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Microbial Communities within Field-Collected *Culiseta melanura* and *Coquillettidia perturbans*

E. S. Andrews[1], **G. Xu**, and **S. M. Rich**
Laboratory of Medical Zoology, Department of Plant, Soil and Insect Science, University of Massachusetts, 102B Fernald Hall, 270 Stockbridge Road, Amherst, MA 01003

Abstract

Although mosquitoes are well known vectors of human and animal diseases, pathogens are only minor components of their total endogenous microbial communities. The midguts of many insects, including mosquitoes, contain diverse microbial communities. In this study, we used denaturing gradient gel electrophoresis to identify the diversity of bacteria in field-collected adult female *Culiseta melanura* (Diptera: Culicidae) (Coquillett) and *Coquillettidia perturbans* (Diptera: Culicidae) (Walker). Few significant differences in bacterial fauna between the two mosquito species were found, but the results suggest that host life history may be a determinant of the endogenous bacterial communities in mosquitoes. The dominant bacteria in the current study are frequently identified as major components of other mosquito species' microbial flora, suggesting the establishment of a stable association between the mosquitoes and the microbes after initial acquisition from the environment.

Keywords

Culiseta melanura; *Coquillettidia perturbans*; DGGE; 16S rDNA; microbial community

Introduction

Studies of environmental microbiomes provide unprecedented insights to how microbial communities function and the roles of individual species. Like the gut of vertebrate species (including humans), the insect gut can be thought of as an environment with a thriving microbial community. Determining microbial contents of insect microbiomes can facilitate a heightened understanding of the ecology of vector-borne diseases and lead to new control methods for the insects that spread plant and human pathogens (Douglas, 2007).

Although some of the non-pathogenic organisms that dominate the mosquito microbiome have important roles in digestion and fecundity (Fouda *et al.*, 2001) and mediating the transmission of human pathogens, comparatively little is known about the overall composition and dynamics of mosquito microbiomes: factors that likely affect the dynamics of the diseases that mosquitoes transmit (Cirimotich *et al.*, 2011). The microbial communities that reside within mosquitoes may be influenced by behavior, genetics, and

[1]Present address: Entomology Department, S-225 Agricultural Science Center North, University of Kentucky, Lexington, KY 40546

mosquito life histories. Hence, the microbiomes may be regarded as proxies for certain aspects of mosquito biology.

Previous studies identified many new types of bacteria associated with mosquitoes and extended our knowledge of mosquito-associated microbial communities, especially with respect to differences before and after blood meals (Demaio *et al.*, 1996) and between lab-reared and field-collected insects (Rani *et al.*, 2009). Bacteria may be found in reproductive tissues and several somatic tissues besides the midgut (Zouache *et al.*, 2009; Saridaki & Bourtzis, 2010) and some bacteria may play important roles in the specific tissues in which they reside. Therefore, the aim of the present study is to examine the overall microbial communities within whole mosquitoes in a series of comparative studies.

Most previous mosquito-microbiome studies to date have focused on *Anopheles* spp., *Culex* spp., or *Stegomyia* (formerly *Aedes*) spp., which are the most common disease vectors in the developing world. In this study polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) was used to describe, characterize, and compare the microbial communities associated with adult females of two mosquito species: *Culiseta melanura* (Diptera: Culicidae) (Coquillet) and *Coquillettidia perturbans* (Diptera: Culicidae) (Walker). Both species are important vectors of North American arboviruses including Eastern Equine Encephalitis virus (EEEV) and West Nile virus. In addition, *Cs. melanura* and *Cq. perturbans* were chosen because of their seasonal and spatial proximity to each other and the differences between their hosts and larval habitats. *Culiseta melanura* feeds primarily on birds and resides in swamps, where its larvae are well protected in crypts formed by the roots of trees (Molaei & Andreadis, 2006). *Coquillettidia perturbans* opportunistically feeds on both birds and mammals and lays its eggs in marshes dominated by dense aquatic plants (Apperson *et al.*, 2004). The body segments of the mosquito compartmentalize organs; reproductive tissues reside solely in the abdomen, the midgut is present in both thorax and abdomen and the salivary glands are present in the thorax (Clements, 1992). Thus, the bacterial communities were examined within the thorax and abdomen separately to determine whether tissue-specific differences could be detected. The goals of this study were to identify and compare the bacteria associated within these lesser-studied mosquitoes. It is anticipated that the findings of the current study will be useful in future efforts to determine the specific ecological roles of different bacteria and how these bacteria compositions may reveal life histories of these important vectors.

