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Diversity of Gut Microbes in Adult *Vespa velutina* (Asian Hornet) Carcasses Killed by Natural Causes

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Abstract: [Objective] This study's objective was to investigate the diversity of intestinal microorganisms in adult *Vespa velutina* (Asian hornet) killed by natural causes. This study investigates the composition of intestinal fungi and bacteria and predicts the pathogenic pathogen in adult *Vespa velutina* (Asian hornet). [Methods] We determined the ITS1 sequence of fungi and the V3–V4 variant region of 16S rRNA of bacteria using Illumina MiSeq technology. Operational taxonomic units (OTU) of gut symbiotic microorganisms were quantified, and the resulting data were subjected to analysis of species abundance, composition, and alpha diversity. OTU function was predicted using PICRUSt2/FUNGuild. In addition, cultured microorganisms from the gut microbiota of adult *Vespa velutina* were isolated and identified. A number of 3610 (fungi) and 8373 (bacteria) were identified via cluster analysis. A total of 13 strains, 51 classes, 126 orders, 285 families, and 586 genera were identified for fungi and 44 strains, 113 classes, 319 orders, 662 families, and 1394 genera were identified for bacteria. *E. shigella*, *Herbaspirillum*, and *Aaaia* were the most abundant classes of bacteria, and *Fusarium*, *Mortierella*, and *Starmerella* were the most abundant classes of fungi. In addition, 16 community genera of fungi and 11 of bacteria were outlined as core taxa. Species diversity and richness for the gut fungal and bacterial communities with VN were found to be higher than those with VA. Furthermore, bacterial species diversity and richness were found to be higher than those of fungi in VA and VN. Functional analysis revealed that *Vespa velutina* gut bacteria exhibited 20 functions, while fungi were classified into three types of nutrient modes. Cultivable bacteria were obtained from two phyla and two classes, but no fungi could be cultivated. [Conclusion] Variations in the species diversity and abundance of both fungi and bacteria in the gut were observed between the VA and the VN. The involvement of bacteria in the death of adult *Vespa velutina* was found to be significant. In addition, VA1 (the self-named strain) may be a pathogenic bacterium derived from the gut of the VA that exhibits virulence.

Keywords: *Vespa velutina*; gut microorganisms; high-throughput sequencing; diversity; function prediction

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1. Introduction

Vespa velutina, also known as a red wasp, tiger bee, or yellow wasp, is an arthropod of Hymenoptera and the family Vespidae and is considered one of the most serious invasive insects in Japan, South Korea, and most of Europe [1]. *Vespa velutina*, an omnivorous insect, is mainly distributed in agriculture and forestry. Young wasps feed on the larvae of other insects. *Vespa velutina* feeds on fruit and trees. It is worth noting that there are reports that its melittin polypeptides are a cause of allergy and even death in humans and animals [2,3]. In addition, due to its high toxicity [4] and wide range of activities, it has caused serious damage to agroforestry ecology [5,6]. There is an urgent need to

control the number of *Vespa velutina* and reduce its range of activities in order to protect the ecological balance of agriculture and forestry and to reduce harm to humans. Controlling *V. velutina* mainly depends on physically removing and eradicating them [2–4]. However, this method is extremely dangerous. It requires a lot of labour and material resources. Therefore, controlling *V. velutina* is very difficult. Biological agents can promote or inhibit the quantitative development of certain organisms under the condition of protecting the ecological balance. It is necessary to use appropriate biological control agents to improve the efficiency of *V. velutina* control. In addition, microbial insecticides have received a lot of attention worldwide. However, there are no reports on the control of wasps.

In order to study the intestinal pathogen *V. velutina* and to develop suitable microbial insecticides for its control, this study investigated the diversity of fungi and bacteria that are present in the adult stages of this insect species. The insect gut is a critical organ that serves as a habitat for numerous microorganisms [7] and plays a vital role in host metabolism, the degradation of toxic substances, and immune response [8,9]. The insect gut is also an important site for the colonisation of pathogens [10–12] given the high adaptability and utility of the wasp, as well as the close relationship between insect gut microorganisms. By studying the diversity of the microbial community, the function of the microbial community can be explored [13]. Traditional methods to study microbial community diversity include pure culture, physical and chemical identification, etc. However, most microorganisms in the natural environment are not culturable and difficult to identify [14,15]. With the development of the metagenomic concept and the rapid development of sequencing technology, 16S rRNA/ITS1 gene sequencing has been widely used in the study of microbial community diversity [16–18]. Zhang conducted a study on the diversity of the gut microbial community in *V. velutina* using high-throughput sequencing [19]. It was shown that Proteus and Lactobacillus (especially Lactobacillus and other lactic acid bacteria) could potentially have a significant impact on the gut of *V. velutina*. However, the study did not reveal any differences in the gut microbiota between naturally dying (VA) and typical (VN) adult *V. velutina*. A Cini [20] used targeted meta-genomics to describe the yeasts and bacteria gut communities of individuals of different reproductive phenotypes (workers and future queens), life stages (larvae, newly emerged individuals, and adults), and colony non-living samples (nest paper and larval faeces). *Bacilli*, *Gammaproteobacteria*, *Actinobacteria*, and *Alphaproteobacteria* were the most abundant classes of bacteria, and Saccharomycetes *Dothideomycetes*, *Tremellomycetes*, and *Eurotiomycetes* were the most represented yeast classes. But the result only provides the resource of the microbiome of *V. velutina* in Europe. Our aim is to determine the differences in species diversity and abundance of gut fungi and bacteria between VA and VN using PICRUSt2/FUNGuild in order to make functional predictions. Furthermore, our research provides data that can be used to investigate the functionality of the adult *V. velutina* gut microbiome. This will be valuable for the development of biocontrol agents for *V. velutina* control. In addition, our results provide the first metagenomic resource of the gut microbiome of *V. velutina* in China.

