

Functional genomics analysis of the implication of mosquito midgut microbiota in the defense against malaria parasites

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Malaria transmitting mosquitoes are continuously exposed to microbes, including their midgut microbiota. Previous studies have shown that this naturally acquired microbial flora can modulate the vectorial capacity by inhibiting the development of *Plasmodium* and other human pathogens. The mechanisms through which the microbiota can reduce *Plasmodium* infection of vector mosquitoes are not well understood. One hypothesis is that the microbial flora stimulates immune responses that inhibit *Plasmodium* development. In order to characterize the mechanism mediating bacteria-associated reduction of malaria infection in mosquitoes, we have undertaken a comprehensive functional genomic approach to elucidate the molecular interplay between the bacterial co-infection and the development of the human malaria parasite *Plasmodium falciparum* in its natural vector *Anopheles gambiae* in the lab-rearing conditions.

We first monitored the dynamic changes of the components of microbiota of female Keele mosquitoes for the 5 consecutive generations and confirmed that even lab-reared mosquitoes harbor many diversified bacteria (Fig. 1). Antibiotic treatment eliminated the microbial flora from adult female mosquitoes and thus mosquitoes became aseptically (Fig. 2A). Aseptic mosquitoes displayed significantly increased susceptibility to *Plasmodium* infection compared to septic mosquitoes (Fig. 2B). Mosquitoes were co-fed of bacteria with *P. falciparum* gametocytes had significant lower infection levels while only a marginal effect observed when mosquitoes were thoracic-injected with live bacteria (Fig. 3), suggesting that tissue specific responses to bacteria are implicated in anti-*Plasmodium* defenses. Confocal microscopy showed that *P. falciparum* development within the mosquito midgut lumen and epithelium wasn't different within septic mosquitoes, or in the presence of additional bacteria in the bloodmeal (Fig. 4), suggesting that the effect of bacterial exposure on mosquito susceptibility to *P. falciparum* happened during ookinete invasion. We then compared gene expression between septic and aseptic mosquitoes using microarrays to identify genes induced by the microbial flora or with additional human pathogenic bacteria (Fig. 5), and used RNAi to screen candidate immune genes for their role in the modulating microbial flora (Fig. 6) or in *P. falciparum* infection (Fig. 7). Through RNAi-mediated gene silencing we have shown that some immune genes mediate anti-*Plasmodium* effects through microbiota, while other factors exert direct effect on the parasite (Fig. 7). Our data suggests the bacteria-mediated anti-*Plasmodium* effect is mediated by the mosquitoes' antimicrobial immune responses, plausibly through activation of basal immunity.

Fig. 1. Dynamics of microbial flora components in 5 consecutive generations of lab-reared *A. gambiae* Keele female mosquitoes

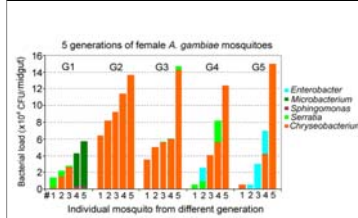


Fig. 2. Antibiotic-treated aseptic mosquitoes became more susceptible to *P. falciparum* infection

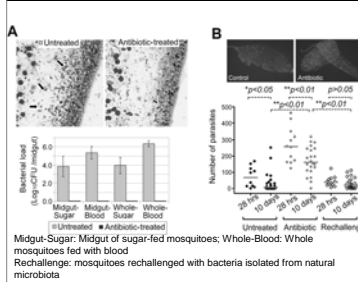


Fig. 3. Co-fed or pre-injection of bacteria decreased the oocysts infection

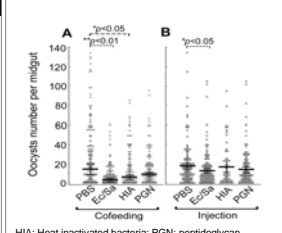


Fig. 4. Ookinetes in the midgut lumen 24 h post infection (upper panel) and ookinetes counts

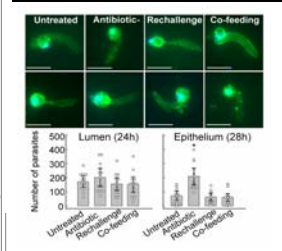


Fig. 5. Global transcription profiling of septic and aseptic mosquitoes or septic mosquitoes fed with additional human pathogenic bacteria

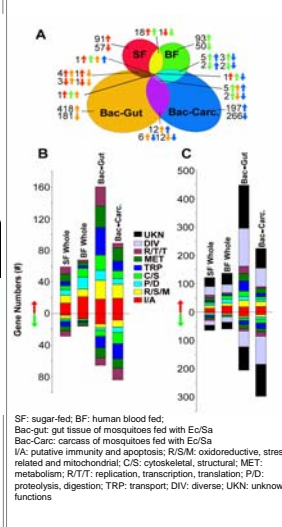


Fig. 6. The natural microbial flora stimulates basal immune activity that controls its proliferation

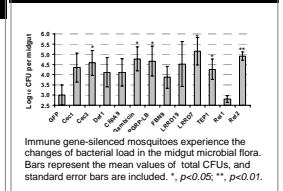
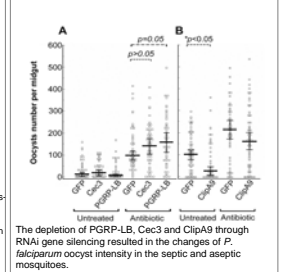


Fig. 7. The anti-*Plasmodium* action of immune genes can be modulated by the presence of the mosquito's endogenous microbial flora.



Dynamics of the microbiota of lab-reared larval and adult mosquitoes and the impact on malaria parasites

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Microbiota are important components of ecosystems and every living being has to deal with a delicate balance between beneficial and pathogen bacteria to survive. Microbiota provide food resource to mosquitoes larvae thus are fundamental during larval development, and in adults mosquitoes microbiota colonize midgut which preventing mosquitoes from pathogen bacterial infections. In the hematophagous insects *A. gambiae*, microbial flora plays another important role in the anti-*Plasmodium* defense.

Dynamics of components of microbiota of larvae mosquitoes and the rearing-water

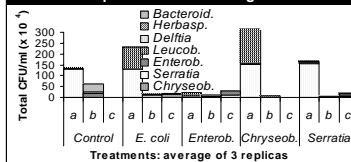


Fig. 1B: microbial flora in larvae-rearing water

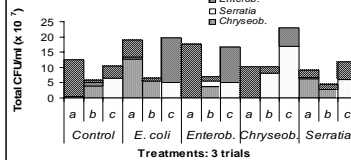
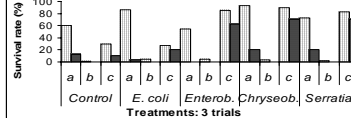


Fig. 1C: survival after incubation



In this study we investigated the bacteria composition of *A. gambiae* mosquitoes from insectary colonies and we monitored their dynamics during larval development and through consecutive generations of adults. In order to see how dominant certain bacteria are, we also artificially introduced several species of bacteria in young larvae which have gone through antibiotic treatments (Fig.1). We observed *Chryseobacterium* sp. was absolutely dominant in adults while in larvae the pattern was more variable and the precise balance between the bacterial species of microbiota and their quantities determined how well larvae could survive to adults (Fig.1). Examination of midgut flora after *Plasmodium* infection revealed the existence of an interesting threshold-effect (Fig. 2).

Strong correlation between components of microbial flora and *P. falciparum* oocysts loads

