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## Mosquito microbiome :implications for mosquito-borne diseases control

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### Abstract

Mosquitoes are home to a large number of microorganisms inside and outside their bodies, forming a diverse and dynamic microbiome. The mosquito microbiome plays an important role in many physiological functions such as nutrition, metabolism, and immunity of mosquitoes. It is a key component of mosquitoes. An indispensable component of growth and development. At the same time, mosquito microbiota significantly affects the infection and transmission of mosquito-borne pathogens, and has important application value in mosquito disease prevention and control. Therefore, the key role of the microbiome cannot be ignored in a comprehensive understanding of mosquito physiology and mosquito-borne disease transmission. This article systematically summarizes the research progress on the formation and influencing factors of mosquito microbiota, the influence and interaction mechanism of microbiota on host physiological functions, and the blocking and control of mosquito-borne diseases by mosquito microbiota. It also discusses the use of microorganisms. The application prospect of preventing and controlling mosquito-borne infectious diseases is put forward.

**Keywords** :microbiota, mosquito-microbe interaction, mosquito-borne infectious diseases, commensal bacteria blocking strategy

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### Introduction

Mosquitoes are the most “deadly” animals in the world. According to a report by the World Health Organization (WHO), mosquitoes serve as vectors to spread parasitic diseases such as malaria and filariasis, as well as dengue fever, yellow fever, Zika, Japanese encephalitis and other viral diseases. Among them, mosquito-borne malaria is the most serious parasitic infectious disease. According to the latest WHO report, about half of the world's population will be at risk of malaria infection in 2020, about 241 million people. A total of 627,000 people have died from malaria (WHO, 2021). Dengue fever spread by Aedes mosquitoes is the most serious viral infectious disease. According to WHO statistics, dengue fever is currently endemic in more than

100 countries and regions around the world, with more than 2 billion people living in areas where dengue fever is endemic, more than 100 million people suffer from dengue fever every year, but the vast majority of dengue fever patients have mild and asymptomatic symptoms (Torresi et al., 2017).

There are currently no highly effective vaccines or specific drugs for most mosquito-borne infectious diseases. Currently, mosquito-borne control mainly relies on chemical insecticides, which has led to an increasingly serious problem of mosquito resistance (WHO, 2021). In addition, mosquitoes are an important part of the natural food chain. It is unrealistic and harmful to the ecological environment to completely eliminate mosquitoes. In recent years, the symbiotic control strategy based on the development of mosquito microbiota is considered to be a new and effective way to block the spread of mosquito-borne diseases (Gao et al., 2020). This strategy blocks the transmission of pathogens from mosquitoes to humans by inhibiting the development and infection of pathogens in mosquitoes (Gao et al., 2020). Therefore, it is important to analyze the impact and role of mosquito microbiota on mosquito physiology and the spread of mosquito-borne pathogenic infections. mechanism, which is crucial for future development and utilization of this strategy.

A wide variety of microorganisms live inside and outside the body of mosquitoes. In the ever-changing environment, the mosquito host is the microbiota, which refers to the collection of all microorganisms that are mutualistic, symbiotic or parasitic living in and on the body of mosquitoes, including bacteria, Fungi, protists, viruses) provide a good habitat, making the microbiome diverse and specific (Guégan et al., 2018). At the same time, the microbiome (microbiome) refers to all the microorganisms living inside and outside the mosquito's body and their genetic information and interactions relationship) plays an important role in various physiological functions such as mosquito nutrition, metabolism, and immunity, such as maintaining mosquito intestinal homeostasis, promoting the digestion of intestinal nutrients, coordinating body metabolism, secreting metabolites with important functions, and conducting immune responses. signals, defense against colonization and invasion of pathogenic microorganisms (Guégan et al., 2018). It is worth noting that these processes mentioned above significantly affect the effectiveness of mosquitoes in transmitting mosquito-borne diseases (Gao et al., 2020). Therefore, the microbiota is a very important "organ" of mosquitoes ", the mosquito is not an isolated entity and should be considered as a complex with close interaction with the microbiota (Guégan et al., 2018).