2. Materials and Methods

2.1. Materials Used

2.1.1. Adult *Vespa velutina* Sampling Method

In June 2022, both naturally decaying (VA) and healthy (VN) adult bodies of *V. velutina* were collected from a wasp farm in Nanning 530004, China. The bodies were surface disinfected with 70% alcohol prior to storage at 4 °C.

2.1.2. Gut Dissection

The worms were aseptically dissected, the intestines were isolated, and the intestines were placed separately in a 5 mL centrifuge tube with 2 mL of sterile water, according to Tu [21]. Then, the intestines were pulverised separately and mixed with a sterile blender. Finally, the intestines were ground separately and mixed using a sterilised blender.

2.2. DNA Extraction, PCR and Data Analysis

According to Jia [22], the total DNA was extracted from the intestine of adult *V. velutina* using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). The extraction steps are described in the manual. The ITS2 (fungal) and 16S rRNA V3–V4 regions (bacterial) were then amplified using universal primers FITS7/RITS4 (fungal) and 333F/806R (bacterial), and our study refers to Jia [22] and Zhang [23]. PCR amplification steps and conditions were adapted from Jia [22], and the combined PCR amplification products were detected by 1% agarose gel electrophoresis. Paired-end sequencing was performed using the Illumina MiSeqPE300 sequencing platform. Finally, the high-throughput sequencing data were processed and analysed using the BMKCloud platform. The SPSS (26.0) analysis system (LSD) was then used to analyse the significance of the differences between treatments. In our study, sequencing data was analysed according to Zeng [24] using the Operational Taxonomic Units (OTU) marker method. To analyse the diversity of the gut microbiota in adult *V. velutina*, Shannon, Simpson, Ace, Chao, and coverage were selected. Ace and Chao reflect sample community richness, and the higher the index values, the higher the sample community richness. Shannon and Simpson reflect on sample species diversity. In addition, the higher the Shannon values and the lower the Simpson values, the higher the species diversity in the sample. The depth of sequencing is reflected in the coverage index. The closer the coverage index is to 1, the more likely it is that all species in the sample are covered by the sequencing depth. The closer the coverage index is to 1, the more reasonable the depth of sequencing is, and the depth of sequencing has basically covered all the species in the sample. Finally, PICRUSt2/FUNGuild was used to predict the function of ITS and 16S gene data and infer the functional gene composition of the sequenced samples, according to Zhang [23] and Kou [25]. Univariate analysis and multiple comparisons were performed using SPSS (26.0).

2.3. Isolation, Purification, and Identification of Cultural Microorganisms

The gut microorganisms of adult *Vespa velutina* were isolated and purified, according to Tu [21]. Simultaneously, the observation and recording of the culture characteristics of the strains were carried out. Preliminary strains were identified according to R.E. Buchanan [26] and Wei [27]. Then, the molecular identification of the strains by the ITS/16S rDNA amplification method and the phylogenetic tree were constructed by MEGA (5.1) according to Li [28].

3. Analysis of Results

3.1. Sequence Mosaic Assembly and OTU Clustering Analysis

Cluster analysis of the gut microbiota of adult *Vespa velutina* yielded a total of 3610 (fungi) and 8373 (bacteria). In addition, the number of gut fungal and bacterial OTUs was higher in VN than in VA. The number of bacterial OTUs was higher in VN or VA than in fungi separately (Table 1).

3.2. Alpha and Beta Diversity Analysis of Gut Bacteria and Fungi

Alpha diversity analysis showed that the sequencing depth covered all species in the sample. The flat curve indicates sufficient sampling for data analysis (Figure 1A,B). Both species diversity and richness were higher in VN than in VA for the gut fungal and bacterial communities. In addition, the species diversity and richness of the bacteria in both the VN and the VA were greater than those of the fungi (Table 2). Among the gut bacteria of adult *Vespa velutina*, the species composition of VA1 and VN1 is more similar, with *Enterobacteriaceae* dominating in VN1 and *Asaia* dominating in VA1; the species composition of VA2 and VA3 is more similar, with *Herbaspirillum* and *Enterobacteriaceae* dominating in VA2 and *Herbaspirillum* dominating in VA3; and the species composition of VN2 and VN3 is more similar, with *Herbaspirillum* dominating (Figure 1C). Among the gut fungi of adult *Vespa velutina*, the species composition of VA1, VA2, and VA3 is more similar, with *Starmerella dominating* in VA1, VA2, and VA3. The species composition of VN1, VN2, and

VN3 is more similar, with *Mortierella* and *Fusarium* dominating in VN1, VN2, and VN3 (Figure 1D).