Materials and Methods

Sampling

A total of 102 *Cs. melanura* and 147 *Cq. perturbans* adult female mosquitoes were collected using a New Jersey light trap (model 1112, John W. Hock Company, Gainesville, FL) in Raynham, Massachusetts, Bristol County, USA on seven dates between June and September 2006. Mosquitoes were transferred into 0.5-ml microcentrifuge tubes and stored at −20°C.

DNA Extraction

Individually mosquitoes were surface sterilized by rinsing them twice with 70% ethanol and once with distilled water. Each mosquito was placed on a sterilized microscope slide and separated the head, thorax, and abdomen using a single-use 20-gauge needle. The abdomen and thorax were placed into separate 1.5-ml microcentrifuge tubes and stored at −80°C. The DNA was extracted from the individual mosquito parts using Epicentre Master Complete DNA and RNA purification kits (Epicentre Technologies, Madison, WI) following the manufacturer's protocols. Total DNA from each insect was dissolved in 30 μl water.

PCR-DGGE

For PCR-DGGE analyses, bacterial 16S-rRNA fragments (Muyzer *et al.*, 1993; Schabereiter-Gurtner *et al.*, 2003) were amplified using a GC-clamped forward primer, 341f (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G CCT ACG GGA GGC AGC AG-3′), and reverse primer 518r (5′-ATT ACC GCG GCT GCT GG-3′). The primers targeted fragments ranging from 150 to 200 bp, which are ideal for DGGE. All reactions were carried out in 40-μl volumes containing 1 μl DNA, 1× buffer, 2.5 μM $MgCl_2$, 0.2 mM dNTP, 0.2 μM of each primer and 2 U of Taq polymerase (Promega, Madison, WI). All master mixes were treated with ultraviolet light for 10 min prior to the addition of Taq polymerase and DNA. DNase/RNAse free molecular-grade water was used as a negative control. PCRs were performed in an Eppendorf ep mastercycler (Eppendorf, Westbury, NY) using the following program: 94°C for 1 min; 3 cycles of 94°C for 15 s, 61°C for 15 s, and 72°C for 1 min; followed by 3 cycles of 94°C for 15 s, 58°C for 15 s, and 72°C for 1 min; then 28 cycles of 94°C for 15 s, 55°C for 15 s, and 72°C for 1 min; and finally, an extension step at 72°C for 6 min. To confirm that the PCR had resulted in the correct-sized fragments, 2 μl of each of the PCR products on a 1% (w/v) agarose gel were separated and stained them with ethidium bromide for visualization. PCR products that did not yield visible bands were not separated using DGGE and the corresponding mosquito samples were removed from the dataset.

The PCR products were separated by DGGE using a D-Code System (Bio-Rad, Hercules, CA) in 10% (v/v) polyacrylamide (37.5:1 acrylamide:bisacrylamide) gels containing a linear denaturing gradient ranging from 40–70% of urea and formamide (100% denaturant contains 7 M urea and 40% v/v formamide). Electrophoresis of the PCR products (300 ng/μl) was performed in 1.25× Tris-acetate-EDTA running buffer at a constant temperature of 56°C at 70 V for 17 h. To facilitate comparisons between gels, a marker was generated using a subsample of bands isolated from the mosquitoes and was run on each gel. After completion of electrophoresis, the gels were stained with 0.1 μl/ml SYBR gold (Invitrogen Corporation, Carlsbad, CA) for 40 min.

Amplicon bands of corresponding electrophoretic mobility were excised and placed them into individual microcentrifuge tubes containing 100 μl water and then eluted the DNA at 4°C overnight. The eluted amplicons via PCR were re-amplified using the same primers and parameters as described above, with 2 μl eluted DNA as template. PCR products were purified and sequenced using a modified 341f oligonucleotide (5′-CCT ACG GGA GGC AGC AG-3′), without the GC-clamp, on an ABI 3130XL genetic analyzer (Applied

Biosystems, Foster City, CA). For identification purposes, bands were assigned an arbitrary number corresponding to their electrophoretic mobility, with the smaller numbers corresponding to the bands of slower mobility. To determine the identity of putatively unique bands, multiple bands of identical mobility were sequenced when present.

Sequence and Statistical Analysis

Sequencher (Gene Codes, Ann Arbor, MI) was used to edit and assemble the nucleotide sequences. The finished nucleotide sequences for matches were compared against known species using the BLASTn algorithm (http://www.ncbi.nlm.nih.gov/BLAST) and assigned identities to the bacterial genera (Table 1). Two bands corresponding to *Cq. perturbans* and *Cs. melanura* 18S rRNA were excluded from the dataset. The presence/absence of the 57 bands was recorded in each mosquito sample. Chi-squared tests were performed to analyze the differences between the microbiomes of *Cs. melanura* and *Cq. perturbans*, and between the thorax and abdomen subsamples within each species.