In view of the important functions of mosquito microbiota and its application value in blocking the transmission of mosquito-borne pathogens, mosquito microbiota has become a research hotspot at home and abroad. By studying the role of mosquito microbiota in mosquito physiological functions, especially on the impact of mosquito nutritional metabolism, immune regulation, environmental adaptation, mosquito pathogen infection and transmission, etc. will help reveal the symbiotic interaction and game confrontation mechanism between microbiota, mosquitoes and mosquito-borne pathogens, and provide information for the development of green prevention and control technologies for mosquitoes. New strategies. Therefore, this article will

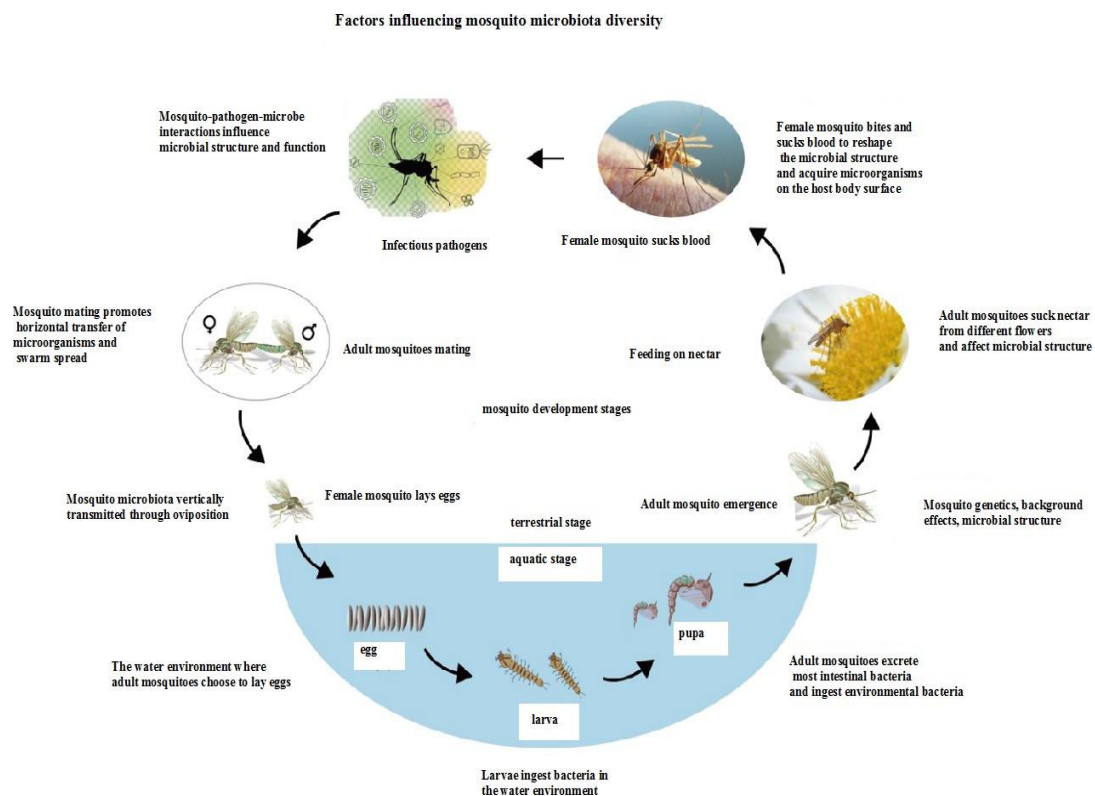
summarize the research progress of mosquito microbiota, focusing on the diversity, biological functions of mosquito microbiota and its application prospects in the prevention and control of mosquito-borne infectious diseases, and provide insights into the future. The research puts forward prospects.

