Journal of Cotton Research

RESEARCH

Open Access

Dynamics and diversity of symbiotic bacteria in *Apolygus lucorum* at different developmental stages

XUE Hui[1,3], ZHU Xiangzhen[1,2], WANG Li[1,2], ZHANG Kaixin[1,2], LI Dongyang[1,2], JI Jichao[1,2], NIU Lin[1,2], GAO Xueke[1,2*], LUO Junyu[1,2*] and CUI Jinjie[1,2*]

Abstract

Background *Apolygus lucorum* is a worldwide omnivorous pest damaging a range of crops and causing great economic losses. Symbiotic bacteria living in insects play a key role in the nutrition, physiology, and behavior of hosts. Here, we present an experiment using Illumina HiSeq sequencing targeting the V3–V4 regions of bacteria's 16S rRNA throughout the entire life cycle of *A. lucorum*.

Results The first and second instar nymphs have the largest alpha diversity compared with other life stages of the insect. Bacterial phyla Proteobacteria (72.29%), Firmicutes (15.24%), Actinobacteria (7.76%) exhibit the largest relative abundance in all developmental stages. *Erwinia* (23.97%) and *Lactococcus* (10.62%) are the two genera with the highest relative abundance. The relative abundance of *Erwinia* in the nymph stage is significantly greater than the adult stage, and the relative abundance of *Lactococcus* in 6-day-old and 9-day-old adult females is higher compared with adult males.

Conclusions These results reveal that microbial community composition and relative abundance shift dynamically at different life stages, implying that different bacterial phyla and genera may have specific roles in specific life stages such as metabolism, nutrition absorption, detoxification, and reproduction. This study reveals for the first time the community composition and ecological dynamics of symbiotic bacteria throughout the life stages of *A. lucorum*, and thus may provide insight to new strategies for pest control.

Keywords Symbiotic bacteria, *Apolygus lucorum*, Life cycle, Pest control, Community composition, Relative abundance, Community richness

*Correspondence:
Gao Xueke
15036138389@163.com
Luo Junyu
luojunyu1818@126.com
Cui Jinjie
aycuijinjie@163.com
[1] State Key Laboratory of Cotton Biology/Institute of Cotton Research, Chinese Academy of Agricultural Sciences, Anyang 455000, Henan, China
[2] Zhengzhou Research Base, State Key Laboratory of Cotton Biology/Zhengzhou University, Zhengzhou 450001, Henan, China
[3] Hubei Insect Resources Utilization and Sustainable Pest Management Key Laboratory/College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei, China

Background

Insects are the most diverse and abundant animals on Earth in terms of species number and physical biomass (Basset et al. 2012). Almost all insects have microbial communities in their bodies. There are a large number of microbial symbionts in insects, including those obtained from the surrounding environment and food (Coolen et al. 2022; Luo et al. 2021). Bacteria that can establish a confirmed symbiotic relationship with the host are regarded as symbiotic bacteria (Kucuk 2020). Insects and symbiotic bacteria have a reciprocal relationship, and microbial symbioses play an important role in the health, survival, and behavior of the host (Dillon and Dillon 2004; Kikuchi et al. 2007; Moran et al. 2008; Santos-Garcia et al. 2017). Endosymbioses are not only related to the coevolution of plants and herbivores, but also play a profound role in the ecology and evolution of insects (Duron et al. 2008; Kikuchi et al. 2012; Pietri and Dangsheng 2018). Specifically, bacterial symbionts can provide nutrients for the host, facilitate host reproduction, protect the host from natural enemies, defend against pathogens, and promote host detoxification and metabolism to improve drug resistance (Broderick et al. 2006; Ceja-Navarro et al. 2015; Cheng et al. 2017; Genta et al. 2006; Gerardo and Parker 2014; Heyworth and Ferrari 2015; Rolff and Siva-Jothy 2003; Scarborough et al. 2005; Sharon et al. 2010).

Hemipteran insects have a needle-like stylet that can be used for sucking plant sap. Some of them are notorious agricultural pests responsible for serious economic losses, because they may not only suck the plant sap and kill crops directly, but also transmit plant viruses (Liu et al. 2018; Qin et al. 2018; Wang et al. 2015). *Apolygus lucorum* belongs to Miridae (Hemiptera) and is an important worldwide agricultural pest (Tan et al. 2018). *A. lucorum* has a wide range of plant hosts and can harm economic crops such as cotton, vegetables, and fruit trees. Due to the high density of wild population and their rapid growth rate, *A. lucorum* outbreaks erupt and spread geographically extremely easily (Lu et al. 2007). Before the 1990s, *A. lucorum* was historically a minor cotton pest in China (Zhen et al. 2016). However, since the large-scale adoption of transgenic *Bacillus thuringiensis* (Bt) cotton beginning in 1997, *A. lucorum* has gradually become a destructive and pervasive cotton pest of serious economic importance with higher frequent outbreaks (Lu et al. 2010). At present, chemical insecticides such as organophosphorus pesticides and pyrethroids are widely used in China to control *A. lucorum* (Zhen et al. 2016). Furthermore, insecticide resistance could easily arise leaving an urgent and difficult to resolve *A. lucorum* outbreak. Moreover, the omnivorous nature of *A. lucorum* increases the difficulty of pest control (Wu et al. 2010). Studies have shown that endosymbiotic bacteria facilitate insect hosts' ability to easily form new feeding habits, expand food sources, and thus enhance the adaptability of insects to the environment (Douglas 2009; Lü et al. 2001). Similarly, there are a great deal of increasing evidence of a link between symbiotic bacteria in insects and evolution of insecticide resistance (Broderick et al. 2006; Engel and Moran 2013; Kikuchi et al. 2012; Xia et al. 2013). Therefore, it is profoundly necessary to understand the composition and ecological dynamics of symbiotic bacteria in *A. lucorum* for the improvement of pest control.

Although the diversity of insect microbes has been extensively studied, most researchers focused on gut microbes (Hulcr et al. 2012; Roh et al. 2008; Salem et al. 2013), and there are few longitudinal studies on the shifts in microbial communities in insect life cycles (Zhao et al. 2019). Therefore, this study focuses on *A. lucorum*, an important pest in cotton fields, and uses 16S rRNA high-throughput sequencing technology to systematically evaluate the microbial diversity of *A. lucorum* at multiple life stages to reveal changes in symbiotic bacterial communities during the development of *A. lucorum*, and to provide a solid theoretical basis for guiding pest control.

Materials and methods

Insect rearing and maintenance

A. lucorum adults were originally collected from the cotton field in Wuhan, Hubei Province, China in July 2014. They were maintained in climate chambers at (75 ± 5)% relative humidity, (26 ± 2) ℃ temperature, 16 h: 8 h light: dark cycle, and fed with beans (*Phaseolus vulgaris*) and 10% (mass fraction) sucrose solution (Tan et al. 2018).

