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The Mosquito Microbiota Interplay in Immune Signalling, Vector competence, and Control Prospects

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ABSTRACT

The area of the mosquito's microbiome has been attracting growing attention in the past decade. However, the direct relationship of collective microbiome-induced immune activation or inhibition on vector competence has not yet been explored in depth. The microbial diversity inside the mosquito host is determined by a variety of factors such as the blood meal, which also contributes to the anti-pathogen immune response inside the mosquito host. The interplay between host microbiota and the immune system actively influences the vector competence and consequently the transmission of pathogens by the arthropod vector. Symbiont bacteria and their key role in mosquito's biological processes are therefore promising candidates to be used in the control of vector-borne disease. Paratransgenesis describes the genetic modification of bacteria to produce effector molecules that can attenuate vector competence after being re-introduced inside the mosquito host. In the current review, we provide an overview of the interaction between microbiota, the immune signalling, and implications of such interplay on the control of mosquito-borne diseases.

INTRODUCTION

Mosquitoes are the most potent arthropod vectors, being responsible for transmitting the infection with the highest worldwide annual fatality rate, namely malaria (Dyer, 2020). The geographic spread of various mosquito vectors, driven by international trade, urbanization, and climate change, has increased concern about vector-borne diseases in the last decade (de La Rocque *et al.*, 2011; Caminade *et al.*, 2019). Insecticides are the mainstay for controlling vector-borne infections due to the lack of effective vaccinations against vector-borne pathogens. Insecticide-based vector control, on the other hand, has evolved resistance in the natural population (Moyes *et al.*,2017).

To limit vector-borne disease transmission, a significant amount of effort is needed to find a viable alternative to the widespread use of insecticides. This would necessitate obtaining data on several aspects of vector physiology and competency (Shaw and Catteruccia, 2019). The term vector competence describes the capability of a vector to acquire, maintain and transmit infection (Sallum *et al.*, 2017).

Mosquitos vigorously fight against the infection and maintenance of invading pathogens. This is achieved by a well-organized immune system that regulates a plethora of invading pathogens ingested through the blood meal, in addition to maintaining balanced homeostasis among existing symbiont microbiota (Guégan *et al.*, 2018). The immune defense against infectious agents does not always end in pathogen elimination, but it may end in a state of disease tolerance. Mosquitoes resort to such a state to decrease infection induced-damage and morbidity without disturbing homeostasis (Talyuli *et al.*, 2021). Though lacking an adaptive immune response similar to that present in mammalian hosts, mosquitoes have a well-orchestrated innate immune response (Lee *et al.*, 2019).

Mosquitoes are challenged not only by invading microbes but also by the existence of symbiotic microbiota that must be maintained in a careful balance at all times. Symbionts utilize self-derived molecules or host-derived factors to achieve equilibrium. They employ a variety of methods and processes to reduce the activation of the hosts' hostile immune system. Hosts modify their immune responses to foster beneficial symbiosis and keep symbiont development under control (Pang *et al.*, 2016).

The mosquito microbiome (symbionts and commensal bacteria, as well as their genomes) is also important for mosquito development, as it can affect vector competence, immune signalling, longevity, insecticide resistance, survival, and reproductive rate (Guégan *et al.*, 2018).

It was reported that some symbionts of the microbiome's makeup shift from commensal to pathogenic status and vice versa (Seitz *et al.*, 1987). Other contributing factors to this altered state include the presence of infections and environmental changes such as temperature (Liu *et al.*, 2019). This indicates that the host-microbe interactions are even more complicated than once believed, given that, the significance of the gut microbiome's composition and diversity in influencing mosquito immunity to various infections is still unknown (Cansado-Utrilla *et al.*, 2021).

On the other hand, the influence of gut microbiota on vector competence, in particular, is critical to infection outcome since pathogen colonization and survival are actively influenced by them. Furthermore, various microbial taxa have been linked to mosquito vectorial potential in both positive and negative ways (Guégan *et al.*, 2018).

The interactions between the microbiota and the vector host will be the subject of this review, with an emphasis on immune signalling. We will describe how gut homeostasis and metabolic interactions shape mosquito vector competence, at least in part; we will then outline the microbiota- and symbiont-based strategies that are used to control mosquitoes' longevity and disease transmission, or that have been proposed but not yet implemented.

