Perspectives de modulation du microbiote de l'anophèle pour limiter la transmission de Plasmodium

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Transmission routes

- Feeding on flower nectars or blood
- Mating
- Moulting

Egg smearing

Trans-stadial transmission

Trans-ovarial transmission

Life cycle stage and diet affect bacterial communities
Microbiota acquisition

The breeding site is a crucial factor shaping the community composition in adults and the season affects microbiota diversity.

Mann-Whitney U= 131; p< 0.0001

Gimonneau et al. 2014

Akorli et al. 2016
Microbiota composition

Changes with physiological state or aging

Mosquito epithelia share a core microbiota

*Pseudomonas, Comamonas, Acinetobacter, Rhizobium, Burkholderia* and members of the Enterobacteriaceae

Large individual variability

Taxonomic classification of bacterial reads retrieved from the mosquito tissues at the different developmental stages.

Tchioffo et al., 2014
Microbiota composition

The bacterial flora was mainly composed of Gram-negative communities.

The microbial communities in the epithelia are structured according to the physiological status of the mosquito.

Some bacteria taxa were more associated with one or another tissue at a particular time point.

Tchioffo et al., 2014
The bloodmeal reshapes bacterial communities

Upon blood feeding:

- reduction in diversity/richness

- bacterial proliferation, mainly Enterobacteriaceae (Klebsiella, Serratia, Enterobacter, ...)

![Graph showing bacterial load over time after blood feeding.](chart.png)
Impact of bacterial communities for the mosquito

From antibiotic treated mosquitoes to clear the microbiota

On development: axenic larvae fail to develop beyond the first instar, but bacterial recolonization rescues larval growth (Coon et al. 2014)

On lifespan and fecundity: supplementation of antibiotics in the blood meal increases fecundity and survival (Gendrin et al. 2015)

On mosquito resistance to insecticides: Antibiotic supplementation via sugar decreased tolerance to the insecticides (deltamethrin and malathion)

On infection: Antibiotic treatment increases mosquito susceptibility to Plasmodium infection (Beier et al. 1994, Dong et al. 2009, Gendrin et al. 2015)

Microbiota protects the mosquito from Plasmodium infection but the natural microbiota proliferation may decrease mosquito population size and time available for parasite transmission
Bacteria that interfere with *Plasmodium* sporogonic development

<table>
<thead>
<tr>
<th><em>Plasmodium falciparum</em></th>
<th><em>Anopheles gambiae</em></th>
<th><em>Chromobacterium</em></th>
<th>Increases infection resistance by forming a protective biofilm against parasite</th>
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<tbody>
<tr>
<td><em>Enterobacter</em></td>
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<td>Inhibits parasite development by stimulating oxidative stress</td>
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<td><em>Escherichia coli</em></td>
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<td>Reduces infection prevalence and intensity</td>
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<td><em>Pseudomonas stutzeri</em></td>
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<td><em>Serratia</em></td>
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<td>Protects against infection</td>
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<td><em>Serratia marcescens</em></td>
<td></td>
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<td>Reduces infection prevalence and intensity Actives IMD/REL2 immune pathway</td>
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*Asaia, Enterobacter, Pantoea, Serratia* and *Pseudomonas* have been proposed as promising symbiotic agents for blocking malaria transmission.
Discrepancies of results between laboratories or models
Contrasting effect according to experimental setups (experimental oral infection versus natural microbiota)

Oral challenge to bacterial cultures

![Graph showing oral challenge results](image)

**Negative impact:** *Serratia marcescens* reduces the *Plasmodium* parasite load

Bando et al. 2013

Correlation on field collected mosquitoes

![Graph showing correlation results](image)

*Serratia* have a **protective role** on natural *P. falciparum* infections

Tchioffo et al. 2014
How mosquito microbiota protects against *Plasmodium* infection

- The natural microbiota stimulates basal immune activity that controls its proliferation, maintaining homeostasis.

