Use of migdut dissections and PCR blockers in the study of the eukaryotic microbiome of the black-legged tick

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Abstract

Bioinformatics is a field that can improve understanding of biological systems. In this study, DNA isolated from the black-legged tick (*Ixodes scapularis*) was analysed using QIIME2 microbiome bioinformatics software on the Cyverse platform. Use used PCR blockers and dissections were tested to reduce the amount of *I. scapularis* reads and thus allow for increased sequencing fungal and protist DNA reads.

Introduction

Tick microbiome research is a growing field that aims to better understand species interactions that facilitate or inhibit pathogen transmission. This research is urgently needed because ticks such as *Ixodes scapularis* transmit pathogens such as *Borrelia burgdorferi* and A*naplasma phagocytophilum*, the agents of Lyme disease and Anaplasmosis, respectively. The eukaryotic microbiome remains understudied due to limitations in DNA sequencing technology. In particular, primer sets designed to amplify eukaryotic microbial species also amplify *I. scapularis* DNA, which is abundant in DNA extracts. The goal of this study is to test two approaches for reducing amplification of *I. scapularis* DNA, thereby allowing for improved sequencing of the eukaryotic microbial community of the black-legged tick. Data analysis of a preliminary dataset was performed by students of BIOL 479 at the University of Bridgeport.

Research Objectives

1. Reduce the number of *Ixodes scapularis* reads that are read by a DNA sequencing machine.
2. Increase the number of fungal and protist (e.g. eukaryotic microbes) reads that are read by a DNA sequencing machine

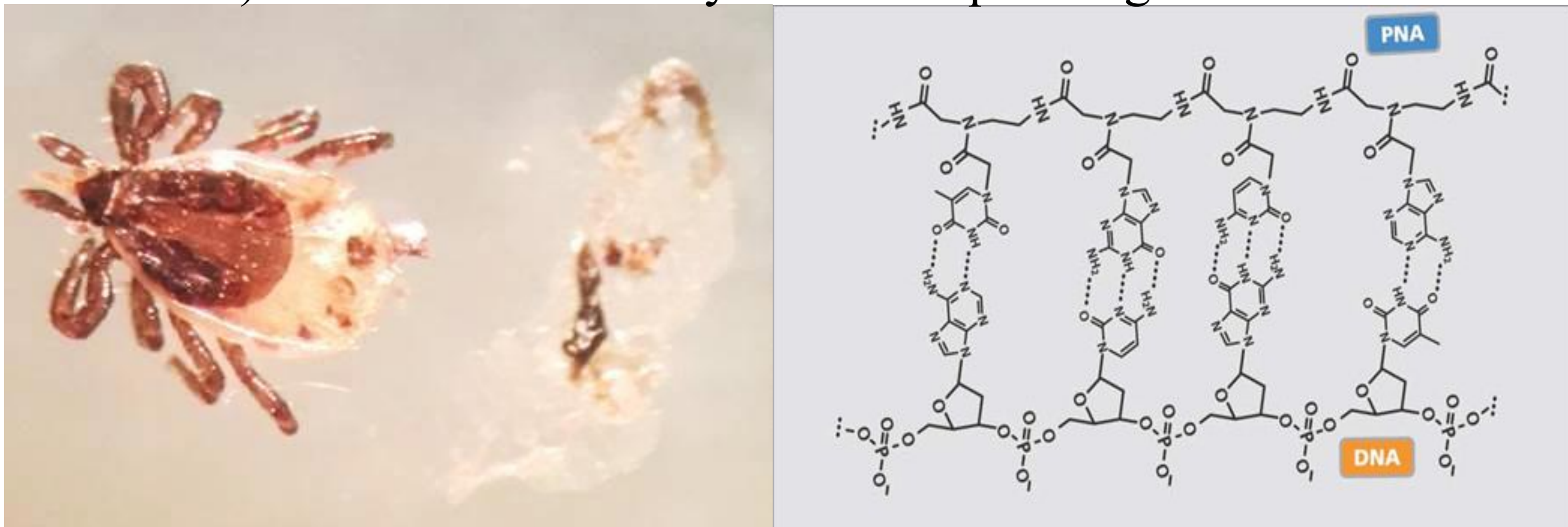

Figure 1. Midgut dissection. Figure 2. PCR blocker.

