Characterizing A Mosquito Gut Bacterial Community

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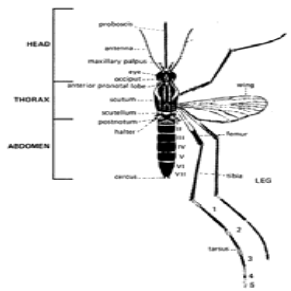
OBJECTIVE
Our goal is to identify culturable bacterial species in the gut community of lab reared female *Aedes aegypti* for paratransgenesis.

ABSTRACT
Mosquitoes are common vectors of devastating diseases that infect both humans and animals. Methods of control, such as insecticides and vaccines, are losing their effectiveness. Alternative strategies need to be considered. Paratransgenesis is one method of blocking transmission of disease from mosquito to host. Paratransgenesis is the genetic engineering of symbiotic or commensal bacteria to produce products that block pathogen transmission. This research project looked at characterizing the bacterial community of adult female *Aedes aegypti* to find candidates for paratransgenesis. We identified 72 diverse culturable bacterial isolates present in the midgut and on the exoskeleton of this mosquito. We examined the difference between the midgut bacterial community of blood-fed and sugar-fed female adults. We also found that approximately 10% of the total bacterial species found in the midgut are culturable, which is consistent with studies from other natural bacteria consortia.

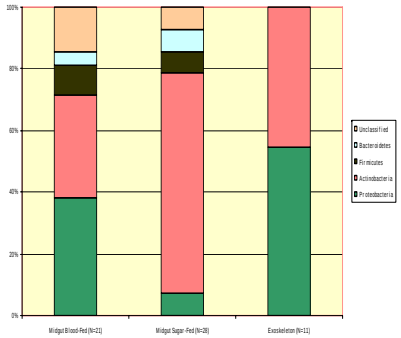
Figure 1. Diagram of a Female Adult Mosquito (NCID 2004)



INTRODUCTION
Our lab is interested in the use of paratransgenesis as an alternative strategy for controlling vector-borne infectious disease. Paratransgenesis is the genetic manipulation of commensal or symbiotic bacteria to alter the host's ability to transmit a pathogen (Aksoy 2000).

The first step in paratransgenesis is identifying and understanding the natural vector gut microbial community. The gut is the site of initial infection of the vector by the pathogen. At present, however, little is known about the mosquito gut microbial community (Whitford et al. 2001). The overall objective of this research project is to use comparative 16S ribosomal RNA analysis to characterize bacterial species in the gut of the dengue and yellow fever virus vector, *Aedes aegypti*. We will compare the bacteria present on the exoskeleton and in the midguts of both blood-fed and sugar-fed adult mosquitoes.

Figure 2. Phylogenetic Distribution of Isolates, N = 60



Phyla	Blood	Sugar
Proteobacteria	8	2
Actinobacteria	7	20
Firmicutes	2	2
Bacteroidetes	1	2
Unclassified	3	2
Total	21	28
TU	0.95	0.63

Table 1. Estimated Cells per Midgut Contents. Cell numbers were calculated based on microscopic counts of DAPI stained midgut contents using the formula of Bloem (1995).

Midgut Sample	Sample Volume (ul)	Estimated Cells per ul of Contents	Estimated Cells per Midgut Contents
Blood Fed	0.25	3.6×10^2	9.0×10^1
Blood Fed	0.50	1.9×10^3	9.7×10^2
Blood Fed	0.50	2.0×10^2	1.5×10^1
Sugar Fed	0.25	2.5×10^2	6.2×10^1

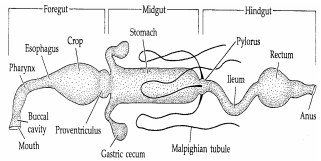
*** Note: This represents counts of bacteria recovered from liquid removed from midguts. Bacteria associated with midgut structures were not counted or isolated**

Table 2. Comparison of Bacterial Diversity at the Genus Level Between Blood-Fed and Sugar-Fed Midgut Isolates Using the Keefe-Bergersen Diversity Index (1977).

Genus of Closest Match	Blood	Sugar
Arthrobacter sp.	2	2
Azospirillum sp.	1	0
Bacillus sp.	2	1
Caulobacter sp	2	0
Chryseobacterium sp.	1	0
Leucobacter sp.	1	4
Matsuebacter sp.	1	0
Microbacterium sp.	3	9
Pseudomonas sp.	2	0
Shingomonas sp.	2	0
Williamsia sp.	1	0
Ancylobacter sp	0	1
Enterobacter sp.	0	1
Kocuria sp.	0	1
Micrococcus sp.	0	1
Nocardioides sp	0	1
Paenibacillus sp.	0	1
Rhodococcus sp.	0	1
Streptomyces sp.	0	1
Taxeobacter sp.	0	1
Total	18	25
TU	0.29	0.02

METHODS
Micromanipulation and processing of DNA from guts
- **Three hours post feed mosquitoes were chilled to 4C**
  - **Swabbed with sterile water to inoculate nutrient agar for exoskeleton communities**
  - **Midguts were dissected out in 70% ethanol**
- **Midgut contents were removed with capillary needles to 100ul sterile water for nutrient agar plates**
- **Plates were incubated in ambient air, room temperature, in the dark for several days**
- **Bloem (1995) Formula, B = (N/X)(A/B)(1/S); where N = number of cells counted, X = number of fields of view (20), A = area of the slide covered by sample, B = area of field of view, and S = amount of sample on slide**
- **DNA was isolated from each distinct colony type using the FAST DNA spin kit**
- **16S rRNA gene was PCR amplified using 63f and 1387r primers (Relman 1993)**
- **PCR products were sequenced using the PCR primers, as well as 515fpl forward and 806r internal primers**

Figure 3. Anatomy of a Mosquito Gut (Brusca and Brusca 2003)



METHODS
Identification of Bacterial Species
- **Forward and reverse sequences were submitted to SeqMan II to generate a consensus sequence (Lasergene, Mayville, CA)**
- **Each sequence was then submitted to the CHECK_CHIMERA PROGRAM of the Ribosomal Database Project (RDP) (Maidak 1999)**
- **Closest matches were identified using BLAST (National Center for Biotechnology Information) to determine the most similar database sequences (Altschul 1997)**
- **Keefe-Bergersen Diversity Index formula (1977), TU = 1-(n/n-1)[Σ{(ni/n)2-(1/n)}]; where ni = number of individuals in each category, n = total number in all categories. The values obtained for the diversity index range between 0 and 1, with 1 representing the most diverse**

RESULTS & DISCUSSION
- **A diverse population of culturable bacterial species can be isolated from the adult female *Aedes aegypti* midgut.**
- **The bacteria identified are from four main Phyla: Actinobacteria, Proteobacteria, Firmicutes, and Bacteroidetes.**
- **We found greater diversity in the blood-fed female adults compared to the sugar-fed adults (Figure 2 and Table 2), with Actinobacteria proportionally greater than Proteobacteria in the sugar-fed.**
- **Estimated cells per midgut liquid contents ranged from 10^1 to 10^3 (Table 1)**
- **Colony forming units from single mosquito midgut contents ranged from 10^0 to 10^2 (data not shown).**
- **Culturable cells represent ~10% of total cells (Table1).**
- **Exoskeleton come from two Phyla, Actinobacteria and Proteobacteria, in ~ equal amounts (Figure 2)**

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