Gut Bacterial Community of the Lone Star Tick (*Amblyomma americanum*)

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Abstract

Ticks are obligate blood feeding ectoparasites and vectors of several animal and human pathogens (Williams-Newkirk et al., 2014). Ticks not only carry pathogens but also a bacterial community with commensal and symbiotic relationships (Bonnet et al., 2017). In other arthropod vectors, the gut microbiome influences their competence for pathogens they transmit. In this study, we used a culturing approach to assess the prevalence, abundance, and diversity of bacteria in the gut of adult lone start ticks (*Amblyomma americanum*) (n = 31) collected in Kansas. We were unable to culture any bacteria from 42% of ticks and the mean bacterial concentration was only 11.8 ± 5.4 CFU/tick. Amplification and sequencing of 16S rDNA of bacterial isolates (n= 36) revealed a low bacterial diversity represented by 3 phyla: Actinobacteria (50%), Firmicutes (40%), Proteobacteria (10%) and 16 genera with a heavy bias toward Gram-positive and catalase-positive bacterial species.

Purpose

To asses the prevalence, abundance, and diversity of the bacterial community in the gut of the lone star adult ticks collected from the field.

Hypothesis: Culturable bacterial communities in wild lone star tick (*A. americanum*) are abundant and include more then 20 genera from about 10 different phyla with equal prevalence of Gram-negative and Gram-positive bacteria and with greater abundance of catalase-positive isolates.

Study System

The lone star tick is a common species in the Midwest and eastern United States (Figure 1.) and a known vector of pathogens causing Rocky mountain spotted fever, tularemia, ehrlichiosis, southern tick associated rash illness and a Lyme-like disease (Sayler et al., 2016). Using a culture-independent approach, several studies have previously shown a diverse and abundant bacterial community in the gut (25 genera from 10 different phyla) as well as several intracellular symbionts (Trout Fryxell and DeBruyn et al., 201). However, the role of these bacteria in the tick fitness and vector competence for pathogens is largely unknown. In this study, we used a culturing approach to: a) assess the bacterial prevalence, abundance, and diversity in the midgut of *A. americanum* and b) obtain bacterial isolates that can be used for: 1) evaluation of their significance in tick biology and vector competence, and 2) studying tick midgut immunity and microbial homeostasis.

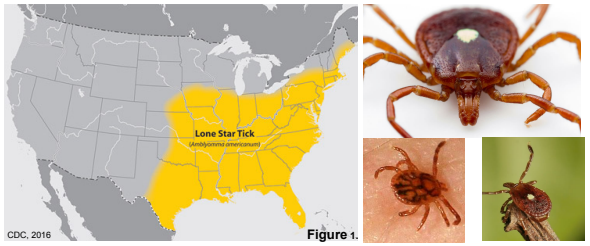


Figure 1.

Methods and Experimental Design

Adult lone star ticks (25 females, 6 males) were collected from northeastern Kansas (Konza Prairie Biological Research Station) and southeastern Kansas (around Pittsburg) in summer 2017. Ticks were surface sterilized using 0.5% sodium hypochlorite, 70% ethanol, and washed with sterile water (mouthparts and anus of ticks were sealed with a glue to prevent access of chemicals to the gut lumen during sterilization). Individual ticks were processed by cutting and homogenizing whole bodies in phosphate buffer saline, spread plated on trypticase soy agar, and incubated at 26˚C for 72 hrs. Bacterial isolates were counted and morphologically distinct colonies were sub-cultured and identified by amplification and sequencing of 16S rDNA using universal eubacterial primers. Catalase was detected in individual isolates by the hydrogen peroxide test. Sequences were manually edited and identified using BLASTn search of the GeneBank database at NCBI. Sequence editing, alignment, and phylogenetic analysis were done in MEGA7: Molecular Evolutionary Genetics Analysis version 7.0.26.