Mosquito Immunity and Immune Signalling:

The process of anti-pathogen defence starts with the recognition of specific microbe-associated molecular patterns (MAMP). Microbial recognition results in the activation of the innate immune response in mosquitoes, which has both cellular and humoral pathways (Kumar *et al.*, 2018) (Fig.1)

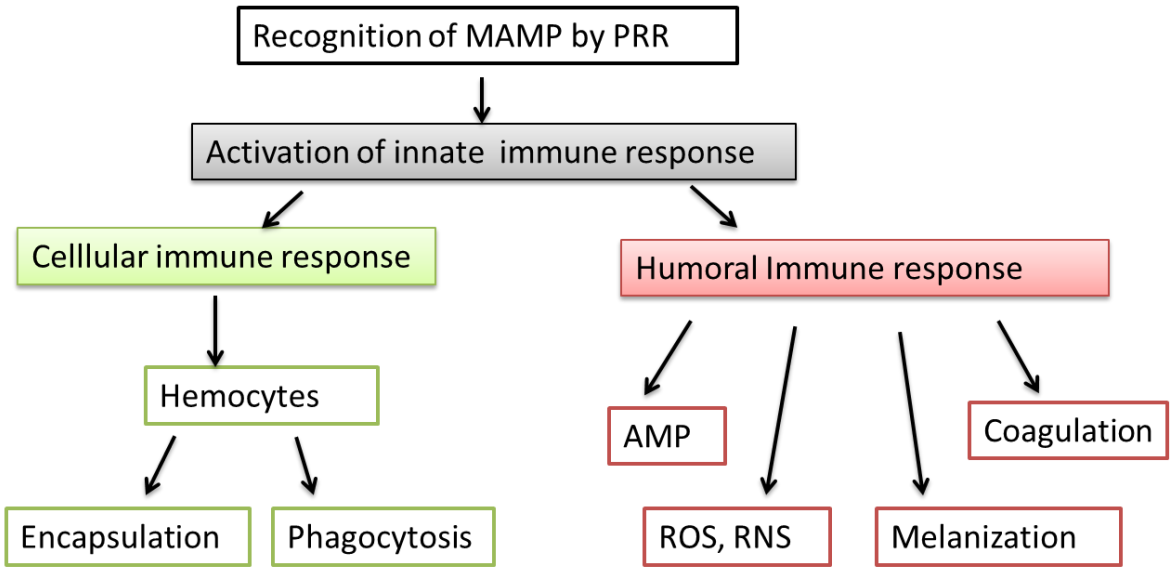

Fig 1. Schematic presentation of the components of the innate immune response in mosquitoes. MAMP: microbe-associated molecular pattern; PRR: pattern recognition receptors; AMP: antimicrobial peptides; ROS: reactive oxygen species; RNS: reactive nitrogen species.

Bacterial cell wall peptidoglycans (PGN) are famous MAMPs, that initiate the immune response after being recognized by specific pathogen recognition receptors (PRR), the peptidoglycan recognition proteins (PGRPs) (Wheeler *et al.*, 2014).

PGRPs can be classified structurally according to their transcript size into short (PGRP-S) and long PGRPs (PGRP-L) (Dziarski and Gupta, 2006). PGRP-S contain only 1 PGN recognition domain, while PGRP-L contains one or more domains. PGRP can also be classified functionally into catalytic and non-catalytic variants, where catalytic PGRP exerts an amidase activity, while non-catalytic variants lack such property (Wang *et al.*, 2018). Amidases are nitrilases that hydrolyze amide groups to yield ammonia and carboxylic acid (Weber *et al.*, 2013). While catalytic PGRPs act as modulators of the immune response by sequestering bacterial PGN, non-catalytic PGRPs include both positive and negative immune regulators (Wang *et al.*, 2018) (Fig. 2).

PGRP-LA2 from the mosquito *Anopheles gambiae*, for example, was expected not to bind PGN, yet demonstrated antiparasitic efficacy against the rodent malaria parasite *Plasmodium berghei* (Meister, 2006; Gendrin *et al.*, 2017). PGRP-LA1 and PGRP-S2/3 were also found to be crucial in the defense against *Plasmodium* infection in the malaria vector *An. Coluzzii* . *Aedes aegypti* mosquitoes treated with both gram-positive and gram-negative bacteria did not produce PGRP-LA or PGRP-LD, (Wang and Beerntsen, 2015).