Sample collection, DNA extraction, and 16S rRNA amplification sequencing

Nymphs of five developmental stages, and male and female adults at 1 day, 6 days and 9 days after eclosion of *A. lucorum* were randomly collected from colonies. Each life stage had six sample groups, and each group included 20 insects. To remove microbial contaminants on the surface of insects, each sample was soaked in 70% (volume fraction) ethanol for 5 min, followed by 10% bleach (mass fraction) for 30 s, and then rinsed with sterile ultrapure water. DNA was extracted from whole insects using MagPure Stool DNA KF Kit B (Magen, Shanghai, China) according to the manufacturer's instructions. DNA was quantified using a Qubit Fluorometer with a Qubit dsDNA BR Assay Kit (Thermofisher, Massachusetts, USA), and the quality was assessed by performing an aliquot on 1% (mass fraction) agarose gel. The variable regions V3–V4 of the bacterial 16S rRNA gene were

amplified with the degenerate polymerase chain reaction (PCR) primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'- GGACTACHVGGGTWTCTAAT-3'). Both forward and reverse primers were tagged with Illumina adapter, pad, and linker sequences. PCR amplification was performed in a 50 μL reaction system containing 30 ng template, fusion PCR primers, and PCR master mix. PCR cycling conditions were as follows: 94 ℃ for 3 min, 30 cycles of 94 ℃ for 30 s, 56 ℃ for 45 s, 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min. PCR products were visualized with electrophoresis in a 1.8% (mass fraction) agarose gel, then the gel was purified with VAHTS DNA Clean Beads (Vazyme Biotech, Nanjing, China), and quantified using the NanoDrop 2000 (Thermo Fisher Scientific, Massachusetts, USA). Validated libraries were sequenced on an Illumina HiSeq platform (BGI, Shenzhen, China) according to standard Illumina procedures and generated 2×300 bp paired-end reads. The sequences obtained in this study were deposited in the GenBank Short-Read Archive (SRA) with accession number PRJNA713416. Sequenced samples were labelled as follows: LL1: 1st instar nymph; LL2: 2nd instar nymph; LL3: 3rd instar nymph; LL4: 4th instar nymph; LL5: 5th instar nymph; LM1D: adult male at 1 day after eclosion; LF1D: adult female at 1 day after eclosion; LM6D: adult male at 6 days after eclosion; LF6D: adult female at 6 days after eclosion; LM9D: adult male at 9 days after eclosion; LF9D: adult female at 9 days after eclosion.

Bioinformatic analysis

Illumina sequencing data was processed with UCHIME (v8.1) and QIIME (v1.9.1) softwares. First, chimeric and low quality sequences were filtered out and removed from downstream analyses. Sequences were spliced using FLASH software (Magoc and Salzberg 2011), paired reads obtained by double-terminal sequencing were assembled into sequences by overlap relationship, and tags of hypervariable regions were obtained. The splicing parameters were as follows: (i) minimum matching length of sequence was 15 bp; and (ii) allowable mismatch rate of overlapping region was 0.1. UPARSE software (Edgar 2013) was then used to cluster sequences at 97% similarity and output the representative sequences of Operational Taxonomic Units (OTU). Chimeric sequences generated by PCR amplification were removed from the representative OTU sequences with UCHIME (v8.1) (Edgar et al. 2011). All tags were compared with representative OTU sequences using usearch_global (Wang et al. 2007). After obtaining representative OTU sequences, taxonomy assignment was performed by comparing OTU representative sequences with the SILVA database using the RDP classifier (v2.2) with a confidence threshold of 0.8.

Differences in community composition were visualized by weighted unifrac and unweighted unifrac Principal Component Analysis (PCA) in R software (v3.1.1). In order to detect the species richness and sequencing depth of each sample, mothur (v.1.31.2) and R (v3.1.1) software were used to calculate coverage, dilution curves, and six alpha diversity indices of community richness: Chao1, ACE, Shannon, Simpson, and Good's coverage. The Kruskal–Wallis test was used to analyze the difference of relative abundance of species in three groups or more, P values < 0.05 were considered statistically significant (*, $0.01 \leq P < 0.05$; **, $0.001 \leq P < 0.01$; ***, $P < 0.001$). In order to best visualize differences in species composition, QIIME (v1.9.1) and R (v3.1.1) softwares were used to cluster the samples by Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to analyze beta diversity. PICRUSt was used to predict bacterial community function based on OTU taxonomy and relative abundance, using on Kegg Orthology (KO).

Results

Sequencing quality analysis

Illumina HiSeq sequencing of 16S rRNA V3–V4 region amplicons from *A. lucorum* yielded a total of 5 336 031 raw reads. Following demultiplexing, quality filtering, and chimera removal, a total of 4 246 604 tags were used in downstream analysis and each of the 66 samples had an average of 64 342 high-quality sequences (Additional file 1: Table S1). High-quality reads were spliced to yield hypervariable region tags, totaling 4 198 796 tags. To each developmental stage of *A. lucorum*, 144 to 505 OTUs were assigned with a similarity cutoff of 97% (Additional file 1: Table S1). Community richness, based on Good's coverage, was 99% in all samples at a 3% dissimilarity cutoff (Additional file 1: Table S1) and rarefaction curves showed saturation, indicating that our sequencing had captured most of the bacterial diversity associated with *A. lucorum* (Additional file 1: Fig. S1). The observed species index dilution curve also tended to flatten, which verified that the depth of sequencing was sufficient to detect all of the bacterial species in the sample (Fig. 1A).

Bacterial community structure among different development stages in *A. lucorum*

Structural composition and classification analysis of microbial samples at different developmental stages of *A. lucorum*, revealed that Proteobacteria was the most abundant phylum. Among all samples, the top three phyla with the highest relative abundance are Proteobacteria (72.29%), Firmicutes (15.24%), and Actinobacteria

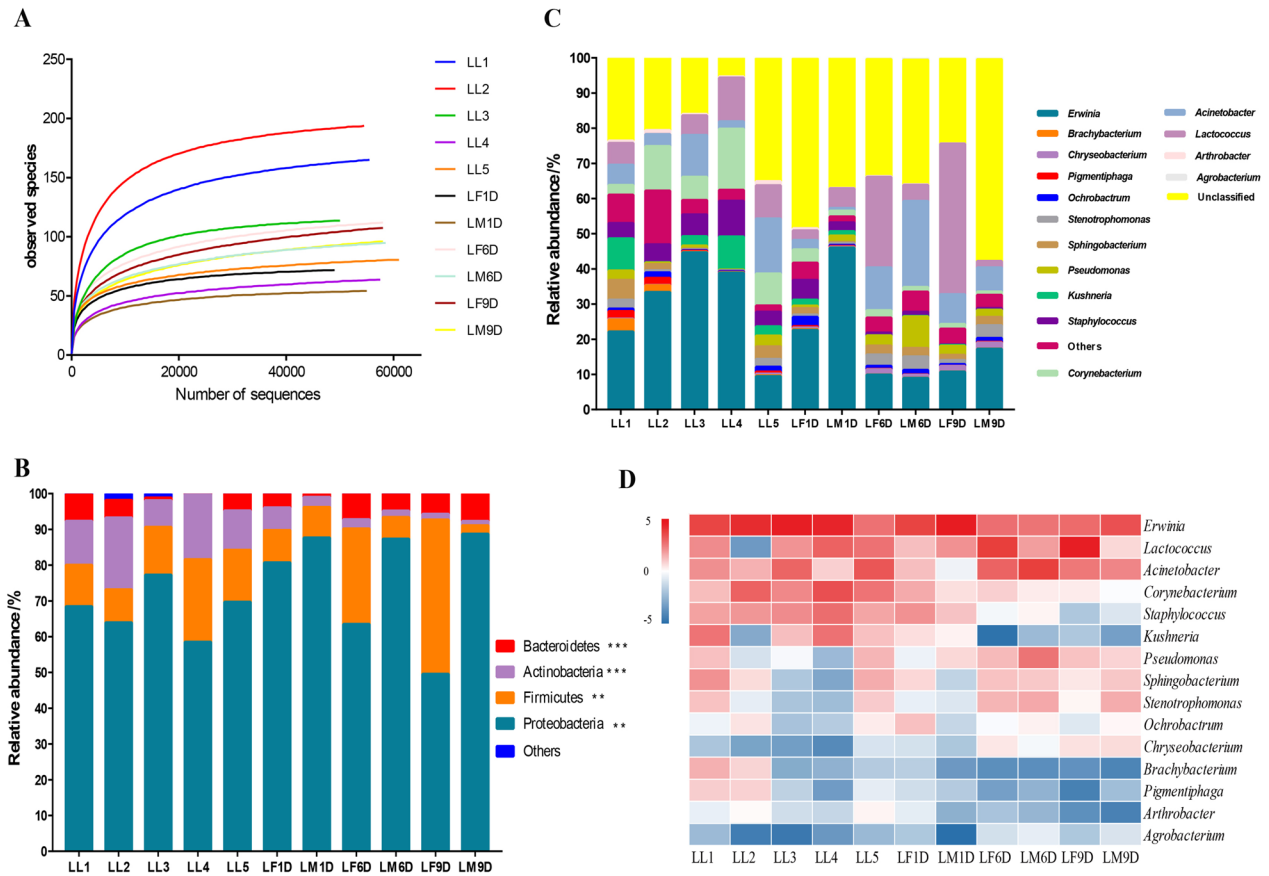

Fig. 1 Bacterial community dynamics among different developmental stages in *Apolygus lucorum*. **A** Observe species dilution curve. **B** Relative abundance of bacteria communities at the phylum level in different groups. **C** Relative abundance of bacteria communities at the genus level in different groups. **D** Heat map analysis of the top 15 microbial populations with relative abundance at different developmental stages, and the data represented by color in the figure are represented by $\log_2$(relative abundance). (Bacteria with relative abundance lower than 0.1% in all samples were all merged into others. The "Kruskal–Wallis test" was used to analyze the differences of phyla at all developmental stages. * $0.01 \leq P < 0.05$, ** $0.001 \leq P < 0.01$, and *** $P < 0.001$)