## 1. Composition of mosquito microbiota and its influencing factors

Mosquitoes belong to the complete metamorphosis of insects, and their life cycle is divided into aquatic stages (eggs, larvae, pupae) and terrestrial stages (adults). At different developmental stages of mosquitoes, the composition and structure of their microbiota are affected by a variety of biological or non-organisms. Under the influence of biological factors, dynamic changes occur (Figure 1). Due to differences in the physical and chemical properties of mosquito body surfaces, intestines, gonads, salivary glands and other tissues and organs, the distribution of microorganisms in different tissues and organs is also different (Figure 1).

**Figure 1**

*Factors of influencing mosquito microflora diversity*



### 1.1 Diversity of mosquito microbiota at different growth stages

Mosquitoes grow from eggs to larvae, undergo four molts, and finally emerge into adult mosquitoes. After each molt, some types of microorganisms are eliminated. Studies have shown that whether it is *Anopheles gambiae* or *E. aegypti* Mosquitoes (*Aedes aegypti*), when mosquitoes emerge from pupae, more than 90% of the

microorganisms in the midgut are eliminated. This may be related to two factors. One is that adult mosquitoes ingest molting fluid containing antimicrobial hydrolytic enzymes. The second is that the intestinal contents are excreted in the form of pupal feces after emergence of mosquitoes (Moll et al., 2001). Taking *Anopheles gambiae* as an example, the microbial diversity of its larvae and breeding sites is significantly higher than that of adult mosquitoes. Some of them originate from water bodies, they are distributed in the intestines of mosquitoes at all stages, such as *Pseudomonas*, *Acinetobacter* and *Bacillus* (Cansado-Utrilla et al., 2021). Compared with larval mosquitoes, the living environment of adult mosquitoes has changed significantly. The proportion of Proteobacteria in the intestinal bacteria of adult mosquitoes has increased, while the proportion of Firmicutes has decreased. Although cyanobacteria exist in larvae, they are not present in adult mosquitoes (Vázquez-Martínez et al., 2002).

### ***1.2 Microbial distribution in different organs of mosquitoes***

In addition to the midgut, microorganisms also colonize other organs or tissues of mosquitoes. For example, the common intestinal bacteria *Asai*, *Serratia*, and *Pseudomonas* also colonize the salivary glands and/or reproductive organs of *Anopheles* and *Aedes* mosquitoes. For mosquitoes, the reproductive organs and salivary glands are important organs for mosquito reproduction and pathogen transmission. The bacterial flora diversity of the reproductive organs of *Aedes aegypti* and *Aedes albopictus* is higher than that of the tract and salivary glands (Scolari et al., 2019),  $\alpha$ -Proteobacteria are the main bacterial flora in the reproductive organs of *Aedes* mosquitoes, and the endosymbiotic bacterium *Wolbachia* is the main microorganism in the ovary of *Aedes albopictus* (Mancini et al., 2018).  $\gamma$ -Proteobacteria are the main bacterial species in the salivary glands of *Aedes* mosquitoes. Common microorganisms mainly include *Serratia*, *Eshilbacter*, *Shigella*, *Pantoea agglomerans*, *Acetobacter*, and *Sphingomonas* (Scolari et al., 2019). Studies have found that the bacterial diversity in the salivary glands of *A. culicifacies* is higher than that in the midgut, and *Chlorobi* are specifically distributed (Minard et al., 2013). Some studies have pointed out that the ovaries of *Anopheles* primordial mosquitoes *Comomonas*, *Acinetobacter* and *Pseudomonas* mainly inhabit the salivary glands, while intestinal bacteria include *Comomonas*, *Serratia*, *Pseudomonas*, *Burkholderia* and *Brevundimonas* (Tchioffo et al., 2016). The composition of microbial flora in different organs has certain selectivity. For example, the genera *Bacillus*, *Staphylococcus* and *Pseudomonas* are widely distributed in the reproductive organs, which may be related to mosquito ovary development and egg laying (Seenivasagan & Vijayaraghavan, 2010).