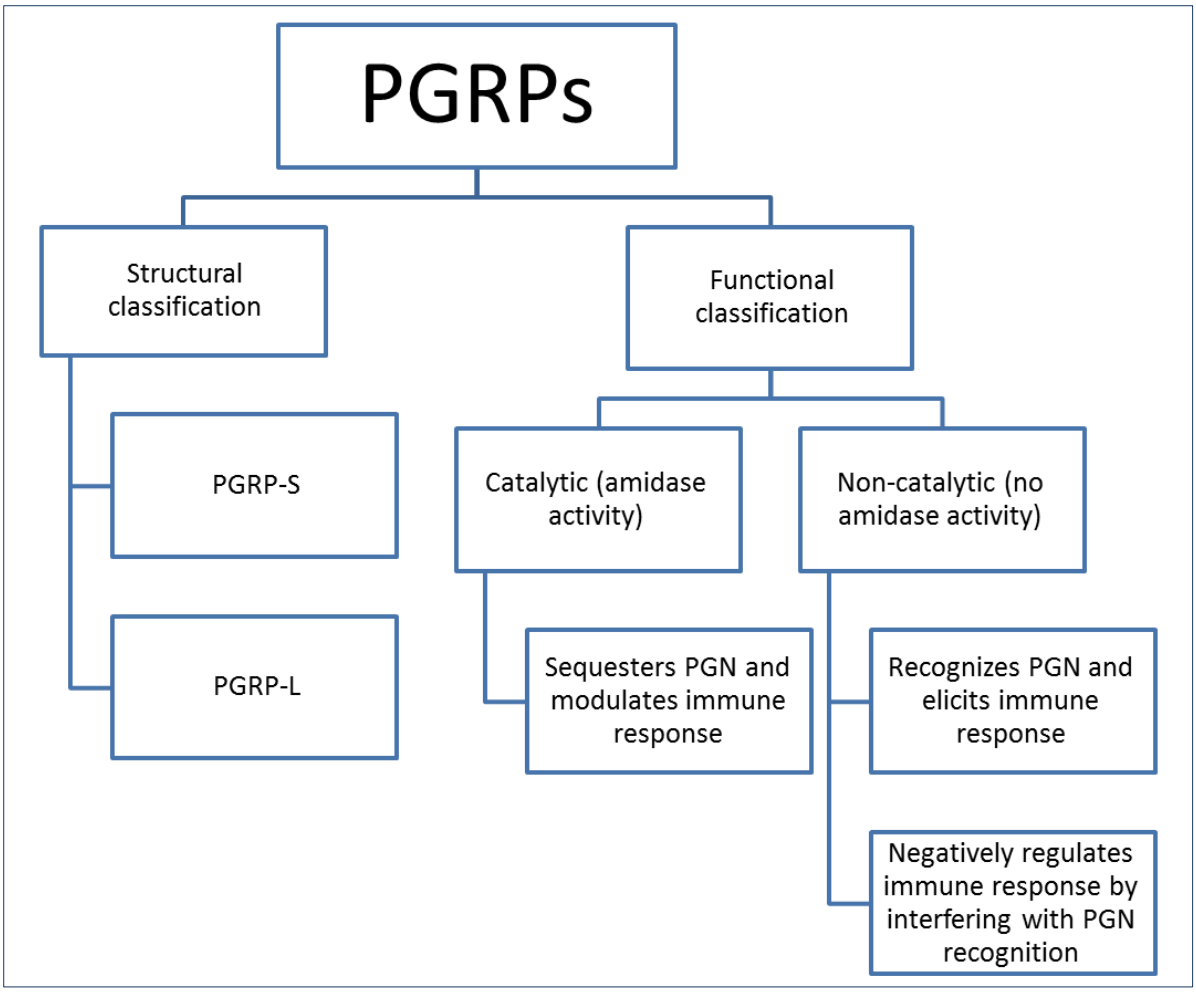

Fig 2. Schematic presentation of the types of PGRP.