Results

A total of 57 unique bands separated by DGGE were identified based on relative mobility. Two of the bands corresponded to *Cq. perturbans* and *Cs. melanura* 18S rRNAs. Although mosquito rRNA bands were from subsequent analyses, their consistent amplification in all mosquito samples served as a positive indicator of the presence of extracted DNA.

Of the 57 unique bands, 47 contained single fragments that could be directly sequenced. All of the sequenced bands yielded strong BLAST hits to known bacterial species. The nucleotide-sequence identities ranged from 88–100%, but most matches had identities greater than 99% (Table 1). Ten of the extracted gel bands contained multiple nucleotide-sequence fragments, as revealed in the chromatograms from the sequencing runs. Bands containing two or more sequence fragments could not be directly sequenced. In four instances, bands with apparently identical DGGE motilities from different mosquitoes yielded unique sequences with substantially different BLAST results (Table 1).

The microbial sequences associated with adult female *Cs. melanura* and *Cq. perturbans* revealed the presence of both gram-negative and gram-positive bacteria representing six classes and 15 families (Table 2). The most ubiquitous bacterial sequences corresponded to the *Enterobacteriaceae* family, which comprised the majority of the unique bands (35%). The genera present in the greatest numbers of individual mosquitoes of both species were *Erwinia* (49.4%), *Acinetobacter* (33.1%), *Asaia* (26.4%), *Pseudomonas* (25.1%), *Enterobacter* (29.7%), and *Enterococcus* (25.9%) (Table 2). *Wolbachia* was the dominant genus in *Cq. perturbans*, having been found in 66% of individuals. *Wolbachia* was only present in *Cq. perturbans* and was confined solely to the abdomen of that species. *Pseudomonas* sp. was only present in *Cs. melanura*, with a similar distribution in the thorax and abdomen. Chi-squared comparisons of frequencies of the remaining bacterial taxa present in both *Cs. melanura* and *Cq. perturbans* revealed no significant differences. Likewise the frequency distribution for these taxa were the same for thorax and abdomen subsamples within each species.

Discussion

Bacteria that associate with insects are involved in key biological processes including generating nutrients to augment poor food resources, detoxifying plant allelochemicals, producing digestive enzymes, and aiding in the prevention of enteric infections (Dillon & Dillon, 2004). Bacteria can also protect insects from parasitoids, parasitic nematodes, and fungal pathogens, and may contribute to overcoming environmental stresses such as heat and suboptimal nutrition (Kikuchi, 2009; Oliver *et al.*, 2010). The recognition of the importance of arthropod symbionts and the increased availability of culture-independent molecular tools has led to many studies investigating the microbial communities associated with insects (Shi *et al.*, 2010). Such studies helped to distinguish obligate symbionts from those that play more transient roles.

Intra- and interspecies bacterial diversity

Using PCR-DGGE, the bacterial diversity was characterized among female *Cs. melanura* and *Cq. perturbans* mosquitoes. The most abundant bacterial genera identified by our study were *Pseudomonas* (25.1%), *Acinetobacter* (33.1%), *Enterobacter* (29.7%), *Enterococcus* (25.9%), and *Asaia* (26.4%). Although reportedly common among other mosquito species, *Bacillus*, *Serratia*, and *Pantoea* were found in only 12.1%, 14.6%, and 10%, respectively, of mosquitoes in our study (Table 2). All of the highly abundant genera identified by our study were previously isolated from mosquitoes using both culture-dependent and culture-independent techniques; and are known to infect a diverse array of mosquito species with vastly different life histories from around the globe (Demaio *et al.*, 1996; Straif *et al.*, 1998; Crotti *et al.*, 2009).

Significant interspecific occurrence was observed for only two bacterial taxa among the set of all detected bacteria. One *Pseudomonas* isolate was more common in *Cs. melanura*, while *Wolbachia* was more frequent in *Cq. perturbans*. Intraspecific comparisons between the thorax and abdomen showed that *Wolbachia* resides exclusively in the abdomens of *Cq. perturbans*. However, most of the bacteria that observed appear in similar abundances in the two mosquito hosts, suggesting that mosquitoes acquire the majority of their bacteria from the same environmental sources.