Results

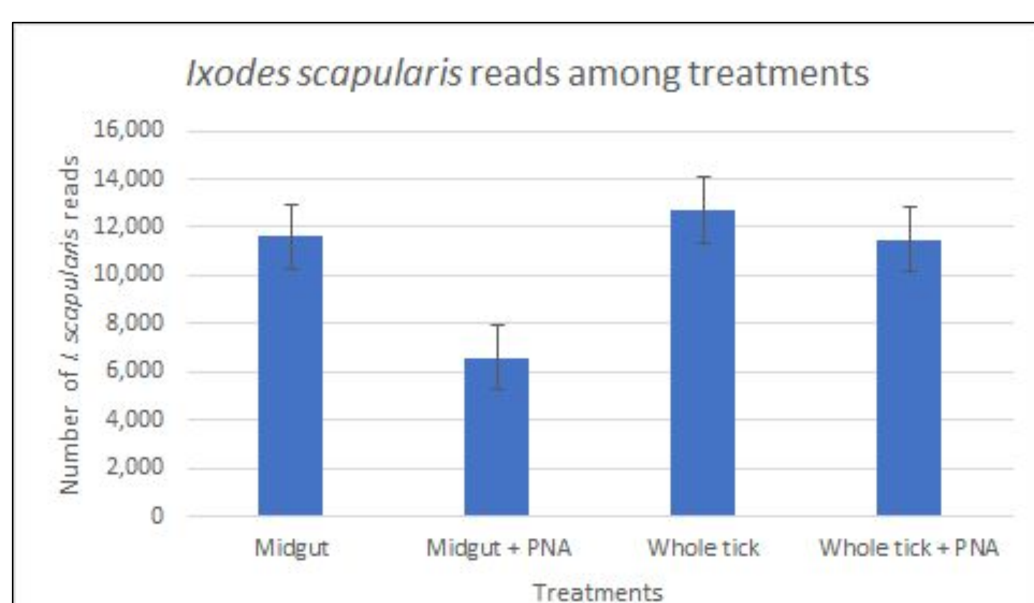

Figure 3. Bar plot analysis of treatment vs. number of *I. scapularis* reads.

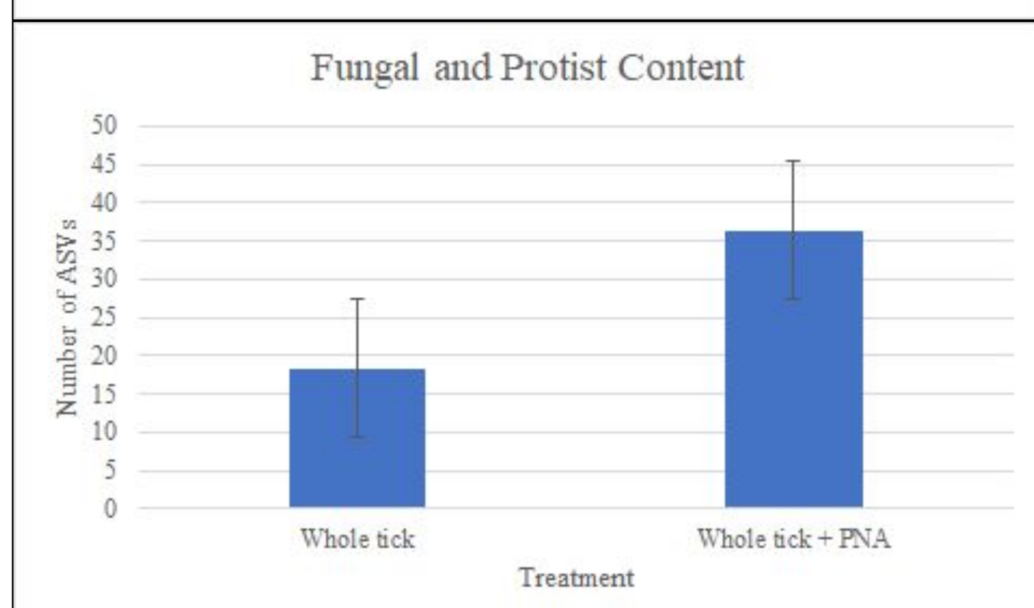

Figure 4. Bar plot analysis of eukaryotic diversity for whole tick vs whole tick + PNA. ANOVA analysis shows a p-value = 0.005.