PRRs also include thioester-containing proteins (TEPs) and leucine-rich repeat proteins (LRRs). TEPs were identified in *Drosophila melanogaster*, *Anopheles gambiae*, and *Aedes aegypti*. TEPs exert a phagocytic activity and interfere with the development of *Plasmodium* in *Anopheles* and impede infection with Dengue virus and West Nile Virus in *Aedes*. LRRs exert an anti-Plasmodium effect by melanization or direct lysis (Waterhouse *et al.*, 2007). Fibrinogen-related proteins (FREPs), C-type lectins, and gram-negative binding proteins (GNBPs) are also PRRs that have been identified in *Anopheles gambia*e and that are reported to exert immunomodulatory effects during infection with *Plasmodium* and bacteria (Kumar *et al.*, 2018).

The three main humoral pathways of immune signalling in mosquitoes are the immune deficiency (Imd) pathway, the Toll pathway, and the Janus Kinase/signal transducers and activators of transcription (JAK/STAT) pathway. Each pathway is stimulated by the recognition of certain pathogens such as Gram-positive and gram-negative bacteria, viruses, fungi, and parasites. Microbial recognition results in the activation of membrane receptors either directly or by binding to specific ligands such as Spaetzle, PGs, Upd (Unpaired family of cytokines), and Vago. Membrane receptor activation leads to the induction of membrane signalling proteins specific for each pathway, resulting finally in the production of effector molecules which include antimicrobial peptides (AMPs), thioester proteins (TEPs), and nitric oxide synthase (NOS) (Gabrieli *et al.*, 2021) (Fig. 3).