There was considerable intraspecific variation in the microbiota of both mosquito species, i.e. between individuals within a species. This may be to due to natural variation in microbial flora or variation in the number or timing of blood meals. A protein-rich blood bolus is an excellent nutrient source for bacteria that usually maintain low population densities in the mosquito midgut. Bacterial population sizes can increases 70 to 16,000-fold in adult mosquitoes 48 h after a blood meal (Demaio *et al.*, 1996). The bacterial populations decrease and return to pre-blood meal levels within a 3-day period (Demaio *et al.*, 1996). The variation in the microbiota within each mosquito species could be due to spikes in the growth of certain species following a recent blood meal. Future studies should measure mosquito age and feeding status to look for correlations between bacterial species and number of blood meals.

Bacteria originating from plant or environmental sources

Our results suggest that many of the bacteria found in mosquitoes are acquired from the environment while feeding on something other than blood. The literature supports the observation that mosquitoes may acquire bacteria from their environment. For example, the bacterium, *Thorsellia anopheles* is the dominant bacterium in the midgut of adult *Anopheles gambiae* captured in Kenya (Briones *et al.*, 2008). This bacterium also resides in an aquatic rice paddy environment nearby to where the mosquitoes were captured, indicating that mosquitoes can acquire bacteria from the environment. In addition, field-collected mosquitoes, as compared to lab-reared mosquitoes, had a higher abundance of the same bacteria types that were observed in our study (Rani *et al.*, 2009). Hence, the microbial community may serve as a record of an individual's feeding habits. Mosquitoes exploit different nutritional sources and habitats throughout their development (Clements, 1992). As larvae, they consume microbes in their aquatic environment. They move to terrestrial habitats and feed upon plant nectar and/or vertebrate blood as adults. Based on previous studies with mosquitoes (Lindh *et al.*, 2005; Rani *et al.*, 2009), the majority of bacteria observed appear to have an environmental or plant origin, indicating that they were most likely acquired directly from the mosquito's habitat.

The current study documents the first report of *Erwinia* in mosquitoes, with half of the sampled mosquitoes being infected (Table 2). *Erwinia* includes phytopathogens that cause soft rot and fire blight in many plants (Permbelon & Kelman, 1980). Some *Erwinia* species can be vectored by *Drosophila* directly feeding on infected fruit, or by pollinators collecting nectar from flowers (Permbelon & Kelman, 1980; Hildebrand *et al.*, 2012). *Erwinia* has been isolated from several plant feeding insects, including thrips and aphids (Harada *et al.*, 1997; de Vries *et al*., 2001). *Erwinia* is most likely acquired while feeding on plants, and consequently, its prevalence among our mosquitoes may reflect the mosquitoes' degree of nectar-feeding behavior. Some *Erwinia* are also obligate endosymbionts, such as "*Ca.* Erwinia dacicola" in the olive fly (Tephritidae: Diptera) (Capuzzo *et al.*, 2005). The exact nature and mode of *Erwinia* infection in *Cs. melanura* and *Cq. perturbans* will require further investigation.

An *Asaia* species with 99% nucleotide identity to flower-dwelling *Asaia bogorensis* and *Asaia siamensis* (Katsura *et al.*, 2001) was in 23.2% of *Cs. melanura* and 28.5% of *Cq. perturbans* (Table 2). This acetic acid bacterium was originally isolated from plants but has since been found to infect a variety of insects that feed on plant tissue, phloem, or nectar (Chouaia *et al.*, 2010). *Asaia* was isolated from several mosquito species; including *An. stephensi*, *An. maculipennis*, *An. gambiae*, *St. aegypti*, and *St. albopicta*; and stably associates with the salivary glands, midgut, and reproductive tissues of adults (Favia *et al.*, 2007; Crotti *et al.*, 2009). The ability of *Asaia* to be transferred both horizontally and vertically makes this bacterium a possible candidate for novel mosquito control strategies, wherein the bacterium could be modified to express molecules that directly target human pathogens within the mosquito (Favia *et al.*, 2008).

Vertebrate host-associated bacteria

Although many mosquito-associated bacteria are likely obtained from environmental sources (e.g., standing water and flower nectar), some bacteria were probably acquired while feeding on blood or tissue of associated vertebrate hosts. One of those bacterial species is *Ewingella americana*, a bacterium originally cultured from Eastern Bluebird feathers (Shawkey *et al.*, 2005). It was present in *Cs. melanura*, an ornithophilic mosquito known to feed on bluebirds (Molaei & Andreadis, 2006). It was also found in the more opportunistic bird ectoparasite *Cq. perturbans*, consistent with the catholic host choice of this species and its role as a bridge vector for avian-borne pathogens. The occurrence of *Ewingella americana* could prove useful as a proxy for determining host feeding behavior.