Results

A large portion (42%) of ticks yielded no bacterial growth. The mean CFU of positive ticks was 11.8±5.4 CFU per tick and only three bacterial phyla (Actinobacteria, Firmicutes, Proteobacteria) were detected (Figure 2). Sixteen bacterial genera were identified from 15 different families (Figure 3). The majority (88%) of the isolates were Gram-positive and 96% were positive for catalase.

Figure 2. Bacterial diversity in the gut of *Amblyomma americanum* on a phylum level

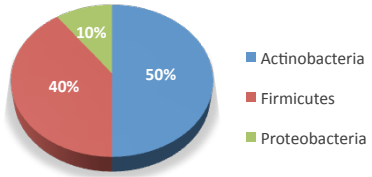
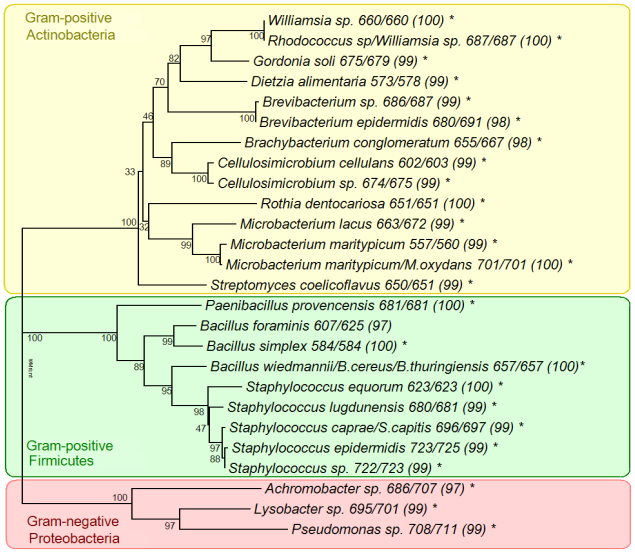


Figure 3. Neighbor-joining phylogenetic tree of bacterial isolates from *A. americanum*

Bootstrap 500. Numbers behind taxa indicate sequence length and % similarity in brackets.
* indicates catalase positive taxa



Discussion and Conclusions

Great prevalence and diversity of bacterial communities in *A. americanum* have been reported previously in several studies using culture-independent approaches (Clay et al., 2008; Trout-Fryxell et al., 2016; Williams-Newkirk et al., 2014)

In this study, we aimed to assess the prevalence, abundance, and diversity of bacteria in the gut of *A. americanum* by a culturing approach in order to obtain bacterial isolates that could be used for manipulation of the gut microbiome and assessment of their importance in tick fitness, gut colonization, gut microbial homeostasis, and vector competence for pathogens.

Surprisingly, the bacterial prevalence (58%), abundance (11.8±5.4 CFU/tick), and diversity (16 genera from 3 phyla) were very low and with a heavy bias (88%) toward Gram-positive taxa. The majority (96%) of detected bacteria were catalase-positive. Most bacterial species were represented by taxa typical for a soil microbiome, suggesting that they were acquired from the environment and not from a host during blood feeding. Our results also indicate that *A. americanum* epithelial immunity functions to control Gram-negative and catalase-negative bacteria and it is therefore likely based on reactive oxygen species (ROS) generated by the NADPH dual oxidase (DUOX) pathway, as suggested in previous studies (Yang et al., 2014).

In addition, we propose that the culture-independent studies likely overestimate the bacterial prevalence and diversity in tick gut due to amplification of DNA fragments from dead cells and/or cells from tick surface.

Future Directions

We plan to collect and screen more ticks with better balance of males and females from different sites in Kansas and from other states in order to asses the viable bacterial community more accurately. In parallel, we will also use a culture-independent approach (total DNA extraction followed by Illumina sequencing) to address the discrepancy between culturing and culture-independent approaches.

We will use the representative bacterial isolates obtained from the gut of wild ticks in laboratory bioassays to assess the tick midgut colonization and homeostasis as well as to study the tick midgut epithelial immunity, specifically focused on the role of ROS using quantitative PCR for selected DUOX pathway markers.

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