Table 1. Basic information for the high-throughput sequencing of ITS1 of intestinal fungi and of 16S rRNA of bacteria in the VN and VA.

Sample Number	Gene	Number of Raw Tags	Number of Valid Tags	Number of OTUs	Number of Taxa of Different Taxonomic Categories				
					Phylum	Class	Orders	Family	Genus
VA1	ITS1	79,892	79,633	95	8	20	33	46	54
	16S rRNA	80,157	79,993	113	15	22	47	69	87
VA2	ITS1	79,967	79,798	120	7	18	34	46	63
	16S rRNA	80,137	80,006	299	21	40	97	145	186
VA3	ITS1	80,060	79,870	84	7	15	27	40	51
	16S rRNA	79,953	79,803	1896	36	82	218	375	688
VN1	ITS1	80,221	79,908	1302	13	44	27	40	51
	16S rRNA	80,323	80,162	73	11	15	30	37	44
VN2	ITS1	80,021	79,655	1302	13	45	100	197	345
	16S rRNA	79,939	79,765	3254	39	101	30	37	44
VN3	ITS1	80,006	79,669	1425	13	51	109	234	406
	16S rRNA	79,947	79,764	3183	41	113	261	504	886

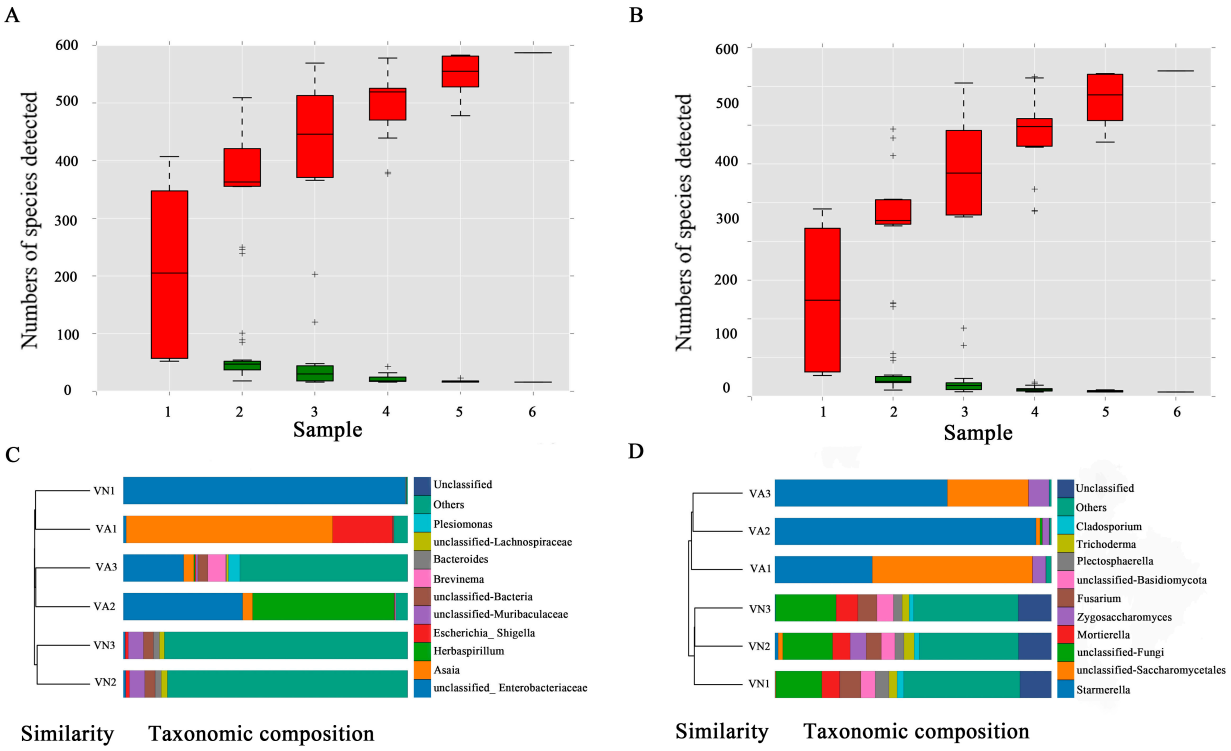

Figure 1. Alpha and Beta Diversity Analysis of Gut Bacteria and Fungi. (**A**): The species accumulation curves of the gut bacteria; (**B**): the species accumulation curves of the gut fungi (at the level of the genus); (**C**): the UPGMA analysis of the gut bacteria; (**D**): the UPGMA analysis of the gut fungi (at the level of the genus).

Table 2. Diversity indices of fungi and bacteria in the gut of *Vespa velutina* Lepeletier in different hosts and at different stages of development.