### 1.3 Factors affecting the composition of mosquito microbiota

Mosquito eggs, larvae and pupae all live in aquatic environments and mainly feed on bacteria, protozoa and plankton. Therefore, the breeding ground is an external factor that affects the microbial composition of larval mosquitoes. Light, temperature, pH, Residual antibiotics and dissolved organic or inorganic salts are important factors in shaping the diversity of mosquito microorganisms (Scolari et al., 2019). Different types of mosquitoes have selective preferences for breeding sites. Anopheles prefers clear water bodies with sunlight, while A. Mosquitoes and Culex mosquitoes prefer turbid water bodies rich in organic matter (Scolari et al., 2019). Oxygen molecules, hydrolytic enzymes, and pH in the intestinal environment will affect the reproduction of microorganisms, but not all microorganisms ingested from the environment can survive in the mosquito intestines (Gimonneau et al., 2014). Therefore, the composition of mosquito microorganisms will also be affected by the interaction between microorganisms. For example, *Serratia marcescens* in *Aedes aegypti* can inhibit *Sphingomonas* and *Burkholderia* (*Burkholderia*) bacterial colonization (Terenius et al., 2012).

Differences in feeding habits are an important factor affecting the composition of mosquito microorganisms. Male adult mosquitoes feed on plant juices and nectar, while female adult mosquitoes not only suck plant juices and nectar, but also suck the blood of vertebrates in order to lay eggs. Therefore, mosquitoes feed on Differences in ingested food will cause changes in the composition of the mosquito microbiota. Some studies have pointed out that when *Aedes aegypti* sucks sugar water, bacteria of the genus *Serratia* or *Asahi* in the diverticula degrade the sugar through metabolism and acidify the diverticula, which can be harmful to certain species. Microbial communities are screened (Minard et al., 2013). At the same time, intestinal bacteria such as *Acetobacter*, *Gluconacetobacter*, and *Asaibacter* are mainly derived from fruits and flowers of plants. Among them, *Asahibacterium* is found in the intestines of *Anopheles* and *Aedes* mosquitoes. Colonization in the tract is closely related to mosquitoes feeding on sugary foods (Crotti et al., 2010). *Aerococcaceae* is only found in adult mosquitoes sucking sugar water (Yadav et al., 2016). In addition, in *A. stephensis*, *Chryseobacterium*, *Pseudomonas* and *Serratia* bacteria mainly appear in the midgut of female adult mosquitoes (Rani et al., 2009). The intestinal bacteria of male adult mosquitoes are mainly composed of Firmicutes, including Grapevine cocci, *Bacillus*, *Paenibacillus* and *Micrococcus* (Rani et al., 2009).

In addition to environmental factors, the composition of microorganisms in mosquitoes with different genetic backgrounds also shows differences. Studies have shown that transgenic *Anopheles gambiae* mosquitoes with enhanced immune activity in the midgut tissue have reduced microbial counts in the midgut and reproductive organs (Pike et al., 2017). Some mosquitoes also contain intracellular symbiotic bacteria, such as *Wolbachia* (Gao et al., 2020). *Wolbachia* can be transmitted vertically when infecting mosquito germ cells. In addition, many *Wolbachia* strains induce Cytoplasmic incompatibility (CI) promotes its spread within mosquito populations. Currently, in wild mosquito populations, *Wolbachia* has been found in *Culex pipiens*, *Aedes albopictus* and *Anopheles gambiae* body (Wilke &Marrelli,

2015). It is present in multiple tissues of mosquitoes, including reproductive organs, salivary glands, head, muscles and Malpighian ducts (Jupatanakul et al., 2014).

## **2. Main functions of mosquito intestinal microbiota**

Mosquitoes and intestinal microorganisms have formed complex and diverse interactions through long-term evolution. The mosquito host shapes the diversity of the microbiome, which in turn profoundly affects the physiological functions of mosquitoes and the infection and spread of pathogens.