Also present was *Bartonella* spp., a gram-negative bacteria that infects mammalian blood cells. Mammals can act as reservoirs of *Bartonella*, and some insects can serve as vectors for members of this genus (Breitschwerdt & Kordick, 2000). *Bartonella washoensis*, previously isolated from California ground squirrels (Kosoy *et al.*, 2003), was found in both *Cs. melanura and Cq. perturbans*. Although *Cq. perturbans* is known to feed regularly on mammals and is therefore likely to have acquired *B. washoensis* while doing so, *Cs. melanura* feeds primarily on birds and would seem less likely to have acquired the bacterium by feeding on an infected mammal. *B. washoensis* may have been transmitted from mammals to birds by *Cq. perturbans* and then acquired from the birds by *Cs. Melanura*, thus demonstrating a bridge vector pattern opposite to that regarded as most important in arbovirus epizootiology.

Wolbachia pipientis, a known mosquito endosymbiont, was present in 66% of *Cq. perturbans* (Table 2). In contrast, there is no evidence of *Wolbachia* in *Cs. melanura*. This is consistent with previous reports that have observed *Wolbachia* within other *Coquillettidia* species, but not *Culiseta* (Ricci *et al.*, 2002). *Wolbachia* is a maternally inherited rickettsia-like α-*Proteobacteria* that has been associated with various arthropods. It causes reproductive abnormalities such as male killing, cytoplasmic incompatibility, parthenogenesis, and feminization (Werren *et al.*, 2008). To our knowledge, the current study is the first documentation of *Wolbachia* in *Cq. perturbans*. Some *Wolbachia* strains are under consideration as control agents for mosquito-borne diseases such as Dengue fever and malaria. *Wolbachia* are known to reduce mosquito population sizes (Brelsfoard & Dobson, 2012). *Wolbachia* also has potential utility to introduce transgenic traits into susceptible mosquito populations and perhaps to interfere with pathogen development (Hoffmann *et al.*, 2011). It has been shown that *Wolbachia* infections can provide a protective effect against West Nile Virus infection within the mosquito (Glaser & Meola, 2010).

Whole microbiome characterization is powerful tool to identify important aspects of the ecology and life history of arthropod vectors. *Cs. melanura* and *Cq. perturbans* are both important arbovirus vectors, and cataloging their microbial communities will help us to better understand the roles that they play in enzootic and epizootic transmission of diseases such as WNV, EEEV and Dengue fever. *Cs. melanura* feeds primarily on birds and is therefore an important maintenance vector of EEEV; while *Cq. perturbans* has a broader

host range and plays a fundamental role as a bridge vector from those enzootic cycles into humans and companion animals. Because the microbial communities within insects are registers of the past activities and habitats of the insects, they can serve as markers for assessing the dynamics of disease transmission and the risk of human exposure to disease. In some cases, the microbiome may reflect past host feeding choices. Other bacteria are possibly environmentally acquired and among these, there may be several for which the association with mosquitoes is merely coincidental. But some of the species detected in our survey, especially a first report of *Wolbachia*, are clearly stable symbionts. How these species interact with each other, the roles they play in the physiology of their hosts, and how they affect the transmission of known infectious agents are subjects for further investigation.