Groups	Sample Code	Diversity Index				
		Ace	Chao1	Shannon	Simpson	Coverage
Fungi	VA1	96.44	95.19	2.05	0.69	0.9999
	VA2	120.62	120.04	0.54	0.11	1.0000
	VA3	84.87	84.16	1.81	0.57	1.0000
	VN1	1326.43	1326.13	8.53	0.99	1.0000
	VN3	1302.41	1302.16	8.62	0.99	1.0000
	VN3	1426.06	1425.10	8.36	0.99	0.9999
Bacteria	VA1	113.00	113.00	1.51	0.44	1.0000
	VA2	303.32	299.72	2.67	0.71	0.9998
	VA3	1896.17	1896.00	8.15	0.98	1.0000
	VN1	73.71	73.04	1.31	0.51	1.0000
	VN3	3254.32	3254.00	10.08	1.00	1.0000
	VN3	3183.23	3183.00	10.26	1.00	1.0000

3.3. Identification of Fungi and Bacteria in the Gut of Adult V. velutina

Sequence alignment (Figure 2) identified the gut microbiota and bacteria of adult *V. velutina*. For the bacterial community, 44 phylum, 113 classes, 319 orders, 662 families, and 1394 genera could be determined. The microbial community composition of the gut samples of adult *V. velutina* is mainly *Bacteroidot*, *Firmicute*, and *Proteobacteria* at the phylum level (Figure 2A); mainly *Bacteroida*, *Alphaproteobacteria*, and *Gammaproteobacteria* at the class level (Figure 2B); mainly *Enterobacterales*, *Burkholderiales*, and *Acetobacterales* at the order level (Figure 2C); at the family level, mainly *Oxalobacteraceae*, *Acetobacteraceay*, and *Enterobacteriaceae* (Figure 2D); and at the genus level, mainly *E. shigella*, *Herbaspirillum*, and *Aaaia* (Figure 2E). For fungi, 13 phylum, 51 classes, 126 orders, 285 families, and 586 genera were identified. The microbial community composition of adult *V. velutina* gut samples is mainly *Asomycota*, *Basidiomycota*, and *Chytridiomycota* at the phylum level (Figure 2F), *Saccharomycetes*, *Sodarmycetes*, and *Mortierellomycetes* at the class level (Figure 2G); at the order level, mostly *Saccharomycetales*, *Hypocreales*, and *Mortierellales* (Figure 2H); at the family level, mostly *Saccharomycetaceae*, *Mortierellaceae*, and *Plectosphaerellaceae* (Figure 2I); and at the genus level, mostly *Fusarium*, *Mortierella*, and *Starmerella* (Figure 2J).

3.4. Differences in Gut Bacteria and Fungi between VN and VA

There were differences in gut bacteria and fungi composition between VN and VA adults. In addition, nine genera of fungi and 186 genera of bacteria were endemic to VA1; five genera of fungi and three genera of bacteria were endemic to VA2; four genera of fungi and six genera of bacteria were endemic to VA3; 73 genera of fungi and one genus of bacteria were endemic to VN1; 63 genera of fungi and 268 genera of bacteria were endemic to VN2; and 134 genera of fungi and 239 genera of bacteria were endemic to VN3. The core flora especially included 16 genera of fungi and 11 genera of bacteria in groups of samples (Figure 3); the common fungi included *Cladosporium*, *Cladosporium*, *Fusarium*, *Trichocladium*, *Mortierella*, *Aspergillus*, *Talaromyces*, *Starmerella*, *Zygosaccharomyces*, etc.; the common bacteria included *Pseudomonas*, *Comamonadaceae*, *Lactobacillus*, *Bacillus*, *Muribaculaceae*, *Brevundimonas*, etc. It is worth mentioning that there was a certain correlation between VN and VA via Source Tracker.