(7.76%) (Fig. 1B) (average of all samples). Bacterial community composition ratios shifted significantly at different developmental stages. The relative abundances of Proteobacteria in the male adult life stages LM9D (88.70%), LM6D (87.31%), and LM1D (87.65%) were the highest. In addition, the relative abundance of Proteobacteria was the lowest in the female adult life stage LF9D (49.52%), and the relative abundance of Proteobacteria notably reduced from the LF1D (80.70%) period when the female adult was just emerging (Fig. 1B and Additional file 1: Table S2).

In the nymph stages, the relative abundance of Proteobacteria was the highest in the third instar nymph stage (77.27%), and the lowest in the fourth instar nymph stage (58.49%). Interestingly, the trend of Proteobacteria's shifts in relative abundances was inverse to the trend of Firmicutes' shifts in relative abundance in female adult stages. At different stages of female development, the relative abundances of Firmicutes increased from 9.04% to 43.14%. The relative abundances of Actinobacteria during the nymph stages were higher than those of the adult stages (Fig. 1B and Additional file 1: Table S2). Proteobacteria, Firmicutes, and Actinobacteria showed significant differences in relative abundances at different life stages (Additional file 1: Table S2).

Proteobacteria was the dominant phylum during the entire developmental period, and the *Erwinia* genus of Proteobacteria, was the most dominant genus (Fig. 1C, D). We combined stacked histograms with heat maps to demonstrate the trend of community changes at the genus level. *Erwinia* (23.97%), *Lactococcus* (10.62%) and *Acinetobacter* (8.57%) were the three most abundant genera overall. Moreover, the relative abundances of *Erwinia* were higher than those of other genera during the development of LL1 (22.14%), LL2 (33.34%), LL3 (44.65%), LL4 (38.87%), LF1D (22.55%), LM1D (45.96%), and LM9D (17.24%) (Fig. 1C and Additional file 1: Table S3). Overall, the relative abundances of *Erwinia* in the nymph stages

were higher than adult stages. However, it is worth noting that the highest relative abundance of *Erwinia* appeared in newly emerged male adults. After 6 days of adult development, the relative abundances of *Erwinia* in each stage decreased (Fig. 1D and Additional file 1: Table S3). The relative abundances of *Lactococcus* in 6-day-old and 9-day-old females were higher than those in males of the same age. *Lactococcus* was also the dominant genus in the LF6D (25.74%) and LF9D (42.81%). In addition, *Acinetobacter*'s relative abundance in the fifth instar nymph stage was much higher than that in the newly emerged adult (Fig. 1C, D and Additional file 1: Table S3).

Comparative analysis of microbiota diversity indices at different developmental stages

We used four metrics to explore alpha diversity: Chao1, Simpson, ACE, and Shannon indices (Fig. 2A–D and Additional file 1: Table S1). There were considerable differences in the bacterial species diversity and richness of *A. lucorum* microbial communities at different developmental stages. The first and second instar nymphs had the highest bacterial community diversity and richness according to the Chao1, ACE and Shannon indices, and were considerably higher than all other developmental stages, showing extremely high complexity. The low Simpson index value of the first and second instar nymphs also reflected the high diversity of bacterial species in these two developmental stages. The community diversity was the lowest in the LM1D stage. Similarly, the community diversity also showed a low level in LF1D. The OTU totals of the first and second instar nymphs, 498 and 505 respectively, were also the highest compared with other developmental stages. The number of total taxa in the LM1D stage was the lowest (Additional file 1: Table S1).

The OTU flower diagram in Fig. 3A visualizes the common and unique microbial OTU numbers of insect host *A. lucorum* at various developmental stages. There were 46 conserved OTUs at different developmental stages. Similarly, first instar nymphs and second instar nymphs had the most unique OTUs, 184 and 168 respectively, followed by third-instar nymphs with 142 OTUs. Bacterial

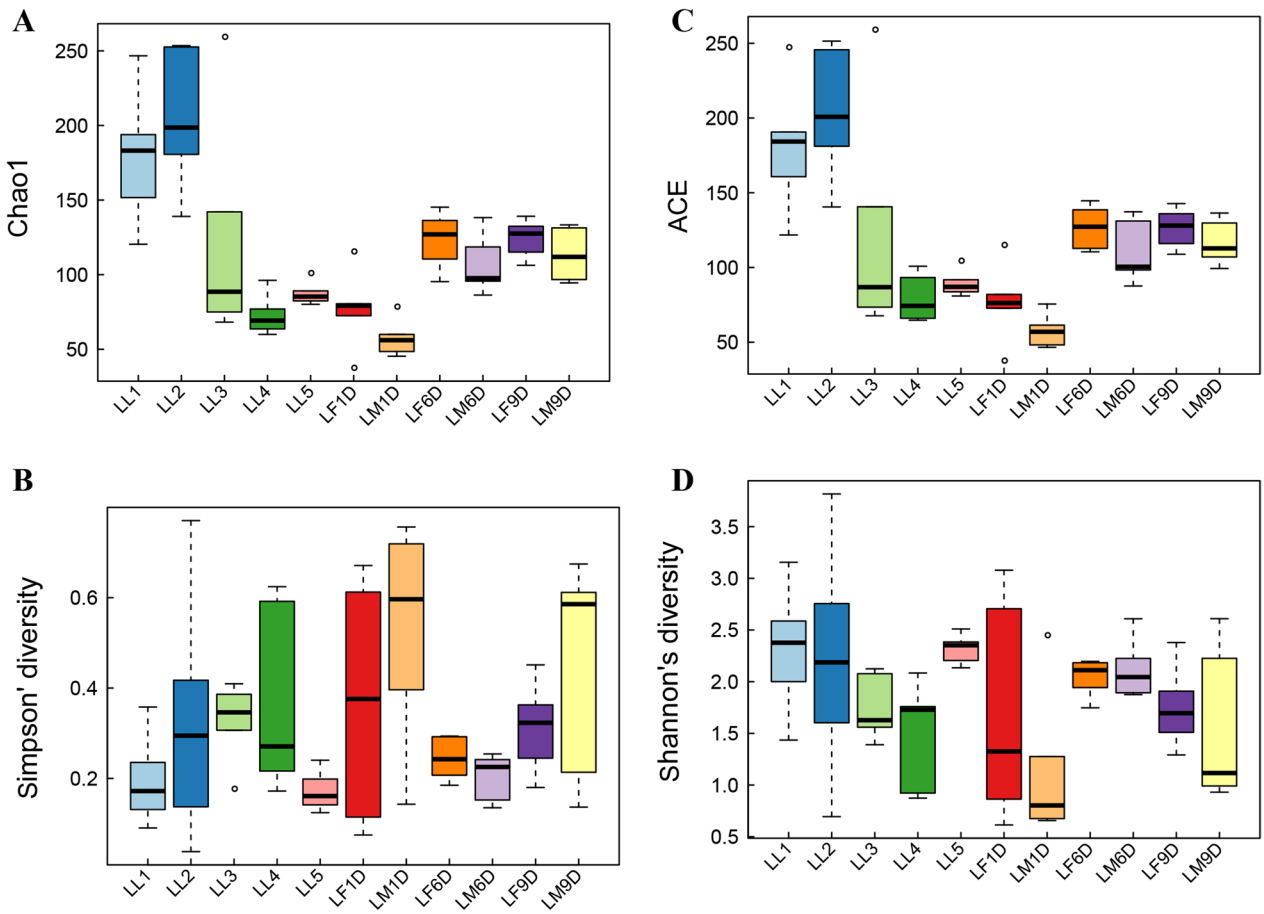

Fig. 2 Sequencing analysis of 16S rRNA gene amplicons of *A. lucorum* with diversity indices. **A** Chao1 index, **B** Simpson' diversity, **C** ACE index, **D** Shannon's diversity. "°" indicates an outlier

