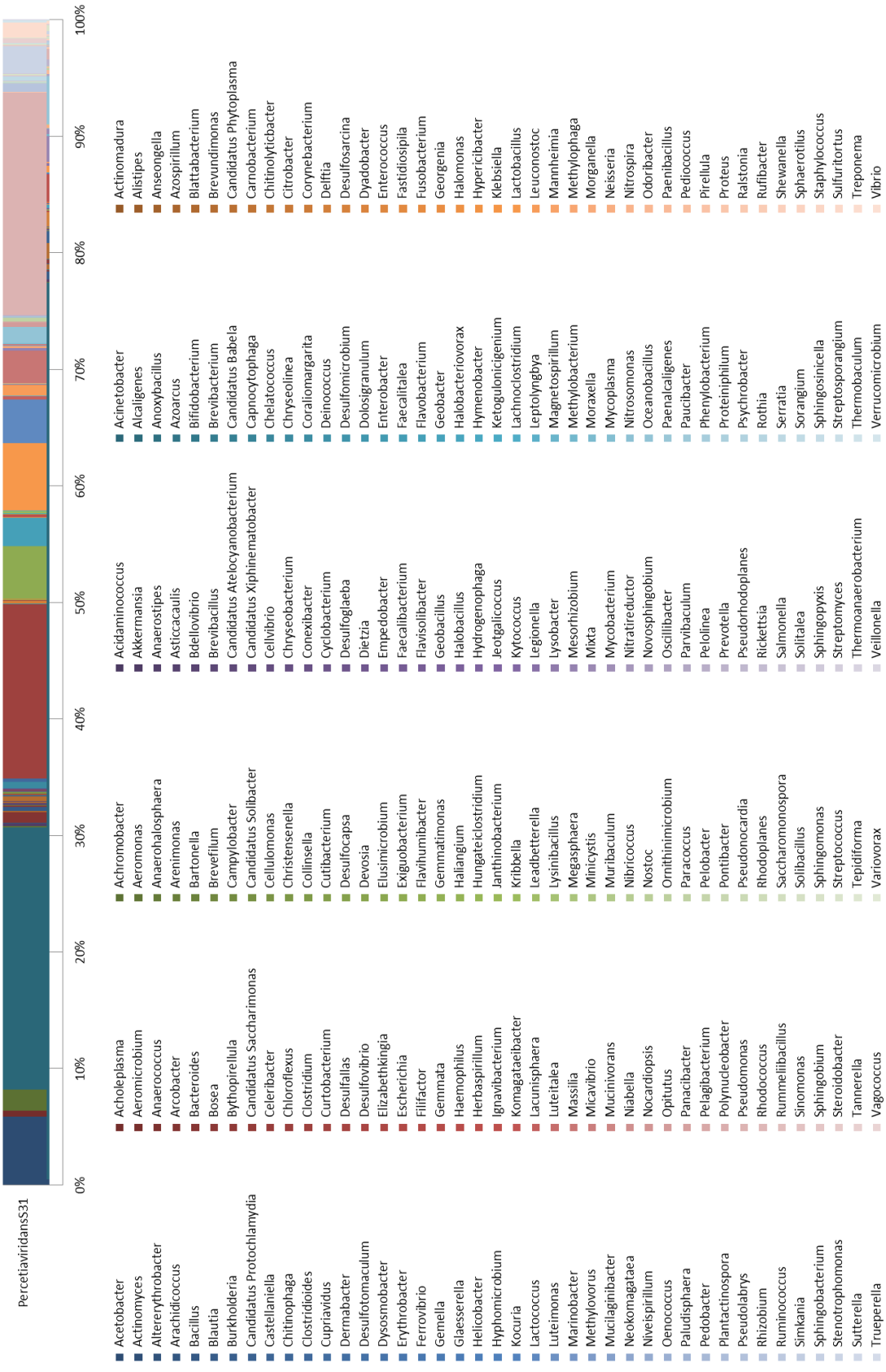

Fig. 6: Genus of *Peucetia viridana* spider species

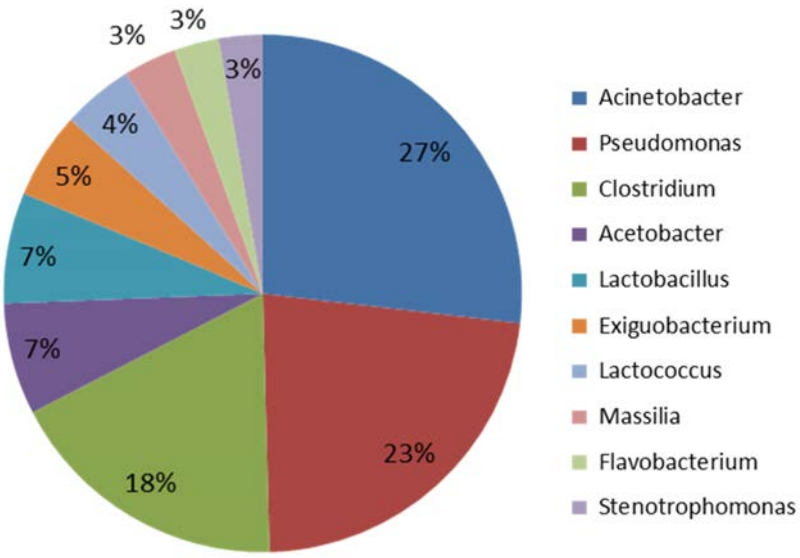

Fig. 7: Top 10 Enriched Genus of *Peucetia viridana* spider species

caused by their various dietary and energetic needs. 40 bacterial classes, 156 family members and 251 genera were identified by analysis as being present in the stomach. To allow for more physiologically significant comparisons, data normalisation attempts to overcome the variability in sample depth and the sparsity of the data. Data was purified to fit into a small library. To scale the data uniformly across all samples, the Total Sum Scaling (TSS) factor was used. The presence of diverse bacterial taxa suggests potential mutualistic interactions between *P. viridana* and its gut microbiota. Similar to other arthropods, these microbes may assist in nutrient digestion, detoxification and pathogen defense(Fig. 2-6)[8].

Acinetobacter, Pseudomonas, Clostridium, Acetobacter, Lactobacillus, Exiguobacterium, Lactococcus, Massilia, Flavobacterium and *Stenotrophomonas* are the top 10 enriched genera of *Peucetia viridana* spider species. The detailed view mode of about <1500 features was used to create the heat map at the genus taxonomic level. This study underscores the possibility of coevolutionary processes shaping the spider-gut microbe relationship. Coevolution between arthropods and their gut microbiota is an evolving field of study and understanding these dynamics can provide valuable insights into host-microbe coevolution[35].

Furthermore, the PICRUSt2 research showed that they are also engaged in processes including fatty acid and sugar metabolism, the breakdown of organic molecules and the creation of vitamin cofactors like vitamin E (tocopherols). *Peucetia viridans*' stomach bacterial populations were grouped apart from those of other spider species, which may be connected to their preferences for prey, their location, or unusual prey consumption prior to capture[36]. In the families Oxyopidae, the Proteobacteria and Firmicutes are two primary dominating phyla. Furthermore, the presence of the Paraclostridium genus (56%) (phylum Firmicutes) in *Peucetia viridans* is responsible for the high abundance of Firmicutes in the family Oxyopidae. Similar findings high phylum Proteobacteria abundance were also found in the guts of a variety of arthropods, including spiders[5], butterflies[37], bugs[38], bees[2,3] and moths[39]. In addition, the differences in prey and ambient settings may account for the variance in bacterial abundance by genus in the guts of seven spider species[5]. In addition, Kennedy *et al.