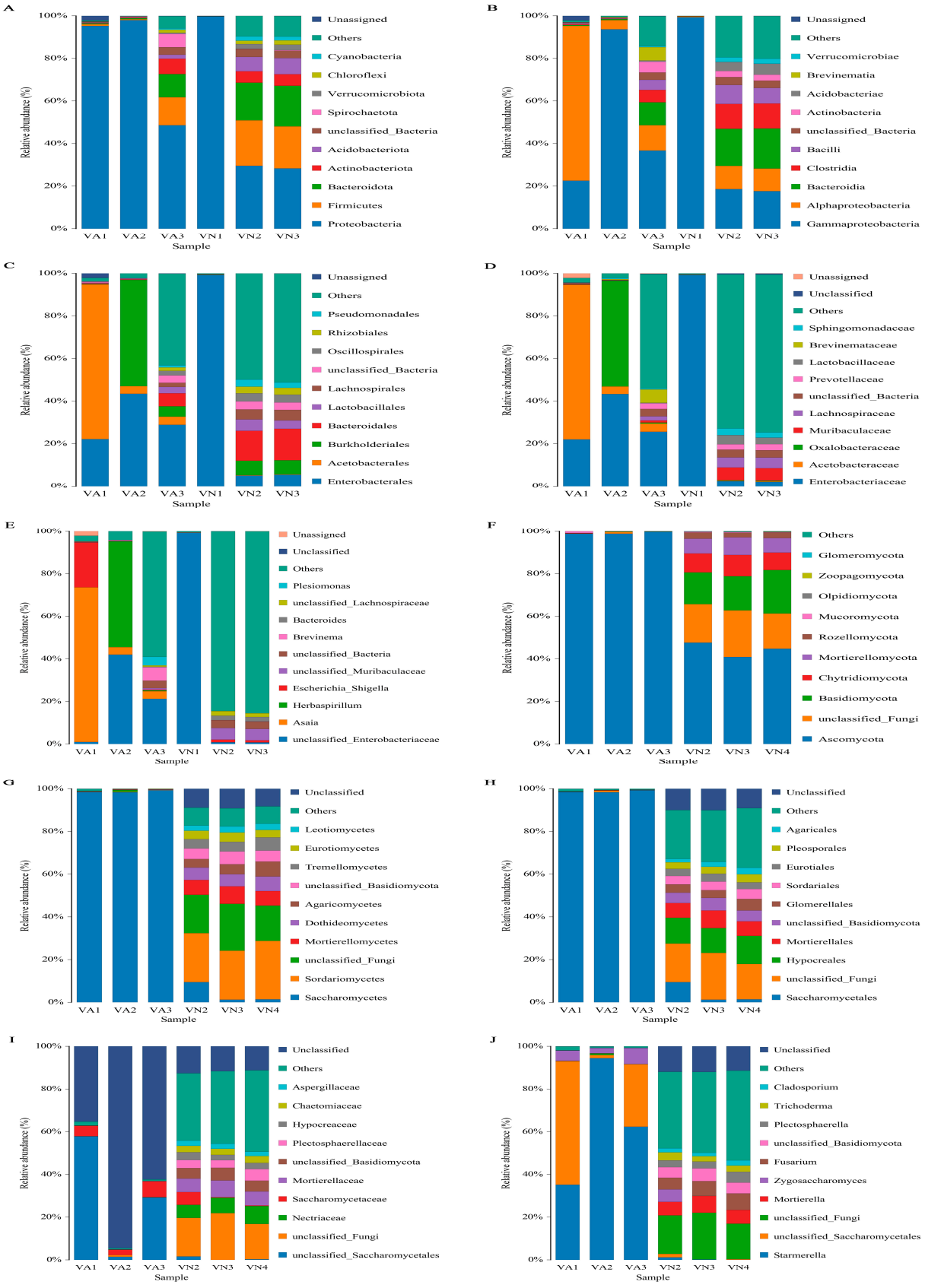

Figure 2. Composition of the top ten gut microbiota of *Locusta migratoria* manilensis Meyen in different geographical populations. (**A**,**F**): Phylum. (**B**,**G**): Class. (**C**,**H**): Order. (**D**,**I**): Family. (**E**,**J**): Genus.

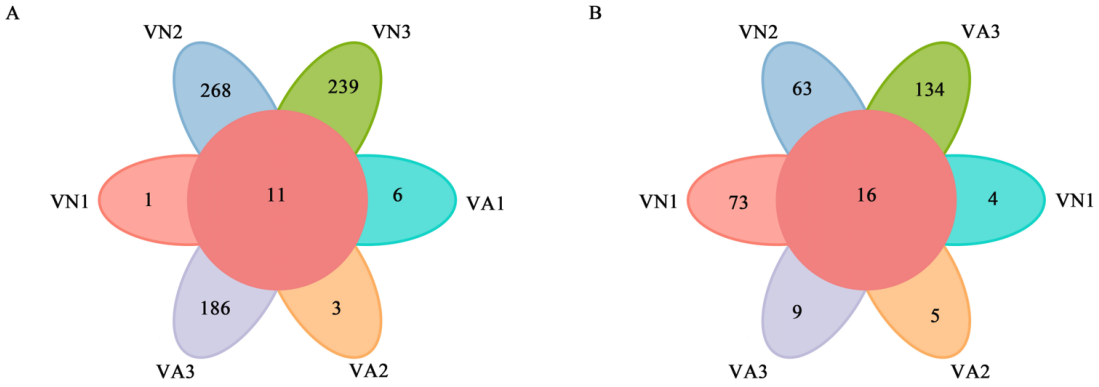

Figure 3. Venn diagram of gut bacteria (**A**) and fungi (**B**).

3.5. Function Prediction

A total of 20 functions were obtained via COG function prediction (Figure 4A), and the functions of bacteria in VN were similar to VA at the genus level (Table 3). Simultaneously, 364 bacterial metabolic pathways were annotated via KEGG metabolic pathway analysis. In addition, nine phenotypes were predicted by Bugbase phenotypes, such as Aerobic, Anaerobic, Gram-Negative, etc. (Figure 4B). There are three types of nutrition (Pathotroph, Saprotroph, and Symbiotroph; Figure 4C), which, in the gut fungi of *V. velutina*, were obtained via FUNGuild's function prediction. In particular, Pathotroph could be subdivided into nine types, including Plant Pathogen, Wood Saprotroph, Fungal Parasite, etc. (Figure 4D).

Table 3. Diversity indices of fungi and bacteria in the gut of *Vespa velutina lepeletier* at different hosts and stages.