### ***2.1 Intestinal microorganisms affect mosquito growth, development and nutritional metabolism***

After long-term co-evolution, mosquitoes and microorganisms have formed an interactive relationship of interdependence and mutual restriction. Microorganisms play an important role in maintaining normal growth, development and nutritional metabolism of mosquitoes. Research has found that mosquito larvae cannot survive successfully in a sterile environment unless introducing live endogenous bacteria. Live bacteria or eukaryotes are necessary microorganisms for the development of *Aedes aegypti* larvae into adult mosquitoes, which may be related to the microorganisms inducing hypoxic signals in the mosquito intestines. Studies have shown the generation of a hypoxic environment in the midgut depends on riboflavin from bacterial sources (Coon et al., 2017). Riboflavin also affects the respiratory metabolism of mosquitoes through flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN) (Wang et al., 2021). In addition, during larval development, bacteria may promote the growth and development of larval mosquitoes by promoting the production and synthesis of folic acid or increasing energy storage (Romoli et al., 2021).

However, some microorganisms can adversely affect the growth of mosquitoes. Khampang et al. isolated *E. amnigenus* from the larval intestine of *Anopheles dirus*. This bacterium can colonize the larval intestine and pass through. High expression of cryIVB toxin causes the death of mosquito larvae. *Serratia marcescens* NMCC46 poisons the larvae of *Anopheles stephensi* and *Aedes aegypti* by producing the toxic metabolite prodigiosin (Patil et al., 2011). In addition, Tong Liangqin and others discovered a strain of pigmented bacteria (*Chromobacterium*) that can cause death of *Aedes aegypti* mosquitoes. Within two days of feeding the bacteria, the mortality rate of *Aedes aegypti* reached 80%. The chromobacterium mainly secretes unknown proteins to destroy the mosquito midgut barrier and affects its passage. Permeability promotes a large number of intestinal microorganisms to enter the mosquito hemolymph and accelerates the death of mosquitoes (Tong et al., 2021).

Female mosquitoes obtain the nutrients necessary for reproduction by sucking the blood of vertebrates, and intestinal bacteria play an important role in helping female mosquitoes digest blood. After sucking blood, the intestinal bacteria of female mosquitoes multiply exponentially, accelerating the digestion of blood meals. , helps

female mosquitoes absorb nutrients in the blood, and the blood digestion speed of female *Aedes aegypti* mosquitoes is significantly reduced after antibiotic treatment (Gaio et al., 2011). In *Anopheles gambiae*, *Elizabethkingia an-ophelis* not only secrete hemolytic enzyme to promote the digestion of red blood cells and resist oxidative stress related to blood digestion (Kukutla et al., 2014). In *Culex pipiens*, *Bacillus*, *Streptococcus*, *Staphylococcus*, *Serratia*, and both *Helicella* and *Acinetobacter* can promote the digestion and absorption of blood meals by mosquitoes, thereby increasing mosquito egg production and fecundity (Fouda et al., 2001). In *Aedes aegypti*, the symbiotic bacteria *Serratia* and *Enterobacter* spp. Bacteria promote the catabolism of red blood cells and proteins by secreting hemolytic enzymes, thereby promoting oviposition by female mosquitoes (Gaio et al., 2011). In the intestine of *Aedes albopictus*, *Acinetobacter baumannii* (*Acinetobacter baumannii*) and *Acinetobacter johnsonii* (*Acinetobacter johnsonii*) helps mosquitoes digest (Minard et al., 2013)

## ***2.2 Intestinal microorganisms affect the immune function of mosquitoes***

The mosquito intestine is both a digestive organ and an "immune organ", which plays an important role in fighting pathogenic infections. Research shows that *Anopheles* mosquitoes use reactive oxygen species and antimicrobial peptides to maintain the homeostasis of the intestinal flora (Kumar et al., 2010). At the same time, intestinal bacteria The swarm has an important impact on the immune system and resistance to pathogenic infections of *Anopheles* mosquitoes. The two regulate and restrict each other to ensure the normal physiological functions of mosquitoes. Studies have shown that *Anopheles* mosquitoes can upregulate the expression of cecropin in the presence of symbiotic bacteria), defensin, and gambicin and other antimicrobial peptide genes, which implies that the presence of intestinal bacteria helps *Anopheles* mosquitoes resist the invasion of pathogenic microorganisms (Cirimotich et al., 2011). In addition, the endosymbiotic bacterium *Wolbachia* Help mosquitoes resist viral infection by inducing an increase in the production of reactive oxygen species (Brennan et al., 2008). *Wolbachia* can also activate the Toll pathway by stimulating the production of reactive oxygen species in *Aedes aegypti*, thereby helping mosquitoes inhibit dengue virus infection (Pan et al., 2012). In addition, *Wolbachia* also helps *Aedes* mosquitoes resist infection by chikungunya virus and Zika virus (Aliota et al., 2016).