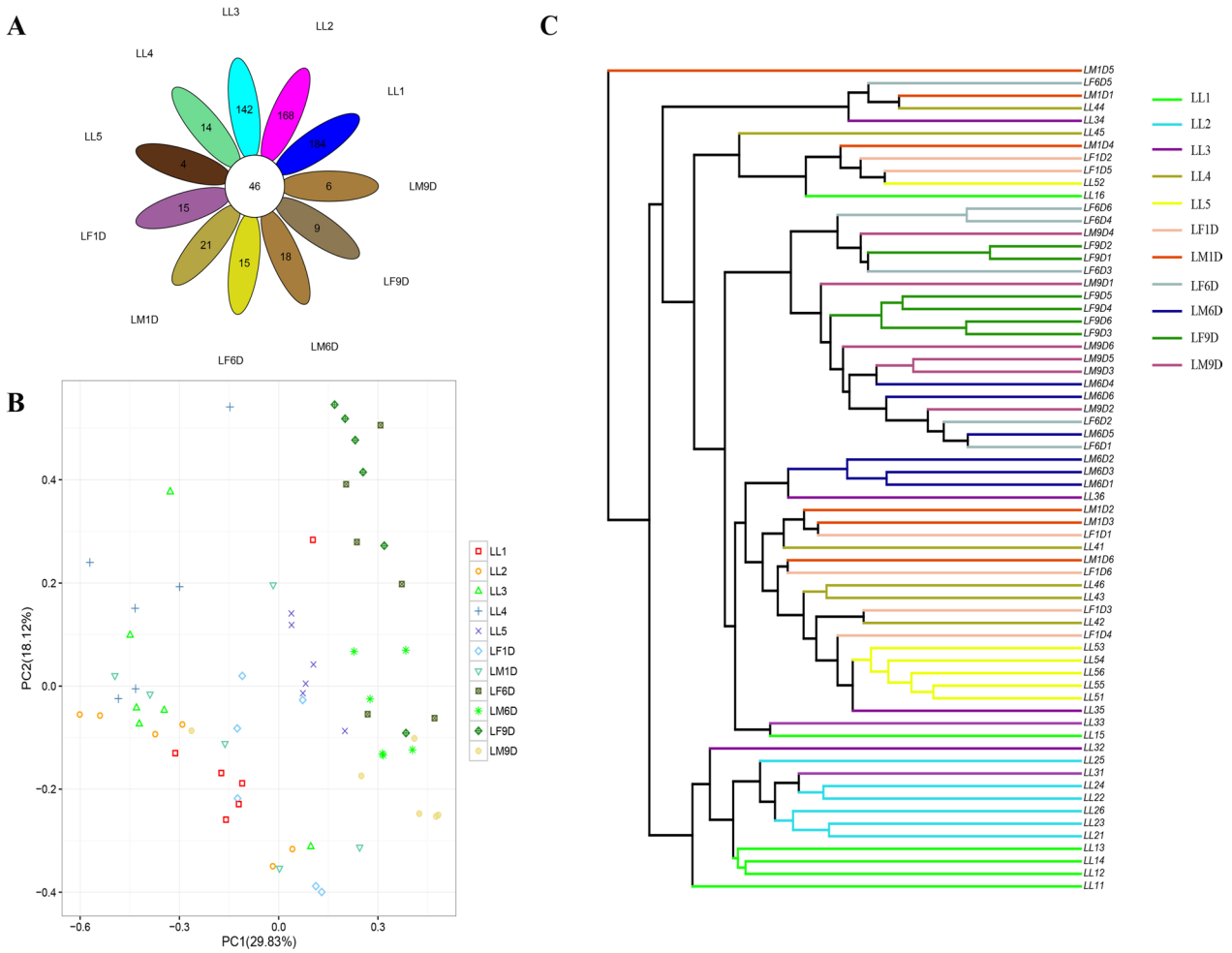

Fig. 3 Bacterial community dynamics among different developmental stages in *A. lucorum*. **A** Flower of OTU to show common and unique OTUs for all samples. **B** Comparison of bacterial community structures in development stages using unweighted uniFrac metrics. **C** Unweighted pair-group method with arithmetic means (UPGMA) analysis of microbial community structure based on 16S rRNA gene amplicon sequencing data

species richness and diversity remained low from fourth instar nymph to adults. Similar results were found in the PCA analysis. The first thru third instar nymphs were clearly distinguished from fourth and fifth instar nymphs and adults (Fig. 3B). UPGMA analysis also verified the above results. The close clustering relationship of first thru third instar nymphs revealed their similarity, and the difference between males and females in different developmental stages of adults was relatively small (Fig. 3C). The results of OTU flower, PCA, and cluster analysis revealed that the bacterial community diversity of the fourth instar nymph and the fifth instar nymph was closer to that of the adult *A. lucorum* microbiota. Both female and male communities were similar in each developmental stage, and the composition of the first thru third instar nymphs community was especially species rich.

Function prediction

Based on the predicted results of KO, the pathway abundance was showed at three levels. In level 1, "Environmental Information Processing" accounted for 16.99% at all developmental stage, followed by "Metabolism" (16.59%) and "Brite Hierarchies" (8.71%) (Fig. 4). In level 2, "Membrane transport" (16.99%) was the pathway with the highest relative abundance. In the metabolic pathways, the relative abundances of "Carbohydrate metabolism" (3.90%), "Amino acid metabolism" (3.65%), and "Nucleotide metabolism" (3.35%) were the three highest pathways. "Transporters" (7.09%) and "ABC transporters" (4.43%) were the two pathways with the highest relative abundance of level 3, followed by "Two-component system" (2.28%) and "Secretion system" (2.19%), both of which belong to "Environmental Information Processing". "Purine metabolism" (1.98%),