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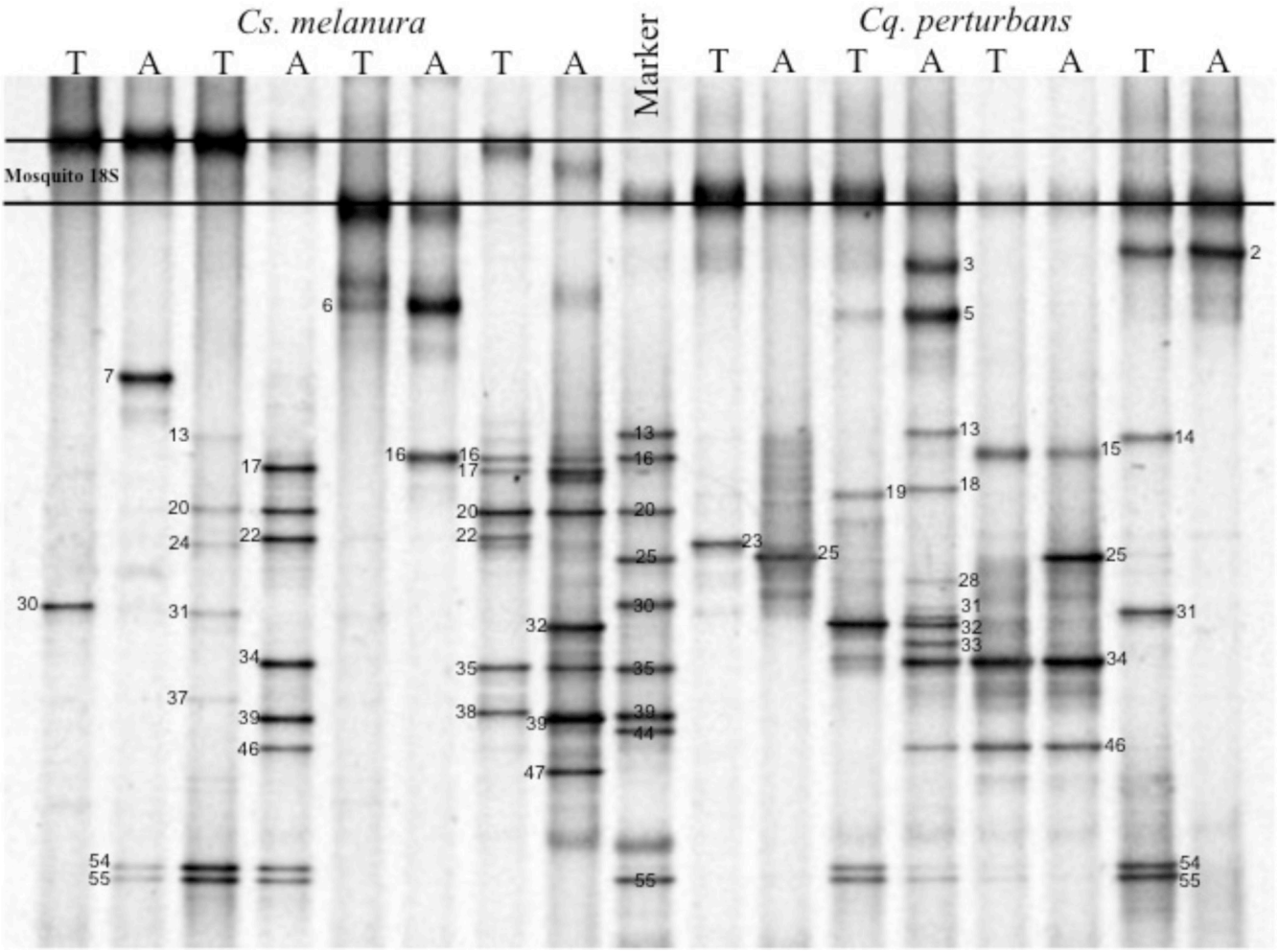

Fig 1.

Denaturing gradient gel electrophoretic profile of 16S rDNA gene fragments amplified by polymerase chain reaction from four representatives of each mosquito species, *Cs. melanura* and *Cq. perturbans*. All bands recovered from the mosquitoes in this study are not represented. The concentration of denaturing agents in the gradient gel range from 40% to 70%, top to bottom. Numbers were arbitrarily assigned to bands based on electrophoretic mobility, and each corresponds to a different bacterial identity (Table 1). T: thorax. A: abdomen

Table 1

Taxonomic identities of bands separated by denaturing gradient gel electrophoresis based on 16S-rRNA gene sequences