Category	Description	Sample (%)					
		VA1	VA2	VA3	VN1	VN2	VN3
R	Genus function prediction only	0.1107	0.1149	0.1148	0.1084	0.1123	0.1112
E	Amino acid transport and metabolism	0.1092	0.0935	0.0937	0.0895	0.1066	0.0986
S	Function unknown	0.0887	0.0750	0.0733	0.0871	0.0934	0.0749
G	Carbohydrate transport and metabolism	0.1050	0.0615	0.0612	0.0841	0.0720	0.0677
C	Energy production and conversion	0.0661	0.0642	0.0647	0.0652	0.0626	0.0648
M	Cell wall/membrane/envelope biogenesis	0.0549	0.0649	0.0659	0.0683	0.0618	0.0639
K	Transcription	0.0671	0.0643	0.0642	0.0581	0.0646	0.0632
J	Translation, ribosomal structure, and biogenesis	0.0456	0.0733	0.0744	0.0598	0.0510	0.0710
P	Inorganic ion transport and metabolism	0.0735	0.0507	0.0502	0.0546	0.0649	0.0553
H	Coenzyme transport and metabolism	0.0448	0.0482	0.0486	0.0486	0.0466	0.0497
L	Replication, recombination, and repair	0.0392	0.0485	0.0489	0.0481	0.0335	0.0466
T	Signal transduction mechanisms	0.0347	0.0419	0.0412	0.0350	0.0449	0.0400
O	Posttranslational modification, protein turnover, and chaperones	0.0335	0.0396	0.0396	0.0416	0.0341	0.0394
I	Lipid transport and metabolism	0.0240	0.0360	0.0361	0.0259	0.0338	0.0331
F	Nucleotide transport and metabolism	0.0242	0.0300	0.0303	0.0287	0.0225	0.0299
U	Intracellular trafficking, secretion, and vesicular transport	0.0222	0.0256	0.0255	0.0321	0.0256	0.0251
N	Cell motility	0.0119	0.0185	0.0180	0.0224	0.0263	0.0193
Q	Secondary metabolites biosynthesis, transport, and catabolism	0.0217	0.0187	0.0186	0.0184	0.0222	0.0184
V	Defence mechanisms	0.0136	0.0190	0.0193	0.0139	0.0122	0.0166
D	Cell cycle control, cell division, and chromosome partitioning	0.0086	0.0109	0.0109	0.0099	0.0082	0.0107
Other	Other	0.0005	0.0006	0.0006	0.0003	0.0009	0.0006