## **3 Research and application of mosquito microorganisms in the prevention and control of mosquito-borne diseases**

### ***3.1 Using intestinal microorganisms to block the spread of mosquito-borne diseases***

The midgut of *Anopheles* mosquitoes is the site of sexual reproduction of *Plasmodium*, so it is a key battlefield for killing *Plasmodium*. The development of synthetic biology and the research of microorganisms have integrated a new vector disease prevention and control strategy—Paratrans-gensis, that is By genetically modifying insect symbiotic bacteria, they can express effector molecules that can inhibit pathogens in the host, helping the host to kill pathogens, thereby blocking the infection and spread of insect-borne pathogens (Gao et al., 2020). At first, researchers

used ordinary *Escherichia coli*, as a chassis bacterium, uses genetic engineering to express anti-*Plasmodium* effector molecules such as single-chain immunotoxin proteins or small peptides to prevent the development of *Plasmodium* in the midgut of *Anopheles* mosquitoes, and has achieved a certain inhibitory effect (Yoshida et al., 2001). However, *E. coli* is not a symbiotic bacterium of mosquitoes and cannot stably colonize the midgut of *Anopheles* mosquitoes. In addition, the use of a single effector molecule can easily cause the development of resistance in *Plasmodium*. In order to solve these problems, Wang et al. (2012) *Pantoea agglomerans*, a common symbiotic bacterium in mosquitoes, serves as a chassis symbiotic bacterium. We designed a strategy to simultaneously secrete five antimalarial effector molecules with different antimalarial mechanisms and targets, achieving the goal of targeting *Anopheles* mosquitoes. Strong inhibitory effect of up to 98% on intestinal *Plasmodium*. However, constitutive expression of antimalarial effectors by commensal bacteria may lead to a reduction in the fitness of commensal bacteria in the intestine and thus affect the antimalarial effect. Recently, Shane et al.(2018) By genetically modifying the *Anopheles* symbiotic bacterium *Bacillus Asai* to drive the expression of the anti-malarial effector molecule scorpine in the blood-inducible promoter, the results show that the genetically engineered bacteria can more effectively colonize the intestines of *Anopheles* mosquitoes. And improve the inhibitory effect against *Plasmodium*.

How to use intestinal symbiotic bacteria to drive the effective diffusion of antimalarial effector molecules into mosquito populations has been a "pain point" in this field. In 2017, Wang et al. (2012) isolated a new strain of *Serratia* from the ovaries of *Anopheles* mosquitoes. AS1. Studies have found that AS1 can be transmitted both horizontally through mosquito mating and vertically through eggs (Wang et al., 2017). Studies have shown that only 5% of AS1-colonized *Anopheles* mosquitoes need to be introduced into the mosquito population, and AS1 will be transmitted within one generation. It can be spread to the entire mosquito population and passed on from generation to generation in the mosquito population. In addition, synthetic biology technology is used to transform AS1 into a "bacterial warrior" with multiple anti-malarial abilities, which can significantly inhibit malaria parasites in the intestines of *Anopheles* mosquitoes. Effectively control the infection and spread of *Plasmodium* from the source (Wang et al., 2017).

