SCIENTIFIC NOTE

THE MICROBIOTA OF THREE ANOPHELES SPECIES IN CHINA

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ABSTRACT. Microbiota are vital for the development, physiology, and vectorial capacity of mosquitoes. The composition and role of microbiota in Anopheles species, especially Anopheles gambiæ and Anopheles stephensi, have been extensively studied, but little is known about the microbiota of Anopheles species in China. We characterized the microbial communities of Anopheles dirus, Anopheles sinensis, and Anopheles lesteri by 16S rRNA sequencing. There were distinct differences in the composition of microbiota in An. lesteri and the other 2 species. The discriminatory genera in the 3 species were analyzed by the linear discriminant analysis effect size method. Our results provide an overview of the population structure of microbiota in 3 native Anopheles species and will pave the way for further understanding of their role in mosquito physiology and vector competence.

KEY WORDS 16S rRNA sequencing, Anopheles, diversity, microbiota

A total of 46 genera and 418 species of mosquitoes (Culicidae) have been reported from China (Fu and Chen 2015), and some of the species are important vectors of human pathogens. In China, malaria is transmitted by Anopheles lesteri Biaisas and Hu, Anopheles sinensis Wiedemann, Anopheles minimus Theobald, and Anopheles dirus Peyton and Harrison (Yin et al. 2014). Anopheles sinensis is the most widespread species, and it is found in 29 provinces (Zhang et al. 2017). Anopheles lesteri, known for its anthropophilic behavior and ability to transmit Plasmodium falciparum (Welch), is the primary malaria vector in central China. Anopheles minimus and An. dirus are mainly found in the southern Chinese provinces of Yunnan and Hainan (Zhu et al. 2013).

Microbiota are involved in regulating development, physiology, and vectorial capacity of mosquitoes. The absence of microbiota can cause delayed development and asynchrony of larval growth (Chouaia et al. 2012). Recolonization of gut microbiota in these larvae eliminates the growth defects (Coon et al. 2014), whereas removing the microbiota of adult mosquitoes reduces the expression of immune-related genes leading to increased susceptibility to Plasmodium and dengue virus infection (Xi et al. 2008, Dong et al. 2009). Due to the vital role of microbiota in the vector competency of mosquitoes and the potential use of paratransgenesis in vector control, many studies have focused on the composition and diversity of mosquito microbiota. High-throughput sequencing has been used to study the population structure of microbiota in different populations of Anopheles species in Africa and southern Asia (Ngo et al. 2016, Berhanu et al. 2019). Many factors affect the diversity of mosquito microbiota including mosquito species and habitat (Muturi et al. 2016, Duguma et al. 2017).

To study the microbiota of the three Anopheles species in China, we obtained An. lesteri and An. sinensis from the Jiangsu Institute of Parasitic Diseases (JIPD), Jiangsu, China) and An. dirus from the National Institute of Parasitic Diseases (NIPD), Shanghai, China). Anopheles lesteri and An. sinensis, maintained at JIPD, were originally field collected from Xuyi County, Jiangsu Province, in the 1980s. Anopheles dirus maintained at NIPD was field collected from Baisha County, Hainan Province, in the 1980s. Total DNA was extracted from surface sterilized, individual 5-day old mosquitoes, using a DNeasy Blood and Tissue Kit (69504, Qiagen). The 16S rRNA sequencing of DNA samples was performed by Novagen (Beijing, China). Specific primers (341F, 806R) were used for the V3–V4 region of 16S rRNA gene amplification. Total effective sequences were clustered into operational taxonomic units (OTUs) with 97% identities, and taxonomic information was annotated using the GreenGene database for each representative sequence. All raw data were archived on NCBI under PRJNA561915.

Based on OTU clusters and annotation, the relative abundance of microbiota was analyzed, and the top 10 OTUs were determined at the phylum and genus levels. The relative abundance of phyla varied among...
the mosquito species (Fig. 1A). Proteobacteria was the dominant bacterial phylum in An. dirus and An. sinensis, contributing to 76.04% and 84.66% of the total OTUs, respectively. The second most common phylum was Firmicutes (16.67%) and An. sinensis (8.69%) (Fig. 1A). In contrast, Proteobacteria was the most abundant phylum in An. lesteri and represented 38.52% of total OTUs, followed by Proteobacteria (30.03%) and Actinobacteria (29.27%) (Fig. 1A). At the genus level, Asaia was the most abundant genus in An. dirus and An. sinensis, but it was only accounted for at a very low level (0.03% relative abundance) in An. lesteri (Fig. 1B). Bifidobacterium predominated in An. lesteri, while it occurred at an extremely low level in An. dirus and An. sinensis (Fig. 1B). Pseudomonas was the second most abundant genus in An. dirus (4.4%), but its relative abundance was less than 0.6% in An. sinensis and An. lesteri (Fig. 1B). The levels of Faecalibacterium and Escherichia-Shigella in An. lesteri were higher than those in An. dirus and An. sinensis (Fig. 1B).

We evaluated the association between mosquito species and microbiota by β-diversity analysis (Segata et al. 2011, Costa et al. 2012). There was a clear separation of microbial composition across the 3 mosquito species (Fig. 2A). A slight difference was observed between An. dirus and An. sinensis. We also identified featured bacteria in each Anopheles species by the linear discriminant analysis (LDA) effect size method (Fig. 2B). Four OTUs, Bifidobacterium, Faecalibacterium, Escherichia-Shigella, and Blautia, were significantly enriched in An. lesteri. An enrichment of Pseudomonas and Ruminococaceae_UCG-005 in An. dirus and Asaia in An. sinensis was also observed (Fig. 2B). These discriminatory genera might contribute to the microbiota divergence across the different species.

These results indicate that the microbiota composition of An. lesteri was different from An. sinensis, although the 2 species were maintained in the same insectary. In contrast, An. dirus and An. sinensis shared most common OTUs, although they were maintained in different environments. The difference of bacterial composition between An. lesteri and the other 2 species suggests that host genetic factors might be more important than environmental factors in influencing the composition of mosquito microbiota.

We found that Bifidobacterium was prevalent in An. lesteri. Previous studies found that Bifidobacterium in the human gut was associated with protection against P. falciparum infection. Also, Asaia is considered to be a leading candidate for use in controlling mosquito-borne diseases (Capone et al. 2013). We showed the dominance of Asaia in An. dirus and An. sinensis, suggesting that it might influence the susceptibility of these mosquitoes to Plasmodium infection. We also found that the bacterial genus Serratia is ubiquitous in the 3
Anopheles species studied. Serratia isolated from field-collected *An. sinensis* in China has been reported to activate the mosquito immune system and increases mosquito resistance to *Plasmodium berghei* Vincke and Lips (Bai et al. 2019). Multiple microbiota species are present in *Anopheles* mosquitoes, but their function(s) are still unknown. Further studies are needed to understand the role of these bacteria in *Anopheles* species.

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