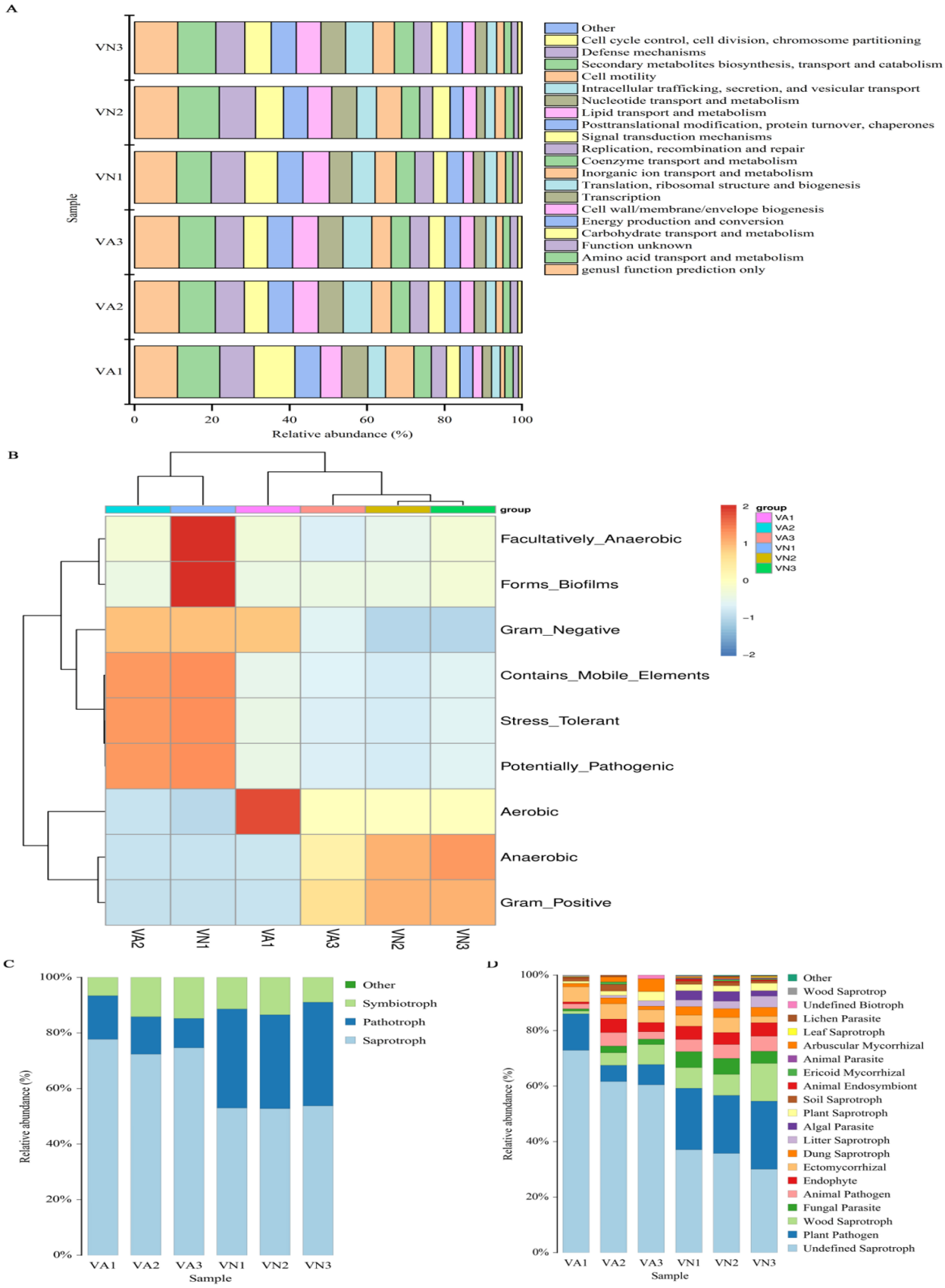

Figure 4. Predicting COG functional genes in gut bacteria (**A**), predicting Bugbase phenotypes in gut bacteria (**B**), and predicting FUNGuild in gut fungi (**C**,**D**).

3.6. Isolation and Identification of Culturable Microorganisms

The isolation and purification of gut microbes from adult *Vespa velutina* lepeletier yielded two genera and two classes of bacteria. However, no culturable fungi were obtained. Figure 5 shows the cultivation characteristics of VN1-VN6 and VA1-VA9. Two groups of culturable bacteria in the gut of adult *V. velutina* were identified in a phylogenetic tree (Figure 5). The first group consisted of Proteobacteria, including *Escherichia*, *Raoultella*, *Shigella*, *Serratia*, *Cedecea*, *Acinetobacter*, *Klebsiella*, and *Erwinia*. The second group consisted of *Firmicutes*, including only *Bacillus*. In addition, VA5, VN2, and VN5 are *Serratia*.;VA1 and VN1 are *Escherichia*; and VN4 and VN5 are *Bacillus*.

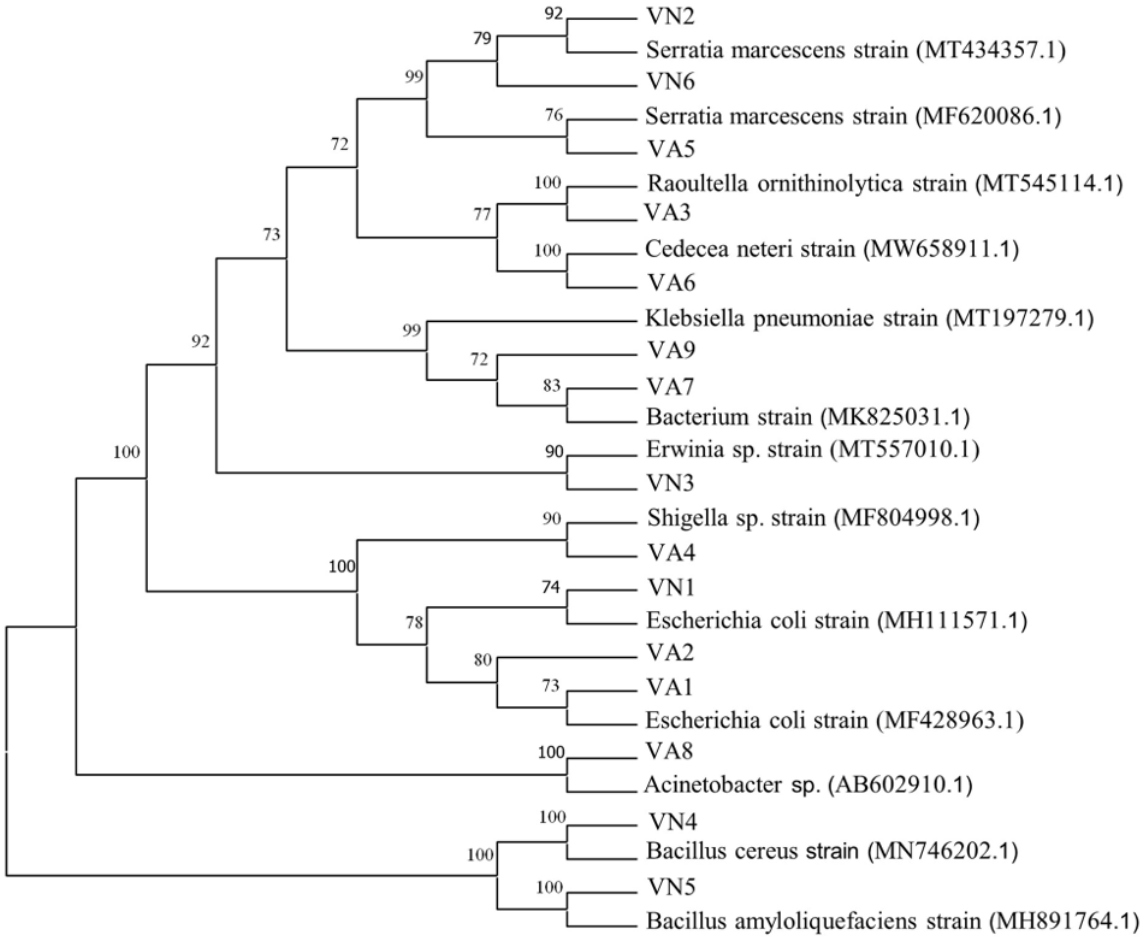

Figure 5. Phylogenetic tree in gut bacteria.