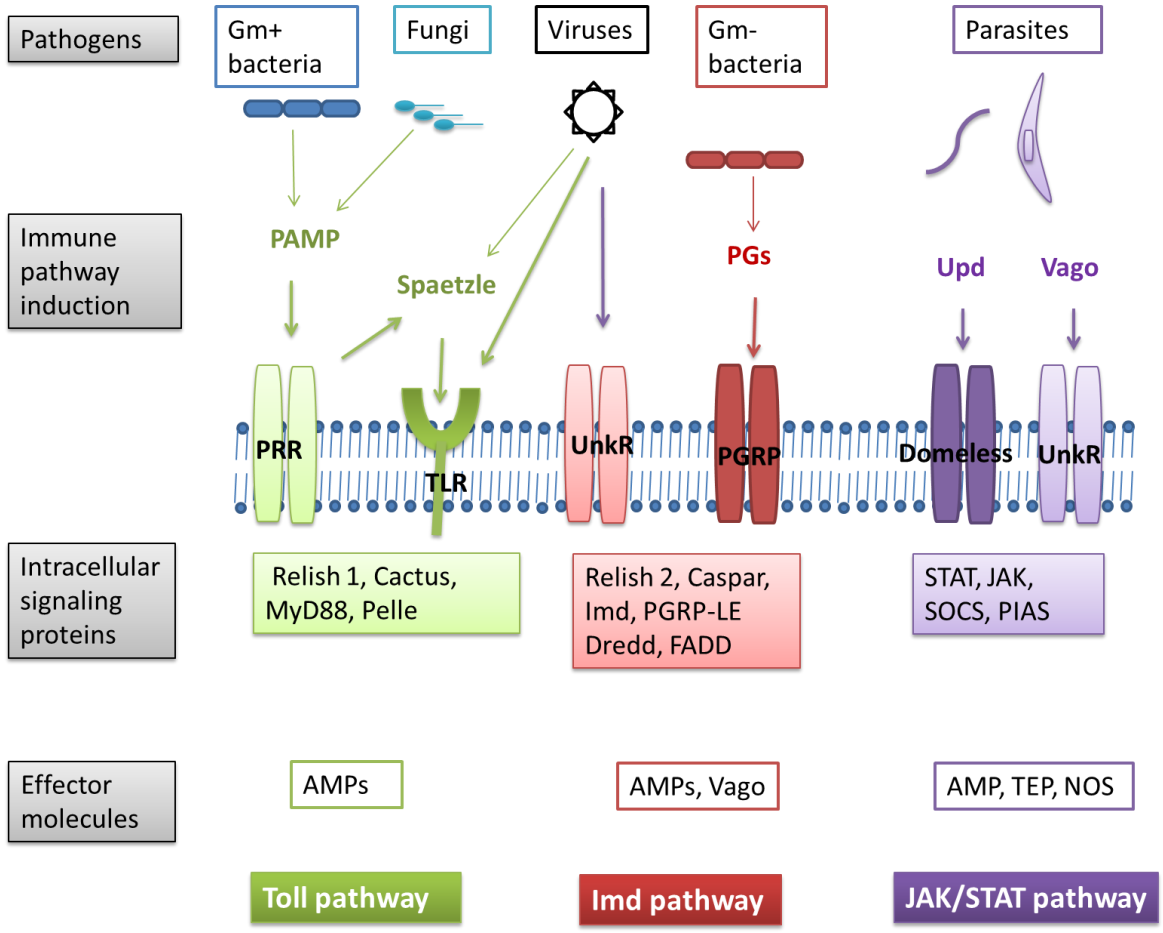

Fig. 3: Schematic presentation representation of the activation of the three main pathways involved in mosquito immunity; the Toll pathway, the Imd pathway, and the JAK/STAT pathway. PAMP: pathogen-associated molecular pattern, PG: peptidoglycan, Upd: the unpaired family of cytokines, PRR: pathogen recognition receptor, TLR: Toll-like receptor, UnkR: unknown receptor, PGRP: peptidoglycan recognition receptor, Imd: immune deficiency, Dredd: death related ced-3/Nedd2-like protein, FADD: Fas-associated death domain protein; STAT: signal transducer and activator of transcription proteins, JAK: Janus kinases, SOCS: suppressor of cytokine signalling, PIAS: protein inhibitor of activated STAT, AMPs: antimicrobial peptides, TEP: thioester-containing protein, NOS: nitric oxide synthase (Gabrieli *et al.*, 2021).

Mosquito Microbiome Composition and Manipulation:

In the last decade, research has focused heavily on the role of mosquito-associated microbiota. These bacteria are involved in immunity as well as key life processes such as food provisioning, reproduction, insect fitness, and pathogen transmission, with nutrition as the most important factor affecting the composition of the gut microbiota (Muturi *et al.*,2016).

Factors that Influence the Microbiota Composition of Mosquitoes:

Diet:

The feeding regime favors the proliferation of specific bacterial taxa over others, as observed with gut bacterial diversity that dropped considerably after sugar feeding or blood-feeding (Muturi *et al.*, 2016). The metabolism of carbohydrate-rich sugar and protein-rich blood may result in various gut conditions, resulting in differential bacterial taxonomic growth,

hence differences in microbial composition and diversity between sugar-fed and blood-fed mosquitos are to be expected (Wang *et al.*,2011).

Chryseobacterium spp. was detected in blood-fed mosquitos regardless of the type of blood meal. *Serratia* and other *Enterobacteriaceae* members are routinely recovered from the midguts of mosquitoes and other hematophagous insects, and their numbers have been shown to increase after a blood meal, probably due to their ability to cope with oxidative stress in the blood bolus, aiding in blood meal digestion (Wang *et al.*, 2011). Gut microbiota are kept under a delicate balance. A study on the *Anopheles coluzzii* mosquito's microbiome indicated that after a blood meal, mosquitoes restore intestinal homeostasis by excreting bacteria with the blood bolus, resulting in a 98 % reduction in bacterial burdens (Rodgers *et al.*, 2017).

Symbiont bacteria associated with sugar meals, such as *Acetobacteraceae*, have evolved to thrive in sugar and ethanol-rich gut environment (Crotti *et al.*, 2010; Muturi *et al.*, 2016). *Elizabethkingia* spp. is a glucose degrader that has been found to thrive in laboratory-reared mosquitoes. This is likely due to the use of sugar as a food source for lab-reared mosquitoes and the low bacterial diversity in lab-reared mosquitoes, allowing this bacterium to thrive in the absence of other bacterial species (Boissiere *et al.*, 2012; Terenius *et al.*, 2012).

Periodicity:

Several factors affect the diversity of mosquito microbiota. These include the behavioral adaptations among the different species such as the periodicity of the biting preference (nocturnal biting in *Anopheles* and *Culex* or diurnal biting in *Aedes*) and the nature of the habitat (clear water in *Anopheles* or turbid water with organic content in *Aedes* and *Culex*) (Clements, 1999).

Localization In the Gut:

Mosquito microbiota also differ according to their localization inside their host, being most abundant in the midgut, where they actively contribute to the process of enzymatic digestion. Other sites colonized by microbiota include the salivary glands, the hemolymph, and the reproductive organs. *Wolbachia* can also be found in the head and thoracic muscles (Minard *et al.*, 2013).