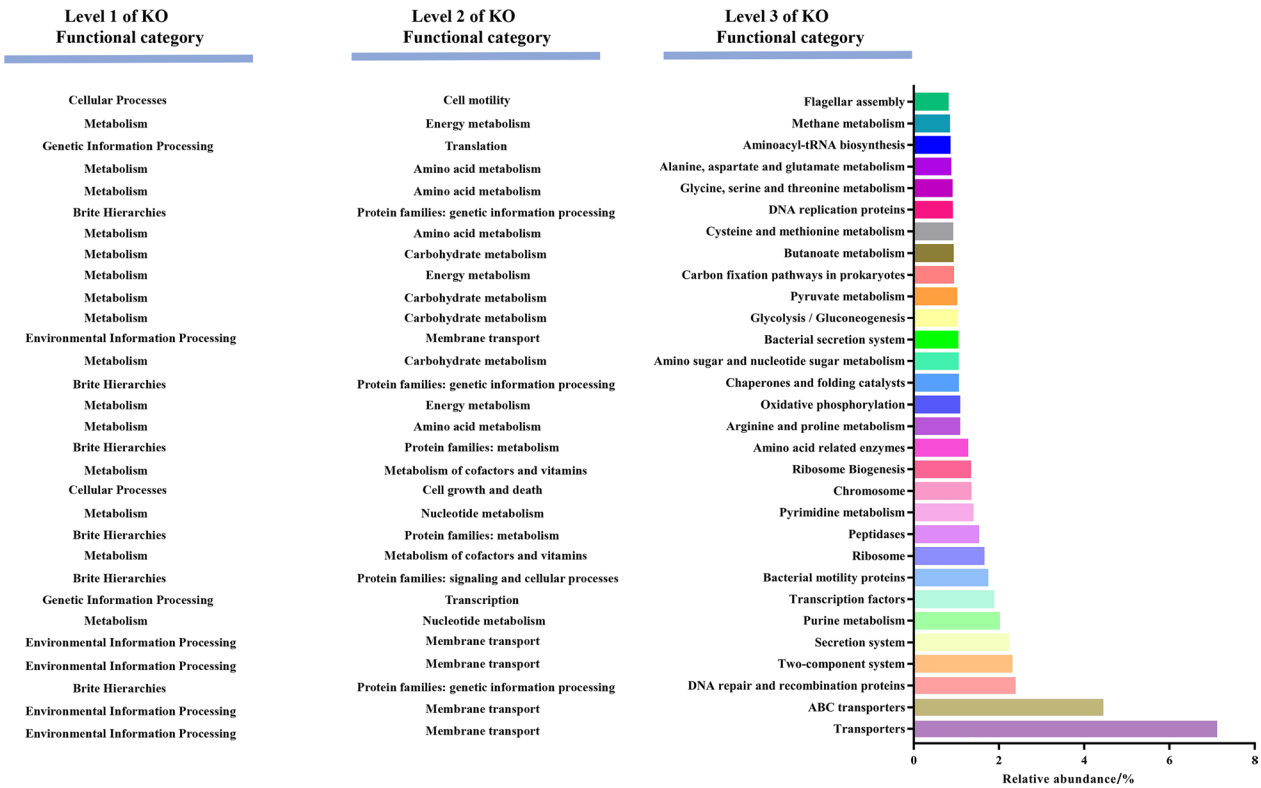

Fig. 4 Relative abundance of the top 30 KEGG Orthology (KO) in all developmental stages of *A. lucorum*

"Ribosome" (1.62%) and "Pyrimidine metabolism" (1.36%) had higher relative abundance in "Metabolism".

Discussion

Although the diversity of microbes associated with insect hosts had been widely studied, most studies were limited to the intestinal tract of insects (Augustinos et al. 2019; González-Serrano et al. 2020; Muratore et al. 2020; Wei et al. 2017; Xia et al. 2018), and there were few longitudinal studies on microbial diversity throughout the life cycle of insects. Here, we present the first research that we know of on microbial composition and ecology during the life cycle of the insect *A. lucorum*. It is well known that the bacterial community in wild-type insects is very different from that raised in the laboratory for a long time (Rani et al. 2009), and different geographical areas will also affect microbial diversity (Zouache et al. 2011). Temperature is also an important factor affecting insect bacterial community (Kikuchi et al. 2016). Although the experimental samples were collected from the field, the bacterial community in *A. lucorum* was relatively stable after countless generations of breeding in the laboratory. In this study, we used the next-generation sequencing technology to comprehensively identify and analyze the microbial communities at different developmental stages of *A. lucorum*. We found that phyla Proteobacteria, Firmicutes, and Actinobacteria dominated at various developmental stages (Fig. 1B), which was similar to microbiota composition of other insect symbiotic communities (Chen et al. 2018; Gao et al. 2020; Mason and Raffa 2014; Zhao et al. 2019). Bacterial community structure and diversity are distinct at different developmental stages of insect host *A. lucorum*. Microorganisms that affect evolutionary and ecological processes such as developmental, physiological, and ecological interactions exist in many insects (Currie et al. 1999; Engel and Moran 2013; Hammer et al. 2017). Although the microbial composition of *A. lucorum* was significantly diverse in the first and second instar nymph stages, the bacterial diversity observed in the later developmental stages was significantly reduced (Fig. 1A). This phenomenon of reduced bacterial diversity has the same evidence in the nymph of *Adelphocoris suturalis*, and the direct influencing factor may be the transformation of a carnivorous diet (Luo et al. 2021). *A. lucorum*'s habit of feeding on eggs or smaller members of the nymph during middevelopment is very similar to that of *A. suturalis*, which may well explain the decrease in microbial diversity (Xue et al. 2021).

A. lucorum is an insect with incomplete metamorphosis. According to PCA and UPGMA analysis, the bacterial community diversity of fourth instar nymphs and fifth instar nymphs was closer to that of adults. During the transition period from nymph to adult, the diversity and structure of microorganisms did not change significantly compared with completely metamorphic insects (Zhao et al. 2019). In general, compared with *Hyalesthes obsoletus* (Iasur-Kruh et al. 2017) and *Myzus persicae* (Xu et al. 2020) of Hemiptera, the microflora of *A. lucorum* showed higher biodiversity. There are great differences in microbiomes of different insects, and the microbial diversity of the same insect species is also affected by many factors, such as in vitro environment (Zouache et al. 2011), artificial feed (Priya et al. 2012) and food sources (Broderick et al. 2004; Priya et al. 2012). Because *A. lucorum* is a highly omnivorous pest, it can feed not only on plants, but also on some small insects or eggs (Li et al. 2016; Lu et al. 2008; Yuan et al. 2013). Complex eating habits may lead to differences in bacterial community diversity and species richness.

Erwinia, which belongs to Gram-negative family Enterobacteriaceae, is both an important intestinal bacterium (Basset et al. 2000) and a symbiote of many arthropods (Estes et al. 2012; Iasur-Kruh et al. 2017). In this study, *Erwinia* populations were found to be continuous throughout the development cycle, indicating that *Erwinia* a persistent symbiont and maybe important in the growth, development, and survival of *A. lucorum*. *Erwinia* can metabolize most nitrogen, sulfur, and phosphorus sources (Friedl et al. 2008), indicating an important role in the digestion and metabolism of insects. Similarly, *Candidatus Erwinia dacicola* may also benefit the larval stage of the fruit fly (Estes et al. 2012). Olives, especially unripe olives, are defended with antimicrobial secondary metabolites (Levinson and Levinson 1984). Fruit fly larvae lacking symbiont *C. Erwinia dacicola* can not survive in unripe olives and few can pupate when olives are ripe (Estes et al. 2012). In this study, the average relative abundance of *Erwinia* in the nymph stage was higher than that in the adult stage, which was consistent with previous studies (Yong et al. 2017). We hypothesize that the nymphs' digestion, metabolism, and drug resistance is weaker than adults, and that *Erwinia* can degrade complete plant tissues and produce antimicrobial agents to help *A. lucorum* larvae grow and develop, thus helping *A. lucorum* have a strong ability to cope with complex environments. Our functional prediction results also give another explanation: most bacterial functions are concentrated in "Environmental Information Processing" and "Metabolism" to meet the adaptability of insects to the environment. Further research might include genome sequencing of *Erwinia* symbionts to explore these metabolic roles. Interestingly, *Erwinia* is also the main pathogenic source of soft rot in fruits, vegetables, and ornamental plants (Grenier et al. 2006). It can secrete a variety of cell wall degrading enzymes, causing potato black leg disease, soft rot, Fusarium wilt, and other plant diseases (Whitehead et al. 2002). In the process of feeding, *A. lucorum* uses its needle tip to pierce plant tissues, inject saliva digestive enzymes, and ingest liquefied plant substances. Polygalacturonase is one of the enzymes that can digest plant tissues and cause damage to plant tissues (Liu et al. 2021). Further research is needed to elucidate if *A. lucorum* spreads the plant pathogen *Erwinia*.