Class and family	Band no.	BLASTn nearest similarity (accession no.)	Query length (bp)	% similarity
α-*Proteobacteria*				
Anaplasmataceae	25	*Wolbachia* endosymbiont (GU592789)	128	100
Bartonellaceae	51	*Bartonella washoensis* (AB519066)	133	100
Acetobacteraceae	54	*Asaia* sp. (FN821397)	135	100
Acetobacteraceae	55	*Asaia* sp. (FN821397)	128	100
Bacilli				
Enterococcaceae	12	*Enterococcus silesiacus* (HM209744)	160	100
Enterococcaceae	13	*Enterococcus silesiacus* (HM209744)	152	99
Carnobacteriaceae	20[a]	*Carnobacterium* sp. (HM141122)	160	100
Streptococcaceae	21	*Lactococcus lactis* (HM218820)	161	100
Enterococcaceae	26	*Enterococcus* sp. (GU905013)	160	99
Bacillaceae	31	*Bacillus* sp. (HQ113214)	160	99
Leuconostocaceae	32[a]	*Fructobacillus ficulneus* (NR025153)	159	97
Mollicutes				
Spiroplasmataceae	2	*Spiroplasma* (FJ57213)	160	97
Spiroplasmataceae	6	*Spiroplasma culicicola* (NR025701)	160	100
Flavobacteria				
Flavobacteriaceae	14	*Chryseobacterium* sp. (EF442766)	155	100
Spirochaetes				
Spirochaetaceae	9	*Spironema culicis* (AF166259)	161	99
Spirochaetaceae	10	uncultured spirochete (AY605143)	161	88
γ-*Proteobacteria*				
Enterobacteriaceae	15	*Erwinia rhapontici* (HM008943)	160	95
Enterobacteriaceae	27	*Enterobacter* sp. (HQ014913)	153	99
Enterobacteriaceae	29	*Enterobacter* sp. (FN555398)	153	100
Enterobacteriaceae	30	*Enterobacter* sp. (AM748792)	157	100
Enterobacteriaceae	32[a]	*Ewingella americana* (FN811904)	158	99
Enterobacteriaceae	33	*Enterobacter* sp. (HQ014913)	153	100
Enterobacteriaceae	34	*Erwinia* sp. (EF016525)	159	99
Enterobacteriaceae	35	*Pantoea* sp. (AY659872)	157	99
Enterobacteriaceae	36	*Brenneria quercina* (HM196337)	158	100
Enterobacteriaceae	37	*Hafnia* sp. (HM489947)	153	99
Enterobacteriaceae	38	*Erwinia rhapontici* (AB087715)	159	100
Enterobacteriaceae	39	*Pantoea* sp. (AY659872)	157	100

Class and family	Band no.	BLASTn nearest similarity (accession no.)	Query length (bp)	% similarity
Enterobacteriaceae	40	*Ewingella americana* (FN811903)	158	100
Enterobacteriaceae	41	*Erwinia persicina* (EF693792)	158	99
Enterobacteriaceae	42	*Serratia* sp. (HM099664)	158	99
Enterobacteriaceae	44	*Yersinia mollaretii* (HM031473)	157	99
Enterobacteriaceae	46[a]	*Erwinia rhapontici* (AB087715)	158	99
Enterobacteriaceae	46[a]	*Erwinia* sp. (HM462346)	158	99
Enterobacteriaceae	47[a]	*Ewingella americana* (DQ383802)	153	100
Enterobacteriaceae	47[a]	*Serratia* sp. (HM242285)	160	100
Enterobacteriaceae	48	*Enterobacter* sp. (GU086387)	160	99
Enterobacteriaceae	49	*Rahnella* sp. (GU068647)	157	98
Halomonadaceae	28	*Zymobacter palmae* (AF211871)	160	95
Moraxellaceae	18	*Acinetobacter* (DQ314740)	160	98
Moraxellaceae	19	*Acinetobacter* sp. (AJ633639)	152	95
Moraxellaceae	22	*Acinetobacter* sp. (GQ464378)	152	96
Moraxellaceae	23	*Acinetobacter* sp. (DQ314740)	155	99
Pseudomonadaceae	16	*Pseudomonas* sp. (AB548846)	154	100
Pseudomonadaceae	17	*Pseudomonas* sp. (HQ166064)	153	100
Pseudomonadaceae	20[a]	*Pseudomonas* sp. (AB548838)	153	100
Pseudomonadaceae	24	*Pseudomonas* sp. (AB548840)	153	100
Uncultured bacteria				
	3	uncultured bacterium (DQ980728)	158	96
	5	uncultured bacterium (DQ343058)	158	96
	8	uncultured bacterium (DQ343058)	159	97
	50	uncultured bacterium (AY627574)	160	99

[a]Bands migrate to identical positions on the gel.

Table 2

Distribution of bacteria genera among *Cs. melanura* and *Cq. perturbans* based on the presence/absence of denaturing gradient gel electrophoresis bands.