Gao et al. (2021) reported that *Serratia urealyticum* Su\_YN1, an intestinal symbiotic bacterium with both natural antimalarial and rapid dispersal capabilities, was isolated from the *Anopheles sinensis* mosquito (*A. sinensis*) in the wild in my country. The study found that Su\_YN1 It directly targets and lyses *Plasmodium* by secreting antimalarial active lipase AmLip, which has strong natural antimalarial activity. In addition, Su\_YN1 has both horizontal and vertical dispersal capabilities in mosquito populations (Gao et al., 2021). These characteristics mean that Su\_YN1 has the ability to spread in epidemic areas It has the potential to spread rapidly in wild *Anopheles* mosquito populations and block the spread of multiple *Plasmodium* infections (Gao et al., 2021). This natural anti-malarial symbiotic bacteria provides a new green prevention and control weapon for curbing malaria epidemics from the source, and will help promote the use of symbiotic bacterial resistance Prevention and control strategies to stop the spread of malaria move towards practical application.



Compared with traditional methods, commensal bacteria blocking strategies have many advantages. (i) Intestinal commensal bacteria stably colonize the mosquito intestines and have the ability to continue to be transmitted across generations, overcoming the adverse effects of reproductive isolation between mosquito species; (ii) Intestinal symbiotic bacteria do not affect various physiological functions of mosquitoes such as blood-sucking, reproduction and lifespan, and there is no "fitness cost" to the host; (iii) Compared with transgenic mosquitoes, the intestinal symbiotic bacteria are genetically modified. It is simple and easy to implement; (iv) In vitro production of intestinal symbiotic bacteria is low-cost and easy to apply in less developed countries; (v) This strategy is well compatible with current mosquito vector control methods and comprehensive mosquito management plans.

### 3.2. *Using entomophilic fungi to control vector mosquitoes*

Different from insecticidal microorganisms such as bacteria and viruses that need to infect insects through the digestive tract, entomophilic fungi infect mosquitoes through invasion through the body wall. They have unique advantages in the prevention and control of adult mosquitoes with sucking mouthparts and can also effectively kill sensitive mosquitoes and resistant mosquitoes can significantly reduce the spread of mosquito-borne diseases. Therefore, it has huge advantages in mosquito biological control and blocking the spread of diseases. It is considered to be one of the most promising next-generation microbial mosquitoicides (Wang et al., 2017). *Beauveria bassiana* and *Metarhizium anisopliae* are two types of entomophilic fungi widely used in biological pest control. They can infect and kill a variety of mosquitoes under suitable conditions (Bukhari et al., 2011). They are environmentally friendly and Advantages of long-term persistence and continuous control of pests. Lovett et al. (2019) reported in *Science* in 2019 that by genetically modifying *M. pingshaense* to express an insecticidal toxin (hybrid), the fungus can be significantly enhanced. The mosquito-killing virulence was tested in a semi-field environment in Burkina Faso, West Africa. Studies have shown that the mosquito population was reduced by more than 99% after 45 days of application of genetically modified fungi (Lovett et al., 2019). In addition, it can also be combined with The aratransgenesis strategy constructs transgenic entomophilous fungi that express antimalarial effector molecules, which can infect mosquitoes and simultaneously kill malaria parasites in the mosquito's hemocoel. Fang et al. (2011) genetically engineered *Metarhizium anisopliae* to express SM1 (- A synthetic small peptide that prevents *Plasmodium* zygotes from invading the midgut of *Anopheles* mosquitoes) and a scorpion toxin protein are two anti-malarial effector molecules. The results show that the transgenic fungus significantly reduces the number of *Plasmodium* sporozoites in mosquitoes after infecting them.

RNA silencing (RNA silencing or RNA interference, RNAi) is a conserved mechanism for regulating gene expression in eukaryotes. Studies have shown that small RNA (sRNA) is transmitted bidirectionally between host and pathogenic bacteria and plays a role in regulating gene expression in recipient cells. During the infection process, pathogenic bacteria release sRNA (miR-NA) effectors similar to