The Sex of the Mosquito*:*

The sex of the mosquito is also an important detrimental factor, since the female mosquitoes feed on both blood and nectar, while male mosquitoes feed on nectar only. Moreover, male mosquitoes tend to keep close to their breeding places, and thus have more limited food sources as compared to their female counterparts (Foster *et al.*, 1995).

The Developmental Stages of Mosquitoes:

The composition of microbiota varies also according to the developmental stage of the mosquito, due to the variability of feeding sources between the aquatic stages and the terrestrial adult stage. Some bacteria, however, are propagated between the different stages of mosquitoes by transtadial (such as *Actinobacter* and *Enterobacter*) and transovarial transmission (such as *Wolbachia*) (Chavshin *et al.*, 2012). In larvae and adult mosquitoes, symbiont bacteria linked with mosquito stages such as *Chryseobacterium*, *Pseudomonas*, and *Serratia* spp. have been identified (Coon *et al*, 2014).

Adults may acquire *Leucobacter* spp. through transstadial persistence since they are highly connected with newly emerging mosquitoes. A recent study found that *Leucobacter* spp. was almost absent in adult mosquitoes, rare in water from the larval habitat, and highly numerous in mosquito larvae, implying that this bacterial species can be acquired transstadially (Coon *et al.*, 2014).

Effect of Different Microbiota on Vector Competence:

Microbiota and pathogens inside a common host are members of a bigger complex community of microorganisms that share a common umbrella of host metabolism, immunity, and other various physiological processes. The different microorganisms actively interact and counteract, synergize and antagonize each other, which consequently reflects on the biology of their host (Guégan *et al.*, 2018).

Blood Digestion:

The presence of midgut microbiota actively contributes to the process of blood digestion and red cell lysis. The proteolytic digestion of hemoglobin provides the necessary amino acids for various anabolic processes, including vitellogenesis (Attardo *et al.,* 2005). The catabolism of the blood meal, in particular, generates reactive oxygen species, which may have an impact on the bacterial composition and diversity in the midgut (Souza *et al.,* 1997). Various host blood meal types contain different amounts of total protein, hemoglobin, and hematocrit, which could contribute to microbial taxonomic growth. By altering the structure and organization of microbial communities, the blood meal promotes a progressive change in oxidative conditions in the gut (Champion, 2017).

Furthermore, the blood meal is made up of cellular and humoral factors as well as exogenous substances that the host receives and absorbs. For example, *An. coluzzii*'s useful life is prolonged when exposed to doxycycline but shortened when exposed to azithromycin (Gendrin,2016), implying that changes in the microbiome are responsible for this phenotype, Similarly, antibiotic clearance by the microbiota has shown that the microbiome plays a role in mosquito metabolism and insecticide sensitivity (Barnard *et al.,* 2019).

The administration of antibiotics was also found to interfere with blood digestion and decrease egg production in *Aedes aegypti*, an effect that was reversed in the gonotrophic cycle that followed the discontinuation of antibiotic treatment (Gaio *et al.,* 2011).

Such studies have shown that the source of host meals can have strong effects on mosquito's microbiome composition and diversity across different developmental stages, which may limit transmission of vector-borne diseases either by inhibiting pathogen development within the vector or by suppressing vector populations through effects on longevity, fecundity, or fertility (Muturi *et al.,* 2018).

Metamorphosis And Development of Premature Stages:

Microbiota are also important for the process of metamorphosis and development of premature stages since they provide a rich source for nutritive elements, especially amino acids (Yamada *et al.,* 2015). Moreover, microbiota in the larval midgut induces hypoxia, which leads to the stimulation of growth signaling pathways such as the insulin/insulin growth factor pathway, via the stabilization of hypoxia-inducible growth factor (HIF) alpha. HIF is also important for the development of the larval midgut and fat body (Valzania *et al.,* 2018).

Stimulating the Immune Response:

Bacteria in the mosquito midgut can be protective against pathogens by stimulating the immune response and initiating the formation of the peritrophic matrix, which isolates pathogens from the midgut epithelium (Kuraishi *et al.,* 2011; Huang et al, 2020). Despite these effects on insect physiology, the effect of microbiota on the infection outcome in mosquitoes is not always predictable. Bacteria can favor either susceptibility or resistance to infection and thus directly impact vector competence. *Wolbachia* has been reported to reduce arboviral transmission in *Aedes aegypti* (Moreira *et al.,* 2009; Dutra *et al.,* 2016) while enhancing densovirus transmission in *Culex pipiens* (Altinli *et al.,* 2018).