4. Discussion

4.1. The Gut Bacteria over Fungi in Adult V. velutina

The number of fungal and bacterial operational taxonomic units (OTUs) in the gut was higher in VN compared to VA, based on the OTU counts obtained. This result can be attributed to the diverse interactions between the insect's gut immune system and the gut microorganisms [29–31]. Consequently, the natural mortality of *V. velutina* gradually eliminated pathogens from other gut microbial groups. Finally, the number of operational taxonomic units (OTUs) of the VA was lower than that of the VN. Furthermore, the number of bacterial OTUs in either VN or VA was higher than that of fungi alone, suggesting that bacteria were the primary beneficial flora in adult *V. velutina*. To further confirm the role of bacteria as beneficial flora, an alpha diversity analysis of the gut microbiota was performed. The gut microbiota of adult *V. velutina* were subjected to alpha diversity analysis. The results of the study showed that the gut fungi and bacteria of adult *Vespa velutina* Lepeletier in VA exhibited both diversity and uniformity. It was observed that the gut bacteria of

adult *V. velutina* were the dominant flora compared to *Chrysomya* [32], megacephala, and *Copris incertus* [33].

4.2. The VA1 Is a Potential Pathogenic Bacteria of Adult V. velutina

In our study, 16 genera of fungi, such as *Cladosporium*, *Cladosporium*, and *Fusarium*, and 11 genera of bacteria, such as *Pseudomonas*, *Comamonadaceae*, and *Lactobacillus*, were present in different samples from both groups (three replicates in each group). In addition, the results were similar to those of Zhang [19]. In particular, *Firmicutes*, *Bacteroidetes* and *Actinobacteria* in gut microorganisms of adult *V. velutina* and *Lactobacillus* were reported to be the dominant genus. Simultaneously, the core flora of our study did not include *Tenericutes* and *Sphingomonas*, as reported by Zhang. Incidentally, *Pseudomonas* bacteria are characterised by their ability to immobilise perforation into tissues, elastin production, and adhesion activity. *Pseudomonas bacteria* are often considered potential pathogenic bacteria [34–36], indicating that under certain conditions, *Pseudomonas* bacteria may play a role in adult *V. velutina* disease. However, whether *Pseudomonas* bacteria form a symbiotic relationship with adult *V. velutina* requires further study. In addition, there are 11 phylum and 21 classes of fungi in our study, but no relevant reports have been found. *Aspergillus* in Fungi is a common pathogen, and most species of Fusarium are plant pathogenic pathogens [37,38].

In our study, we categorised fungi into three groups using FUNGuild, namely Symbiotroph, Pathotroph, and Saprotroph. It is noteworthy that symbiotrophs had the highest abundance across the different samples, whereas undefined saprotrophs and plant pathotrophs had significant numbers in the pathotroph group. The results indicate that fungi are not the main pathogen in mature *V. velutina*. However, they may be important in disease progression. Furthermore, the comparison of bacteria with PICRUSt2 in our study showed that there is a greater diversity of gut bacterial species between different samples. However, the function of the gut bacteria remains consistent, so we can speculate that this function belongs to a specific group of bacteria in the *Vietnamese* and *American* guts, which is the result of the long-term evolution of the microorganisms and their respective hosts. A total of nine types of bacteria were identified according to the Bugbase phenotype prediction. Incidentally, the pathogenic potential of VA was found to be higher than that of VN. For the village, the alpha diversity analysis showed that intestinal bacteria were the dominant flora. Therefore, it can be hypothesised that the pathogenic bacteria in adult *V. velutina* are bacterial in nature. To clarify the origin of these bacteria, a source tracker analysis was performed. The results indicate that the pathogenic bacteria could originate from either the gut or the environment, although the latter is more likely. To obtain culturable pathogens, gut microorganisms were isolated and identified. Two phylum and two classes of bacteria were obtained. No fungi were found. Furthermore, *Escherichia* and *Serratia*, which are common pathogenic bacteria [34–36], were detected in the gut of both adult VN and VA. The presence of *Escherichia* bacteria in VA and VN, however, differed significantly. VA1 was found to be more abundant than VN1 in the digestive tracts of adult *V. velutina*. It has been suggested that VA1 may be the causative agent of *V. velutina* in adults, but further authentication is required.

5. Conclusions

There were discrepancies in species diversity and prevalence of gut fungi and bacteria between VA and VN. In particular, both species diversity and frequency of VN were greater than VA alone. In addition, both species diversity and abundance of bacteria exceeded that of fungi in both VN and VA. The results indicate that bacteria are a critical component of the decomposition process. Furthermore, microbial isolation and identification revealed a potentially virulent bacterial strain (VA1), which is believed to be the pathogen causing infestation in adult *V. velutina*.

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