At the same time, we also found that the average relative abundance of *Lactococcus* was higher in female adults than in male adults. Six-day-old female adults we selected were samples obtained based on the preoviposition data of *A. lucorum* (Zhen et al. 2018). *Lactococcus* is abundant in the insect intestinal tract (De Jonge et al. 2020), and can decompose sugars, produce organic acids, reduce the pH value of the environment, and resist some acid-sensitive pathogenic bacteria (Evans and Armstrong 2006). These bacteria are well-known for the ability to ferment complex carbohydrates and produce lactic acid, which can be oxidized to pyruvate and participate in the tricarboxylic acid cycle, and which can also be converted into glucose in the liver, thus playing an important role in the insect nutritional pathway, and the nutritional pathways of some insects are closely related to reproduction (Carpenter et al. 2012; Hansen et al. 2004; Koyama et al. 2013; Lu et al. 2016; Pérez-Hedo et al. 2013; Roy and Raikhel 2011; Sancak et al. 2008). Therefore, we speculate that *Lactococcus* may have an important connection to the reproduction of *A. lucorum*. However, *Wolbachia* and *Rickettsia* that are ubiquitous in several other arthropods and play an important role in insect reproduction (Duguma et al. 2013; Rasgon and Scott 2004), were not detected during multiple developmental stages of *A. lucorum* in this study.

In this study, we provide an understanding of the symbiotic biodiversity and community composition dynamics in different life cycle stages of *A. lucorum*. This knowledge of the *A. lucorum* microbiome may lead to important practical applications in the development of microbially based strategies for the management of insect pests (Crotti et al. 2012). For example, future pest control might be based on targeting essential microbial symbionts to insect host survival and thus eliminating host insect pest. Similarly, this research might inform future pest control which may involve preventing microbe-facilitated insecticide resistance in

host microbe.These results provide an important theoretical basis for the control of *A. lucorum*, including the theoretical relationship between drug resistance and reproduction of microorganisms in *A. lucorum*, and thus provide novel approaches for guiding new pest control strategies.

Conclusion

The bacterial community composition and diversity of *A. lucorum* changed at different developmental stages, and these bacteria have potentially important functions in different developmental stages. The high abundance of *Erwinia* reflects that it plays an important role in the physiology and biochemistry of *A. lucorum*, involving important functions such as metabolism, nutrition absorption and detoxification. *Lactococcus*, the dominant genus in LF6D and LF9D period, may be the key genus in the reproduction of female *A. lucorum*. More research is needed to verify the function of these bacteria. This research reveals for the first time the community composition and ecological dynamics of symbiotic bacteria throughout the life stages of *A. lucorum*, and thus may provide insight to new strategies for pest control.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42397-023-00142-1.

Additional file 1. Table S1. 16S rRNA gene sequencing data. **Table S2**. Relative abundance of bacteria communities at the phylum level in different group. **Table S3**. Relative abundance of bacteria communities at the genus level in different group (Top 15). **Fig. S1**. Alpha diversity dilution curve.

Acknowledgements
Thanks for the support of experimental technology and equipment provided by the Institute of Cotton Research of Chinese Academy of Agricultural Sciences.

Author contributions
Conceptualization, Cui JJ and Gao XK; Methodology, Gao XK; Software, Xue H, and Niu L; Validation, Cui JJ; Formal analysis, Xue H; Investigation, Xue H and Zhu XZ; Resources, Luo JY; Data curation, Xue H, Ji JC, and Gao XK; Writing—original draft preparation, Xue H; Writing—review & editing, Cui JJ and Gao XK; Visualization, Wang L and Li DY; Supervision, Zhang KX; Project Administration, Zhang KX; Funding acquisition, Cui JJ. All authors read and approved the manuscript.

Funding
This research was supported by Agricultural Science and Technology Innovation Program of Chinese Academy of Agricultural Sciences.

Availability of data and materials
All the sequencing data of this experiment have been uploaded to NCBI, the accession number is PRJNA713416.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

Received: 8 October 2022 Accepted: 1 March 2023
Published online: 22 March 2023

References

Augustinos AA, Tsiamis G, Cáceres C, et al. Taxonomy, diet, and developmental stage contribute to the structuring of gut-associated bacterial communities in tephritid pest species. Front Microbiol. 2019;10:2004. https://doi.org/10.3389/fmicb.2019.02004.

Basset A, Khush RS, Braun A, et al. The phytopathogenic bacteria *Erwinia carotovora* infects *Drosophila* and activates an immune response. Proc Natl Acad Sci U S A. 2000;97(7):3376–81. https://doi.org/10.1073/pnas.070357597.

Basset Y, Cizek L, Cuénoud P, et al. Arthropod diversity in a tropical forest. Science. 2012;338(6113):1481–4. https://doi.org/10.1126/science.1226727.

Broderick NA, Raffa KF, Goodman RM, et al. Census of the bacterial community of the gypsy moth larval midgut by using culturing and culture-independent methods. Appl Environ Microbiol. 2004;70(1):293–300. https://doi.org/10.1128/aem.70.1.293-300.2004.

Broderick NA, Raffa KF, Handelsman J. Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. Proc Natl Acad Sci U S A. 2006;103(41):15196–9. https://doi.org/10.1073/pnas.0604865103.

Carpenter VK, Drake LL, Aguirre SE, et al. SLC7 amino acid transporters of the yellow fever mosquito *Aedes aegypti* and their role in fat body TOR signaling and reproduction. J Insect Physiol. 2012;58(4):513–22. https://doi.org/10.1016/j.jinsphys.2012.01.005.

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

Chen B, Du K, Sun C, et al. Gut bacterial and fungal communities of the domesticated silkworm (*Bombyx mori*) and wild mulberry-feeding relatives. ISME J. 2018;12(9):2252–62. https://doi.org/10.1038/s41396-018-0174-1.

Cheng D, Guo Z, Riegler M, et al. Gut symbiont enhances insecticide resistance in a significant pest, the oriental fruit fly *Bactrocera dorsalis* (Hendel). Microbiome. 2017;5(1):13. https://doi.org/10.1186/s40168-017-0236-z.

Coolen S, Magda RD, Welte CU. The secret life of insect-associated microbes and how they shape insect-plant interactions. FEMS Microbiol Ecol. 2022. https://doi.org/10.1093/femsec/fiac083.

Crotti E, Balloi A, Hamdi C, et al. Microbial symbionts: a resource for the management of insect-related problems. Microb Biotechnol. 2012;5(3):307–17. https://doi.org/10.1111/j.1751-7915.2011.00312.x.

Currie CR, Scott JA, Summerbell RC, et al. Fungus-growing ants use antibiotic-producing bacteria to control garden parasites. Nature. 1999;398:701–4.

De Jonge N, Michaelsen TY, Ejbye-Ernst R, et al. Housefly (*Musca domestica* L.) associated microbiota across different life stages. Sci Rep. 2020;10(1):7842. https://doi.org/10.1038/s41598-020-64704-y.

Dillon RJ, Dillon VM. The gut bacteria of insects: nonpathogenic interactions. Annu Rev Entomol. 2004;49:71–92. https://doi.org/10.1146/annurev.ento.49.061802.123416.

Douglas AE. The microbial dimension in insect nutritional ecology. Funct Ecol. 2009;23(23):38–47.