*[24] found that prey has a significant impact on the gut microbiota of spider species, which changes over time depending on the prey diet. It might be explained by the stress that is brought on when spiders are raised and given a set diet of prey. Additionally, substantial sampling from various environmental settings has been necessary to better understand the basic gut bacterial populations in spiders. Insects can acquire microbiota from their surrounding habitats[40] and environmental variables have been shown to play essential roles in the assembly of the gut microbiota in arthropods[7,8]. The composition and diversity of gut bacteria in *P. viridana* may be influenced by environmental factors. Investigating how environmental conditions impact the spider's gut microbiota can deepen our understanding of the adaptability of these communities[41]. The gut microbiome of *P. viridana* may have broader ecological implications, influencing the spider's role in its ecosystem. Spider gut microbes can contribute to nutrient cycling and impact predation dynamics[42]. Further research can explore the functional roles of specific bacterial taxa within *P. viridana's* gut. Functional metagenomics and experimental studies can elucidate how these microbes contribute to the spider's physiology and ecology[43-47].

CONCLUSION

In conclusion, the research on the composition and diversity of the bacterial community in the stomach of the interesting arachnid species *Peucetia viridana*has shed important light on its microbial ecology. We have uncovered a rich and varied community of bacteria living in the spider's intestines using extensive molecular research and sequencing tools. The findings of this study underscore the significance of the gut microbiota in *Peucetia viridana* and potentially other spider species. The presence of a diverse bacterial community suggests possible mutualistic relationships, aiding in nutrient digestion, immunity and overall fitness of the spider.

Furthermore, this research contributes to our broader understanding of the intricate interactions between spiders and their gut microbes, shedding light on the coevolutionary processes that have shaped these relationships over time. As we delve deeper into the complexities of the spider gut microbiome, we open up avenues for further research. Future studies can explore the functional roles of specific bacterial taxa, their potential contributions to the spider's ecology and how these relationships might be influenced by environmental factors. In summary, this investigation enhances our knowledge of the microbial world within *Peucetia viridana* and underscores the importance of studying gut microbiota in arachnids and other organisms. It is our hope that this research will serve as a foundation for future inquiries into the fascinating interplay between spiders and their bacterial partners, providing insights into the broader field of arachnid biology and ecology.

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