- Blood feeding induces high levels of oxidative stress, released heme reduces ROS generation and contributes to bacterial proliferation.

- A dityrosine network (Duox/Peroxidase) decreases midgut permeability to bacterial elicitors, protecting the microbiota but also favoring *Plasmodium* development.
Fighting malaria with paratransgenesis

« An ideal paratransgenenic candidate is one that can be genetically modified, colonizes vector mosquitoes in the same body compartments where pathogens develop, spreads through mosquito populations, and is not a human pathogen”

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<th>Basic requirements for paratransgenesis</th>
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</table>
**Elizabethkingia anophelis,**

a bacterium in the microbiome of lab-reared Anopheles mosquitoes

*E. anopheles*, Flavobacteria, is the predominant bacterial species in insectary-reared *Anopheles*, also found in field mosquitoes

Cultivable and amenable to genetic manipulation

Could be a good candidate for paratransgenesis BUT

Outbreaks of *E. anophelis* infections in humans have been described in Singapore, in the Midwestern United States, Congo, (meningitis and respiratory infections in new born babies and people with weakened immune systems) ...

A multiresistant bacteria, an emerging nosocomial pathogen
Wolbachia and Anopheles

- Field populations of anophelines were thought to be resistant to Wolbachia infections
- In 2014: first evidence of Wolbachia sequences in Anopheles gambiae in Burkina Faso (Baldini et al. 2014)
- A new strain, wAnga, highly divergent from Wolbachia strains isolated in other insects

Baldini et al. Nature Com 2014

Gomes et al. PNAS 2017
**Wolbachia prevalence in Anoph eles**

*Wolbachia* infection prevalence varies greatly among *Anopheles* species, country, season....

*Wolbachia* infection is commonly lower than 15% and *Wolbachia* is present at low density (<1% of mosquito genome copies)

- Shaw *et al.* Nature Com 2016 : In BF, Vallée du Kou, wAnga at a frequency of 33% (16/49) in *A. arabiensis*, 46% (275/602) in *An. coluzzii*
- Baldini *et al.* Nature Com 2014: Prevalence of Wolbachia from *An. gambiae*: in VK7, 7.1% (3/42), Soumousoo 4.2% (1/24), VK5 86% (6/7)
- Gomes *et al.* PNAS 2017 : In Mali, prevalence of Wolbachia infection in *A. gambiae* (53.6%)
- Niang *et al.* Mal J 2018 : In Senegal: frequency of Wolbachia infection in *A. funestus* is of 1.2% (3/121)
- Ayala *et al.* Mal J 2019 : In Gabon, 10.8% (70/648) *Wolbachia*-positive specimens that belonged to 16 different *Anopheles* species and *An. moucheti* and *An. nili* have higher infection rates (>50%)
- Baldini *et al.* Parasites & Vectors 2018 : In Tanzania: wAnga infection prevalence in *An. arabiensis* was 3.1% (n = 65, 2014) and 7.5% (n = 147, 2016); no infection was detected in *An. funestus* (n = 41)
- Sawasdichai *et al.* 2019 : in Myanmar, *Wolbachia* DNA was detected in 3.5% (13/370) samples
Phylogeny of *Wolbachia* from *Anopheles*

Anopheles-infecting *Wolbachia* bacteria show high genetic diversity

*Anopheles* species are infected by different *Wolbachia* strains

*Wolbachia* acquisition seems to be independent of the mosquito phylogeny

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Gomes *et al.* PNAS 2017

Niang *et al.* Mal J 2018

Ayala *et al.* Mal J 2019
Wolbachia and Plasmodium infections

Wolbachia infection negatively impacts Plasmodium sporozoite infection in A. coluzzii but the effect is opposite at the oocyst stage, with an enhanced oocyst infection.

Gomes et al. PNAS 2017
**Wolbachia and Anopheles**

In *Anopheles* (BF, VK5):
- *Wolbachia* infection significantly reduces the prevalence and intensity of sporozoite infection
- BUT no cytoplasmic incompatibility, just accelerates egg laying

Shaw et al. 2016

The native mosquito microbiota can impede *Wolbachia* transmission in *Anopheles*

Hughes et al. PNAS 2014
Is *Anopheles gambiae* s.l. a natural host of *Wolbachia*?

- From data generated in the Ag1000G project, (Illumina sequences of 765 wild-caught mosquitos from 8 African countries, 79G reads total): less than 500 reads map to *Wolbachia*

- No single *Wolbachia* strain associated with *Anopheles gambiae*

- Conflicting phylogenetic affiliations of the sequenced strains (span at least two *Wolbachia* supergroups)

- The presence of *Wolbachia* sequences in *An. gambiae* tissues can be a contamination

Chrostek mBio, 2019

The mosquito microbiota can impede *Wolbachia* transmission?
Asaia spp. and Anopheles

Asaia inhibits Wolbachia transmission in Anopheles (Hughes et al., PNAS 2014), No Wolbachia/Asaia co-infections have been reported so far.

Asaia (Acetobacteraceae) : a genus of acetic acid bacteria, also isolated from tropical plants
Dominant bacteria colonizing mosquito midgut, ovaries/testis and salivary glands
Asaia is transmitted vertically from mother to progeny and horizontally through feeding on nectars
An easily cultivable and transformable bacterium (Favia et al. 2007)
Asaia, a suitable agent for the paratransgenic control?

Asaia sp. SF2.1 isolated from An. stephensi
- Cultivable and transformable (Favia et al. 2007)
- Express antiplasmodial effector molecules constitutively (Bongio et al. 2015)
- Constructs with blood meal induced (BMI) promoters inhibit Plasmodium infection and transgenic bacteria compete effectively with wild type Asaia (Shane et al. 2018)

Next:
- Effector genes to be inserted into the Asaia chromosome of field strains where they can be inherited stably
- Express more than one antiplasmodial effector gene simultaneously to suppress the evolution of resistance by Plasmodium to the antiplasmodial products
Microbiota and insecticide resistance

Mosquito microbiota is colonized by insecticide-degrading bacterial species: *Pantoea, Pseudomonas, Sphingomonas, Burkholderia, Bacillus, Klebsiella* and *Serratia*

Insecticide exposure leads to shifts in microbial composition (Dada et al. 2019)

Insecticide resistant strains have lower bacterial diversity, but enrichment of insecticide degrading bacteria such as *Klebsiella* an OP-degrading species (Dada et al. 2018, Barnard et al. 2019)

Microbial communities have a role in insecticide resistance phenotype
Bacteria could be manipulated to produce toxins with novel insecticidal activity
Eco-holobiont approach, the microbiome of an entire ecosystem

An. gambiae s.s. has a preference for specific plants: *Mangifera indica, Thevetia neriifolia, Senna siamea, Parthenium hysterophorus, Ricinus communis* (Manda et al. 2007, Gouagna et al. 2010)

The source of plant sugar meal affects
- Survival and fecundity
- Biting frequency

Pollen and nectar microbiomes may be important microbial contamination sources for mosquitoes (Asaia)

Plants can serve as a reservoir for horizontal transmission of bacteria (Caspi-Fluger et al. 2012, Kenney et al. 2017)
Plant bacteria could be used to express effector molecules interfering with the mosquito physiology
The source of plant sugar meal affects mosquito fitness and also *Plasmodium* infection level (Hien *et al*. 2016)

Plant secondary metabolites may impact on the gut microbiota dynamics and contribute to pathogen development/inhibition

Can mosquitoes use medication to increase resistance or tolerance to infection? (de Roode *et al*. 2019)
The best microbiota: to cure or kill the mosquito?

Common Symptoms of Malaria:
- Diarrhea
- Cough
- Headache
- Body Ache
- Fever
- Sweat
- Chills and Shivers
- Nausea and Vomiting

Thanks for attention!