Duguma D, Rugman-Jones P, Kaufman MG, et al. Bacterial communities associated with *culex* mosquito larvae and two emergent aquatic plants of

bioremediation importance. PLoS ONE. 2013;8(8):e72522. https://doi.org/10.1371/journal.pone.0072522.
Duron O, Bouchon D, Boutin S, et al. The diversity of reproductive parasites among arthropods: *Wolbachia* do not walk alone. BMC Biol. 2008;6:27. https://doi.org/10.1186/1741-7007-6-27.
Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods. 2013;10(10):996–8. https://doi.org/10.1038/nmeth.2604.
Edgar RC, Haas BJ, Clemente JC, et al. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194–200. https://doi.org/10.1093/bioinformatics/btr381.
Engel P, Moran NA. The gut microbiota of insects - diversity in structure and function. FEMS Microbiol Rev. 2013;37(5):699–735. https://doi.org/10.1111/1574-6976.12025.
Estes AM, Hearn DJ, Burrack HJ, et al. Prevalence of *Candidatus* Erwinia dacicola in wild and laboratory olive fruit fly populations and across developmental stages. Environ Entomol. 2012;41(2):265–74. https://doi.org/10.1603/en11245.
Evans JD, Armstrong TN. Antagonistic interactions between honey bee bacterial symbionts and implications for disease. BMC Ecol. 2006;6(1):4.
Friedl MA, Kubicek CP, Druzhinina IS. Carbon source dependence and photostimulation of conidiation in *Hypocrea atroviridis*. Appl Environ Microbiol. 2008;74(1):245–50. https://doi.org/10.1128/aem.02068-07.
Gao X, Li W, Luo J, et al. DNA sequencing reveals bacterial communities in midgut and other parts of the larvae of *Spodoptera exigua* Hubner (Lepidoptera: Noctuidae). FEMS Microbiol Lett. 2020;367(4):fnaa002. https://doi.org/10.1093/femsle/fnaa002.
Genta FA, Dillon RJ, Terra WR, et al. Potential role for gut microbiota in cell wall digestion and glucoside detoxification in *Tenebrio molitor* larvae. J Insect Physiol. 2006;52(6):593–601. https://doi.org/10.1016/j.jinsphys.2006.02.007.
Gerardo NM, Parker BJ. Mechanisms of symbiont-conferred protection against natural enemies: an ecological and evolutionary framework. Curr Opin Insect Sci. 2014;4:8–14. https://doi.org/10.1016/j.cois.2014.08.002.
González-Serrano F, Pérez-Cobas AE, Rosas T, et al. The gut microbiota composition of the Moth *Brithys crini* reflects insect metamorphosis. Microb Ecol. 2020;79(4):960–70. https://doi.org/10.1007/s00248-019-01460-1.
Grenier AM, Duport G, Pagès S, et al. The phytopathogen *Dickeya dadantii* (*Erwinia chrysanthemi* 3937) is a pathogen of the pea aphid. Appl Environ Microbiol. 2006;72(3):1956–65. https://doi.org/10.1128/aem.72.3.1956-1965.2006.
Hammer TJ, Janzen DH, Hallwachs W, et al. Caterpillars lack a resident gut microbiome. Proc Natl Acad Sci USA. 2017;114(36):9641–6. https://doi.org/10.1073/pnas.1707186114.
Hansen IA, Attardo GM, Park JH, et al. Target of rapamycin-mediated amino acid signaling in mosquito anautogeny. Proc Natl Acad Sci U S A. 2004;101(29):10626–31. https://doi.org/10.1073/pnas.0403460101.
Heyworth ER, Ferrari J. A facultative endosymbiont in aphids can provide diverse ecological benefits. J Evol Biol. 2015;28(10):1753–60. https://doi.org/10.1111/jeb.12705.
Hulcr J, Rountree NR, Diamond SE, et al. Mycangia of ambrosia beetles host communities of bacteria. Microb Ecol. 2012;64(3):784–93. https://doi.org/10.1007/s00248-012-0055-5.
Iasur-Kruh L, Naor V, Zahavi T, et al. Bacterial associates of *Hyalesthes obsoletus* (Hemiptera: Cixiidae), the insect vector of bois noir disease, with a focus on cultivable bacteria. Res Microbiol. 2017;168(1):94–101. https://doi.org/10.1016/j.resmic.2016.08.005.
Kikuchi Y, Hayatsu M, Hosokawa T, et al. Symbiont-mediated insecticide resistance. Proc Natl Acad Sci U S A. 2012;109(22):8618–22. https://doi.org/10.1073/pnas.1200231109.
Kikuchi Y, Hosokawa T, Fukatsu T. Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. Appl Environ Microbiol. 2007;73(13):4308–16. https://doi.org/10.1128/aem.00067-07.
Kikuchi Y, Tada A, Musolin DL, et al. Collapse of insect gut symbiosis under simulated climate change. Mbio. 2016. https://doi.org/10.1128/mBio.01578-16.
Koyama T, Mendes CC, Mirth CK. Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. Front Physiol. 2013;4:263. https://doi.org/10.3389/fphys.2013.00263.
Kucuk RA. Gut bacteria in the holometabola: a review of obligate and facultative symbionts. J Insect Sci. 2020. https://doi.org/10.1093/jisesa/ieaa084.
Levinson HZ, Levinson AR. Botanical and chemical aspects of the olive tree with regards to fruit acceptance by *Dacus oleae* (Gmelin) and other frugivorous animals. Zeitschrift Für Angew Entomol. 1984;98:136–49.
Li W, Wyckhuys KAG, Wu K. Does feeding behavior of a zoophytophagous mirid differ between host plant and insect prey items? Arthropod-Plant Interact. 2016;10(1):79–86.
Liu W, Hajano JU, Wang X. New insights on the transmission mechanism of tenuiviruses by their vector insects. Curr Opin Virol. 2018;33:13–7. https://doi.org/10.1016/j.coviro.2018.07.004.
Liu Y, Liu H, Wang H, et al. *Apolygus lucorum* genome provides insights into omnivorousness and mesophyll feeding. Mol Ecol Resour. 2021;21(1):287–300. https://doi.org/10.1111/1755-0998.13253.
Lu K, Chen X, Liu WT, et al. TOR pathway-mediated juvenile hormone synthesis regulates nutrient-dependent female reproduction in *Nilaparvata lugens* (Stål). Int J Mol Sci. 2016;17(4):438. https://doi.org/10.3390/ijms17040438.
Lu Y, Qiu F, Feng H, et al. Species composition and seasonal abundance of pestiferous plant bugs (Hemiptera : Miridae) on Bt Cotton in China. Crop Prot. 2008;27(3):465-47.2.
Lu Y, Wu K, Jiang Y, et al. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. Science. 2010;328(5982):1151–4. https://doi.org/10.1126/science.1187881.
Lu YH, Liang GM, Wu KM. Advances in integrated management of cotton mirids. Plant Prot. 2007;33(33):10–5.
Lü ZX, Yu XP, Chen JM, et al. Role of endosymbiote in virulence change of the brown planthopper to rice resistant varieties. Acta Entomol Sin. 2001, 44(2): 197-204. https://doi.org/10.16380/j.kcxb.2001.02.012.
Luo J, Cheng Y, Guo L, et al. Variation of gut microbiota caused by an imbalance diet is detrimental to bugs' survival. Sci Total Environ. 2021;771:144880. https://doi.org/10.1016/j.scitotenv.2020.144880.
Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957–63. https://doi.org/10.1093/bioinformatics/btr507.
Mason CJ, Raffa KF. Acquisition and structuring of midgut bacterial communities in gypsy moth (Lepidoptera: Erebidae) larvae. Environ Entomol. 2014;43(3):595–604. https://doi.org/10.1603/en14031.
Moran NA, McCutcheon JP, Nakabachi A. Genomics and evolution of heritable bacterial symbionts. Annu Rev Genet. 2008;42:165–90. https://doi.org/10.1146/annurev.genet.41.110306.130119.
Muratore M, Sun Y, Prather C. Environmental nutrients alter bacterial and fungal gut microbiomes in the common meadow katydid, *Orchelimum vulgare*. Front Microbiol. 2020;11:557980. https://doi.org/10.3389/fmicb.2020.557980.
Pérez-Hedo M, Rivera-Perez C, Noriega FG. The insulin/TOR signal transduction pathway is involved in the nutritional regulation of juvenile hormone synthesis in *Aedes aegypti*. Insect Biochem Mol Biol. 2013;43(6):495–500. https://doi.org/10.1016/j.ibmb.2013.03.008.
Pietri JE, Dangsheng L. The links between insect symbionts and insecticide resistance: causal relationships and physiological tradeoffs. Ann Entomol Soc Am. 2018;3(3):92–7.
Priya NG, Ojha A, Kajla MK, et al. Host plant induced variation in gut bacteria of *Helicoverpa armigera*. PLoS ONE. 2012;7(1):e30768. https://doi.org/10.1371/journal.pone.0030768.
Qin F, Liu W, Wu N, et al. Invasion of midgut epithelial cells by a persistently transmitted virus is mediated by sugar transporter 6 in its insect vector. PLoS Pathog. 2018;14(7):e1007201. https://doi.org/10.1371/journal.ppat.1007201.
Rani A, Sharma A, Rajagopal R, et al. Bacterial diversity analysis of larvae and adult midgut microflora using culture-dependent and culture-independent methods in lab-reared and field-collected *Anopheles stephensi*-an Asian malarial vector. BMC Microbiol. 2009;9:96. https://doi.org/10.1186/1471-2180-9-96.
Rasgon JL, Scott TW. An initial survey for *Wolbachia* (*Rickettsiales: Rickettsiaceae*) infections in selected California mosquitoes (Diptera: Culicidae). J Med Entomol. 2004;41(2):255–7. https://doi.org/10.1603/0022-2585-41.2.255.
Roh SW, Nam YD, Chang HW, et al. Phylogenetic characterization of two novel commensal bacteria involved with innate immune homeostasis