Class and family	Genus	Both mosq. (%) n=239	*Cs. melanura* (%) 95	Thorax (%) 90	Abdomen (%) 89	*Cq. perturbans* (%) 144	Thorax (%) 132	Abdomen (%) 141
α-*Proteobacteria*								
Anaplasmataceae	*Wolbachia*	95 (39.7)	0	0	0	95 (66)	0	95 (67.3)
Bartonellaceae	*Bartonella*	10 (4.2)	4 (4.2)	1 (1.1)	4 (4.5)	6 (4.2)	3 (2.3)	5 (3.5)
Acetobacteraceae	*Asaia*	63 (26.4)	22 (23.2)	19 (21.3)	13 (14.8)	41 (28.5)	40 (30.3)	26 (18.4)
Bacilli								
Bacillaceae	*Bacillus*	29 (12.1)	13 (13.7)	11 (12.4)	8 (9.1)	16 (11.1)	14 (10.6)	5 (3.5)
Carnobacteriaceae	*Carnobacterium*	20 (8.4)	14 (14.7)	3 (3.4)	13 (14.8)	6 (4.2)	4 (3.0)	5 (3.5)
Enterococcaceae	*Enterococcus*	62 (25.9)	29 (30.5)	14 (15.7)	22 (25.0)	33 (22.9)	21 (15.9)	18 (12.8)
Leuconostocaceae	*Fructobacillus*	24 (10)	8 (8.4)	5 (5.6)	8 (9.1)	15 (10.4)	10 (7.6)	12 (8.5)
Streptococcaceae	*Lactococcus*	16 (6.7)	9 (9.5)	6 (6.7)	7 (8.0)	7 (4.9)	3 (2.3)	5 (3.5)
Mollicutes								
Spiroplasmataceae	*Spiroplasma*	5 (2.1)	2 (2.1)	1 (1.1)	1 (1.1)	3 (2.1)	1 (0.8)	3 (2.1)
Flavobacteria								
Flavobacteriaceae	*Chryseobacterium*	21 (8.8)	11 (11.6)	8 (9.0)	6 (6.8)	11 (7.6)	8 (6.1)	6 (4.3)
Spirochaetes								
Spirochaetaceae	*Spironema*	1 (0.4)	1 (1.1)	0	1 (1.1)	0	0	0
Spirochaetaceae	uncultured spirochete	4 (1.7)	2 (2.1)	0	2 (2.3)	2 (1.4)	2 (1.5)	1 (0.71)
γ-*Proteobacteria*								
Enterobacteriaceae	*Brenneria*	8 (3.3)	4 (4.2)	2 (2.2)	4 (4.5)	4 (2.8)	2 (1.5)	3 (2.1)
Enterobacteriaceae	*Enterobacter*	71 (29.7)	36 (37.9)	17 (19.1)	29 (33)	42 (29.1)	31 (23.5)	30 (21.3)
Enterobacteriaceae	*Yersinia mollaretii*	22 (9.2)	11 (11.6)	7 (7.9)	8 (9.1)	11 (7.6)	8 (6.1)	3 (2.1)
Enterobacteriaceae	*Erwinia*	118 (49.4)	57 (60.0)	28 (31.5)	52 (59.1)	61 (42.4)	50 (37.9)	46 (32.6)
Enterobacteriaceae	*Ewingella americana*	40 (16.7)	17 (17.9)	7 (7.9)	16 (18.2)	22 (15.3)	15 (11.4)	16 (11.3)
Enterobacteriaceae	*Hafnia*	8 (3.3)	4 (4.2)	4 (4.5)	4 (4.5)	4 (2.8)	4 (3.0)	4 (2.8)
Enterobacteriaceae	*Pantoea*	24 (10.0)	12 (12.6)	6 (6.7)	9 (10.2)	12 (8.3)	7 (5.3)	10 (7.1)

Class and family	Genus	Both mosq. (%) n=239	*Cs. melanura* (%) 95	Thorax (%) 90	Abdomen (%) 89	*Cq. perturbans* (%) 144	Thorax (%) 132	Abdomen (%) 141
Enterobacteriaceae	*Rahnella*	17 (7.1)	6 (6.3)	3 (3.4)	6 (6.8)	11 (7.6)	7 (5.3)	10 (7.1)
Enterobacteriaceae	*Serratia*	35 (14.6)	22 (23.2)	8 (9.0)	31 (35.2)	13 (9.0)	10 (7.6)	8 (5.7)
Halomonadaceae	*Zymobacter palmae*	8 (3.3)	2 (2.1)	0	2 (2.3)	6 (4.2)	4 (3.0)	4 (2.8)
Moraxellaceae	*Acinetobacter*	79 (33.1)	37 (38.9)	24 (27)	26 (29.5)	42 (29.1)	36 (27.3)	23 (16.3)
Pseudomonadaceae	*Pseudomonas*	60 (25.1)	38 (40.0)	10 (11.2)	35 (39.8)	22 (15.3)	11 (8.3)	16 (11.3)
Uncultured bacteria								
	uncultured bacterium	33 (13.8)	13 (13.7)	7 (7.9)	11 (12.5)	20 (13.9)	13 (9.8)	9 (6.4)