Characterization of the Bacterial Midgut Community of Wild-Caught *Aedes aegypti*

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ABSTRACT

It is estimated that 10^{14} microbes make the human gut home, outnumbering the total number of cells in the body. The importance of microbes in the digestive system of mammals is fairly well known. However the microbial flora inhabiting the gut of insects, particularly mosquitoes, is less well studied. Understanding the microbial flora of the mosquito may provide valuable information for understanding why some mosquitoes are more effective vectors for human disease than others. Genetic alteration of these gut microbes may also lead to novel methods for controlling mosquito populations or blocking disease transmission. In this study, the microbial gut community of field-caught larval *Aedes aegypti* (the major vector of yellow fever and dengue) was analyzed. PCR products of bacterial 16S ribosomal RNA genes, amplified from larval DNA extracts, were cloned. 68 clones were then digested with *Msp1*. 57% of the clones had unique fragment patterns, while the other 43% shared one of 11 patterns, suggesting that the mosquito gut contains a diverse consortium of bacteria.

INTRODUCTION

Mosquitoes are an important vector of disease, carrying numerous maladies ranging from dengue to lymphatic filiariasis to malaria. While insecticides are the method of choice for mosquito control, its widespread use has resulted in resistant mosquitoes. Thus, other ways to control the mosquito population must be found.

Although the life cycle of each pathogen is different, all must survive ingestion, exposure to the midgut, and migration to the salivary glands for transmission to a new host. If the pathogen cannot survive one of these steps, the mosquito cannot transfer the disease (Beerntsen *et al.* 2000.) This presents a new angle to mosquito control. Instead of controlling the mosquito population, we may be able to control the ability of the mosquito to transmit pathogens. This may release the selective pressure on mosquitoes to become resistant to control methods.

The midgut presents several barriers to pathogen transmission including dodging the mosquito's digestive enzymes, escaping the peritrophic matrix, and infecting and escaping from the gut epithelial cells (Beerntsen *et al.* 2000.) Not much is known about the microbial flora associated with the mosquito midgut and whether it might be involved in blocking the ability of the pathogen from infecting the gut epithelium (Straif *et al.* 1998.) Also, the gut flora may contain ideal candidates to be genetically modified to produce anti-pathogen compounds (paratransgenesis).

HYPOTHESIS

The bacterial gut community of wild larval *Aedes aegypti* will contain a diverse array of species and subspecies with a few being more dominant than others.

RESULTS

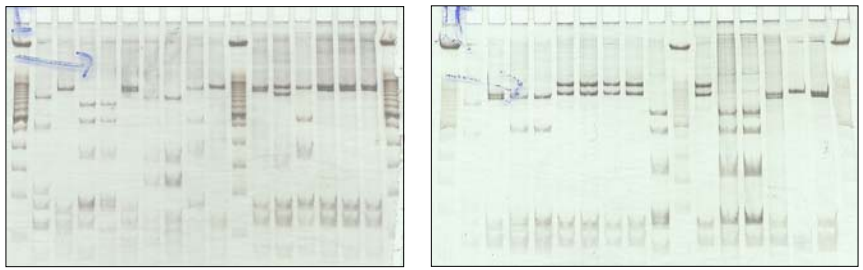


Figure 1a and b. Fragment patterns produced by clones after digestion with *Msp1* run on native polyacrylamide gels

Table 1. Example of pattern scoring sheet. Fragment patterns were compared and assigned a number. Fragment patterns from 68 clones were analyzed.

		50 bp Ladder																	
		2652-800	500-450	at 450	450-400	at 400	400-350	at 350	350-300	at 300	300-250	at 250	250-200	at 200	200-150	at 150	150-100	at 100	100-50	at/below 50	Pattern #
SNALE ID	11	1					1				1				1		1		1		1
	12		1		1												1		1		2
	13		1		1																3
	14						1				1				1		1		1		4
	15						1				1				1		1		1		4

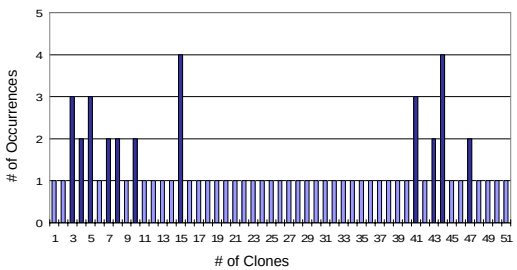


Figure 2. Pattern of clone distribution. Out of 68 clones, 39 clones had unique patterns. The other 29 clones shared one of 11 patterns..

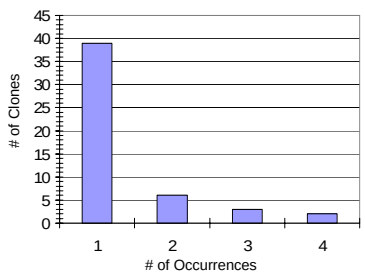


Figure 3. Number of occurrences by clone. Most clones were rare while few were abundant

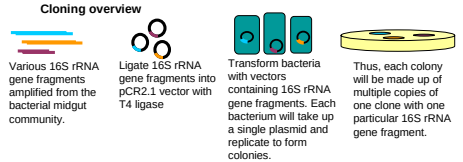
CONCLUSIONS

- 57% of the clones analyzed had unique fragment patterns suggesting that they are different species/subspecies of bacteria
- This data suggests that the bacterial flora of the mosquito gut is diverse but does contain a few species that are relatively abundant

METHODS

- DNA was isolated from larvae of *Aedes aegypti* during the summer of 2004 by J. Bushanam using the FastDNA Kit (Bio101 Systems, Carlsbad). Larvae were from Emma's Pond in Pingree Park.
- Bacterial 16S rRNA gene was amplified via polymerase chain reaction (primers 1387 reverse and 63 forward) using Ex Taq (Takara Biomed, Japan)
- Chimeras were removed by digesting 16S rRNA gene fragments with T7 endonuclease (New England BioLabs, Beverly)
- 16S rRNA gene fragments were cloned using the TA Cloning Kit (Invitrogen, Carlsbad)

Cloning overview



- Successful clones were chosen based on blue-white screening and amplification of the 16S rRNA gene fragment with toothpick PCR (primers M13 reverse and T7)
- 16S rRNA gene fragments from successful clones were cut using the *Msp1* restriction enzyme (Fisher, Fair Lawn)
- Samples were run on native polyacrylamide gels for approximately 3 hours at 10°C. A 50 bp ladder (Invitrogen, Carlsbad) was run beside samples for comparison.
- Gels were silver stained (Black & DuTeau 1997)
- Gels were rinsed in dH2O and allowed to dry overnight before pattern analysis

FUTURE DIRECTIONS

- Compare RFLP patterns among lab-reared adult and larval *A. aegypti*, wild-caught adult and larval *A. aegypti*, and environmental samples. This will allow us to see if there is a difference between the bacterial flora of adult and larval mosquitoes. This may also give us an idea of whether the bacterial flora is co-evolved with the mosquito or if the flora is acquired from the environment during larval development.
- Identify clones by comparing RFLP patterns from this study with a database of RFLP patterns from previously sequenced 16S rRNA gene fragments.

SOURCES

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