in *Drosophila melanogaster*. Appl Environ Microbiol. 2008;74(20):6171–7. https://doi.org/10.1128/aem.00301-08.
Rolff J, Siva-Jothy MT. Invertebrate ecological immunology. Science. 2003;301(5632):472–5. https://doi.org/10.1126/science.1080623.
Roy SG, Raikhel AS. The small GTPase Rheb is a key component linking amino acid signaling and TOR in the nutritional pathway that controls mosquito egg development. Insect Biochem Mol Biol. 2011;41(1):62–9. https://doi.org/10.1016/j.ibmb.2010.10.001.
Salem H, Kreutzer E, Sudakaran S, et al. Actinobacteria as essential symbionts in firebugs and cotton stainers (Hemiptera, Pyrrhocoridae). Environ Microbiol. 2013;15(7):1956–68. https://doi.org/10.1111/1462-2920.12001.
Sancak Y, Peterson TR, Shaul YD, et al. The Rag GTPases bind raptor and mediate amino acid signaling to mTORC1. Science. 2008;320(5882):1496–501. https://doi.org/10.1126/science.1157535.
Santos-Garcia D, Silva FJ, Morin S, et al. The all-rounder sodalis: a new bacteriome-associated endosymbiont of the Lygaeoid bug *Henestaris halophilus* (Heteroptera: Henestarinae) and a critical examination of its evolution. Genome Biol Evol. 2017;9(10):2893–910. https://doi.org/10.1093/gbe/evx202.
Scarborough CL, Ferrari J, Godfray HC. Aphid protected from pathogen by endosymbiont. Science. 2005;310(5755):1781. https://doi.org/10.1126/science.1120180.
Sharon G, Segal D, Ringo JM, et al. Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proc Natl Acad Sci U S A. 2010;107(46):20051–6. https://doi.org/10.1073/pnas.1009906107.
Tan YA, Zhao XD, Sun Y, et al. The nuclear hormone receptor E75A regulates vitellogenin gene (AI-Vg) expression in the mirid bug *Apolygus lucorum*. Insect Mol Biol. 2018;27(2):188–97. https://doi.org/10.1111/imb.12365.
Wang H, Wu K, Liu Y, et al. Integrative proteomics to understand the transmission mechanism of Barley yellow dwarf virus-GPV by its insect vector *Rhopalosiphum padi*. Sci Rep. 2015;5:10971. https://doi.org/10.1038/srep10971.
Wang Q, Garrity GM, Tiedje JM, et al. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261–7. https://doi.org/10.1128/aem.00062-07.
Wei G, Lai Y, Wang G, et al. Insect pathogenic fungus interacts with the gut microbiota to accelerate mosquito mortality. Proc Natl Acad Sci U S A. 2017;114(23):5994–9. https://doi.org/10.1073/pnas.1703546114.
Whitehead NA, Byers JT, Commander P, et al. The regulation of virulence in phytopathogenic *Erwinia* species: quorum sensing, antibiotics and ecological considerations. Antonie Van Leeuwenhoek. 2002;81(1/2/3/4):223–31. https://doi.org/10.1023/a:1020570802717.
Wu D, Lin FM, Lu Y, et al. Selective preferences of *Apolygus lucorum* and *Adelphocoris suturalis* (Hemiptera:Miridae) to cotton plants with different resistance levels and damaging treatments. Acta Entomol Sin. 2010;53(6):696–701.
Xia X, Sun B, Gurr GM, et al. Gut microbiota mediate insecticide resistance in the diamondback moth, *Plutella xylostella* (L.). Front Microbiol. 2018;9:25. https://doi.org/10.3389/fmicb.2018.00025.
Xia X, Zheng D, Zhong H, et al. DNA sequencing reveals the midgut microbiota of diamondback moth, *Plutella xylostella* (L.) and a possible relationship with insecticide resistance. PLoS ONE. 2013;8(7):e68852. https://doi.org/10.1371/journal.pone.0068852.
Xu S, Jiang L, Qiao G, et al. Diversity of bacterial symbionts associated with *Myzus persicae* (Sulzer) (Hemiptera: Aphididae: Aphidinae) revealed by 16S rRNA Illumina sequencing. Microb Ecol. 2020;81(3):784–94. https://doi.org/10.1007/s00248-020-01622-6.
Xue H, Zhu X, Wang L, et al. Gut bacterial diversity in different life cycle stages of *Adelphocoris suturalis* (Hemiptera: Miridae). Front Microbiol. 2021;12:670383. https://doi.org/10.3389/fmicb.2021.670383.
Yong HS, Song SL, Chua KO, et al. High diversity of bacterial communities in developmental stages of *Bactrocera carambolae* (Insecta: Tephritidae) revealed by Illumina MiSeq sequencing of 16S rRNA gene. Curr Microbiol. 2017;74(9):1076–82. https://doi.org/10.1007/s00284-017-1287-x.
Yuan W, Li W, Li Y, et al. Combination of plant and insect eggs as food sources facilitates ovarian development in an omnivorous bug *Apolygus lucorum* (Hemiptera: Miridae). J Econ Entomol. 2013;106(3):1200–8. https://doi.org/10.1603/ec13016.
Zhao C, Zhao H, Zhang S, et al. The developmental stage symbionts of the pea aphid-feeding *Chrysoperla sinica* (Tjeder). Front Microbiol. 2019;10:2454. https://doi.org/10.3389/fmicb.2019.02454.
Zhen C, Miao L, Gao X. Sublethal effects of sulfoxaflor on biological characteristics and vitellogenin gene (*AlVg*) expression in the mirid bug, *Apolygus lucorum* (Meyer-Dür). Pestic Biochem Physiol. 2018;144:57–63. https://doi.org/10.1016/j.pestbp.2017.11.008.
Zhen C, Miao L, Liang P, et al. Survey of organophosphate resistance and an Ala216Ser substitution of acetylcholinesterase-1 gene associated with chlorpyrifos resistance in *Apolygus lucorum* (Meyer-Dür) collected from the transgenic Bt cotton fields in China. Pestic Biochem Physiol. 2016;132:29–37. https://doi.org/10.1016/j.pestbp.2016.04.008.
08">Zouache K, Raharimalala FN, Raquin V, et al. Bacterial diversity of field-caught mosquitoes, *Aedes albopictus* and *Aedes aegypti*, from different geographic regions of Madagascar. FEMS Microbiol Ecol. 2011;75(3):377–89. https://doi.org/10.1111/j.1574-6941.2010.01012.x.