microRNA (miRNA) and transport them into host cells, using the host RNAi system to interfere with immune pathways and inhibit host immune genes. At the same time, the host also The delivery of conserved miRNAs to interacting pathogen cells inhibits the expression of pathogenic genes and affects pathogenicity. Recent studies on different plant and animal hosts and their interacting pathogens have revealed the role of extracellular vesicles (EVs) as Vector for sRNA exchange in cross-species or cross-kingdom RNAi. Cui et al. (2019) found that *Beauveria bassiana* upregulates the expression of a micro-RNA-like RNA (bba-milR1) during infection of mosquitoes, through vesicles Transport transports it into mosquito cells and silences mosquito antifungal immune genes through the cross-kingdom RNAi mechanism, thereby promoting a new mechanism of fungal infection. Similarly, mosquitoes can also transfer their own conserved miRNAs (Let-7 and miR-100) are delivered into invading fungal cells to silence fungal virulence genes to resist fungal infection (Wang et al., 2021). Lai et al. (2020) studied that *Metarhizium robertsii* (Metarhizium robertsii) mediated through histone modification The KMT2-Cre1-Hyd4 signaling pathway regulates the fungal infection structure-the development and differentiation of appressoria and thereby promotes fungal infection. Interestingly, insecticidal fungi can also manipulate intestinal bacteria to coordinately kill mosquitoes by suppressing intestinal immunity (Wei et al., 2017). The above epigenetic research not only provides new mechanisms for the interaction between entomophilic fungi and host insects, but also provides new targets and transformation strategies for the genetic improvement of efficient mosquito-killing fungi.

#### 4. Future prospects

So far, some progress has been made in research on the diversity and functions of mosquito microbiota. Relevant studies have shown that mosquito microbiota plays an important role in affecting various physiological functions such as mosquito nutrition, immunity, metabolism, and pathogen infection and transmission. Since most symbiotic microorganisms are not easy to culture in vitro, and the study of mosquito microbiota diversity lacks unified research methods, analysis strategies and evaluation systems, these have greatly affected the discovery and research of mosquito-borne microorganisms. In addition, typical Mosquito intestinal microorganisms contain dozens of species of microorganisms, and each intestinal microorganism may contain multiple subspecies and strains with different biological functions. This complex bacterial community structure requires an in-depth understanding of intestinal microorganisms. Therefore, in the future, there is an urgent need to conduct more research on the in vitro isolation and culture of mosquito intestinal microorganisms and the analysis of the structure and energy of intestinal flora based on bioinformatics, which will help to further explore the structural diversity and specific characteristics of mosquito microbiota. Functional mechanism.

Current research progress has opened a door for us. However, in view of the complexity and diversity of mosquitoes and their living habits, there is currently no understanding of the interactions within the mosquito microbiota, the mosquito microbiota and the host or pathogen, and the mosquito The functions of the microbiota are still poorly understood. For example, what is the molecular basis for

mosquitoes to distinguish between commensal and pathogenic bacteria? What mechanisms do mosquitoes use to maintain commensal bacteria and resist pathogenic bacteria? Stable colonization of intestinal commensal bacteria is closely related to the intestinal What are the molecular mechanisms of intestinal immune homeostasis regulation? These are important scientific questions in the field. Although research on intestinal microorganisms in mammals and model animals *Drosophila melanogaster* is in full swing, based on the inherent systematic differences in different biological systems and mosquitoes The research results of model organisms cannot be applied to the characteristics of blood-sucking, and some effective analysis strategies and research methods will also be "acclimated" to mosquitoes.

In the future, it is urgent to establish a set of scientific and efficient methodology and biological research methods to provide more effective means for research in the field of mosquito intestinal microorganisms. Strengthening the research on mosquito microbiota will not only develop the understanding of mosquito intestinal flora Cognition is also conducive to mining valuable microbial and genetic resources for the control of vector diseases. Vector disease prevention and control strategies based on symbiotic microorganisms currently shine in the laboratory stage, but this strategy cannot be used in semi-field or wild environments. The effect is still unknown. In addition, there are still some challenges that need to be addressed before practical application. For example, how to release symbiotic microorganisms into wild populations more effectively? How efficient is the diffusion of symbiotic microorganisms in wild mosquito populations? How effective are symbiotic microorganisms in the wild? How effective is it in blocking vector diseases? Assessment of the ecological safety of symbiotic microorganisms in the wild environment, etc., are the focus of future research.

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