Resource Competition:

Pathogens exploit mosquito nutritional elements to support their development. *Plasmodium* oocysts sequester and incorporate the mosquito lipoprotein lipophorin (Atella *et al.,* 2009). Dengue virus uses mosquito lipids to re-arrange its cell membrane for efficient replication (Dennison *et al.,* 2014). Invading microorganisms are not the only competitors for the mosquitoes' nutritive repertoire, since symbiont microbiota also use the host nutritional elements for their own needs. *Wolbachia* for example utilizes cholesterol and lipids, and thus reduces their availability for *Plasmodium* and Dengue virus, which impedes their

development and consequently decreases their infective ability.

Microbiota As Valuable Tools in The Control of Vector-Borne Diseases:

Insecticide resistance is a serious challenge to the chemical control of infectious diseases transmitted by mosquitoes. Biological control and the exploitation of the microbial environment of the insect vectors has thus emerged as a promising alternative (Benelli *et al.,* 2016). Microbiota constitute an efficient tool in the biological control of mosquitoes since they share a common location with pathogenic organisms inside the mosquito gut, and they are also a rich source of antimicrobial effector molecules (Wang and Jacobs-Lorena, 2013). Potential microbial candidates for the control of vector-borne diseases must be able to efficiently maintain themselves in their mosquito host and propagate across generations by trans-ovarian and trans-stadial transmission. They should have an evident anti-pathogen effect, and finally, they should be easy subjects for genetic interference (Huang *et al.,* 2020).

Paratransgenesis is an approach that exploits symbiotic bacteria by genetically manipulating them and then re-introducing them inside the vector to produce certain targeted immune effectors. These bacteria may act by attenuating the host's fecundity or fertility or decreasing its vector competence (Wilke *et al.,* 2015). One of the symbiotic bacteria used in this technique is the midgut inhabitant *Pantoea agglomerans*, which has been genetically engineered to produce two anti-malarial effector proteins in *Anopheles gambiae*. *Pantoea agglomerans* are a very appropriate candidate to deliver the genes expressing these effector proteins as they proliferate in large numbers following the blood meal and more importantly, they share the same location with the malaria parasite after the host acquires the infected meal, i.e. the midgut. The expression of these anti-malarial peptides in the mosquito leads to a 98% reduction in *Plasmodium* development and the number of mosquitoes carrying the parasites became reduced by 84% rendering *P. agglomerans* a promising tool in vector-borne disease control (Wang *et al.,* 2012).

Another symbiont used in para-transgenesis is the Gram-negative bacterium *Asaia*. It has also been employed in the delivery of antimalarial peptides and a significant reduction of parasite development was observed (by 80.1%). *Asaia* is advantageous over *P. agglomerans* in that it propagates better and longer within a mosquito population. Additionally, it is present not only in the midgut but also in the salivary glands and reproductive organs, which are all organs involved in disease transmission (Bongio and Lampe, 2015).

Paratransgenesis, though a promising approach for the control of vector-borne diseases, faces certain challenges that have to be addressed. For example, the action of commensal bacteria can vary according to the transmitted pathogen and the mosquito species. *Serratia* displays an anti-plasmodial effect in *Anopheles* while promoting the transmission of the dengue virus by *Culex spp*. Another limitation is that *Wolbachia* is the only symbiont that can successfully propagate inside the mosquito and simultaneously inhibit pathogen infection. Therefore, the identification of a symbiont that can decrease disease transmission by *Anopheline* remains an urging target (Huang *et al.,* 2021).

Conclusion And Future Implications :

The key to disease transmission is the hematophagous nature of mosquitoes that require blood meals to continue their life cycle. Though, in the context of the complicated host-microbe symbioses, the host's genetic susceptibility and the timing of infection are both crucial. Manipulation of the microbiome composition and diversity through paratransgenesis (the engineering of many blocking factors into a single microbial species) is a powerful method for vector-borne disease management. Thus, disrupting or enhancing mosquito-host symbionts may have an impact on disease control, with possible ecological implications. The identification of suitable microbial candidates

that can affect mosquito vector competence remains a challenge in the control of vector-borne diseases.

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