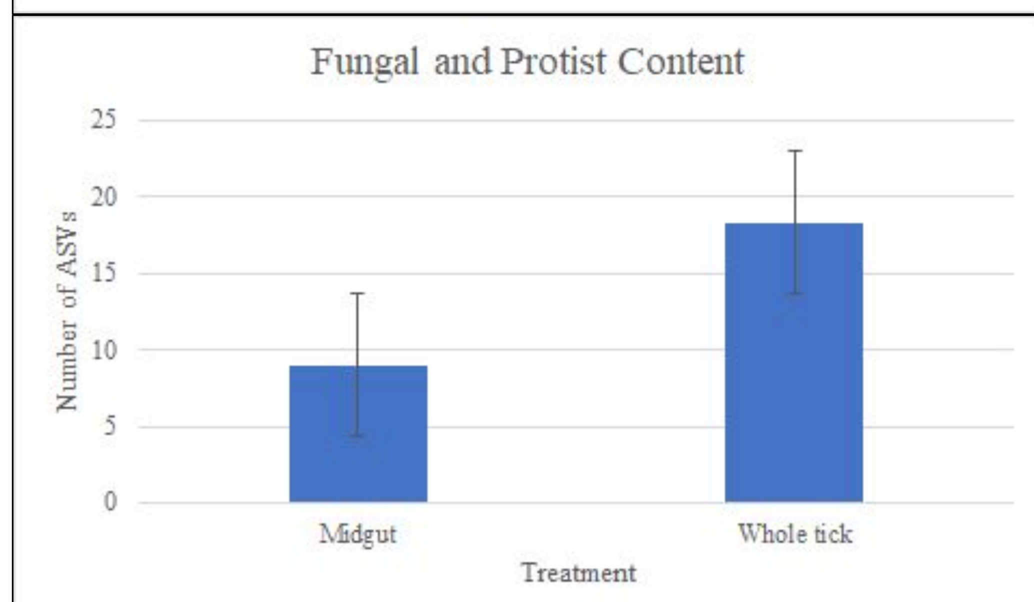

Figure 5. Bar plot analysis of eukaryotic diversity for midgut vs. whole tick. ANOVA analysis shows a p-value = 0.20.

Methods

DNA was extracted from 12 nymphal-stage *I. scapularis* collected in southern Vermont, USA. A section of the 18S rDNA gene was amplified using a universal primer set and these amplicons were sequenced on an Illumina MiSeq machine by the University of Illinois Urbana-Champagne. We used three approaches to reduce the number of *I. scapularis* 18S genes that were amplified:

1. Three whole ticks were compared the three midguts separated from the tick (Figure 1).

2. Three whole ticks were compared to three whole ticks for which a PCR blocker (Figure 2) was used to reduce PCR amplification of *I. scapularis.*

3. Three tick midguts were compare to three midguts for which a PCR blocker was used.

Bioinformatics: Within the QIIME2 software, predefined Python codes were used to rarefy and filter the data acquired through sequencing. One analysis was performed by only including *I. scapularis reads.* Another analysis was performed by including only protists and fungi reads.

Discussion

The PCR blocker showed to successfully reduce the amount of *I. scapularis* reads (Fig. 3). The PCR blocker also increased the diversity of species acquired from the samples. Figure 4 shows a significant difference from Whole tick vs. Whole tick + PNA ($p = 0.005$). Midgut dissections did not increase the diversity of species, and therefore, the result is not significant (Fig 5). The comparison of midgut vs. midgut + PNA was not significant.

Conclusion

- The use of PCR blocker to acquire a reduced amount of *I. scapularis* reads was successful.
- The PCR blocker led to an increase in microbial diversity, which is an important factor to consider in this study, since a goal was to have a better understanding of the tick microbiome.
- These findings pave the way for a larger-scale studys of the eukaryotic tick microbiome that may reveal species important in pathogen transmission.

References

[1] Bolyen, Evan, et al. "Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2." Nature biotechnology 37.8 (2019): 852-857.
[2] