Symbiotic Bacterial Delivery of RNA Interference to Mosquitoes to Inhibit Diverse Arbovirus Transmission

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Hughes lab research interests

- Host-microbe interactions
- Vector biology
- Arbovirology

Tripartite interactions

- Microbes
- Insect
- Pathogen

Bacterial symbionts to delivery RNAi and CRISPR to mosquitoes

Factors that shape microbiome
Exploiting the gut microbiota to interfere with arboviruses in Aedes mosquitoes

**Mechanism to block virus**

1. Bacteria has native anti-viral activity
2. Target virus directly with dsRNA.
3. Inhibit mosquito genes essential for virus replication with dsRNA.
4. Up-regulate mosquito immunity with dsRNA
The microbiome of field caught and lab-read *Aedes aegypti*. 
The mosquito microbiome is highly variable between individuals.

Field caught *Ae. aegypti*

Field caught and lab-reared *Ae. aegypti*

Lab-reared mosquitoes have a distinctly different microbiome compared to field caught counterparts.
Mosquitoes fed FP labeled bacteria in sugar meal.

Blood fed mosquitoes and oviposition site provided.

Mosquitoes fed clean sugar.

Culture whole mosquito

Culturing ovary and carcass

Microscopy on ovaries

Culturing oviposition water

Experimental timeline

Reintroduction of Symbionts

DsRED

AntiR

2x10^7

N=50

N=50

Sugar
E. coli
Enterobacter Serratia

Culture whole mosquito

4 days

Days 0 5 8 9 10 15

N=50
Gut microbes can be re-introduced to mosquitoes via sugar feeding.

24 hours on sterile sugar

5 days on sterile sugar

N=15
Serratia localized to the ovarian duct

Eggs smeared with bacteria when laid.
Bacterial delivery of RNAi to mosquitoes

![Diagram showing bacterial delivery of RNAi to mosquitoes]

- **DsRED**
- **AntiR**
- **Pro**
- **shRNA**
- **T**

**COP-I**
**GFP**

**RNA extraction and transfection**

**Survival Post Treatment**
**COP-I/GFP**

**Survival**
**PBM**

**Sugar fed**

**Blood fed**

- **GFP**
- **COP-I**

Unpublished data
Bacterial expression and delivery of long RNA hairpins to mosquitoes to block Zika virus

Transferred to expression of IhRNA
- Easy to verify expression by PCR
- Processed into multiple siRNAs
  - Greater silencing efficiency
  - Mitigate against evolution of resistance.

IhRNA hairpins to target
- PIAS – JAK-STAT pathway
- Cactus - Toll pathway
- NS4B – ZIKV genome
- NS5 – ZIKV genome
- GFP – control.

Unpublished data
Evaluate bacterial-produced dsRNA affect on ZIKV

RNA extraction and transfec onto insect cells → Challenge cells with ZIKV → Quantify infectious virus with focus forming assay

IhRNA produced in bacteria that target ZIKV suppress viral titer in cell lines.

![Graph showing the effect of RNAi treatments on ZIKV titer with significant differences marked by asterisks.](image)
Bacterial delivery of RNAi to mosquitoes

Fed ZIKV and quantify virus in mosquito
Inhibition of Zika virus in *Ae. aegypti* subjected to *E. coli* expressing dsRNA

*Ae. aegypti* carcasses 14 dpi

Prevalence

- **Mock**
- **GFP**
- **PIAS**
- **Cactus**
- **NS4B**
- **NS5**

*Infected* | *Uninfected*
Summary

• Gut symbionts can administered to mosquitoes in a sugar meal.

• Transmission between generations was observed.

• Mechanism could be coating the eggs with bacteria.

• Bacterial can be engineered to deliver dsRNA to silence genes in mosquitoes.

• Bacterial delivery of dsRNA to mosquitoes is a possible strategy to inhibit Zika virus in *Aedes aegypti*.
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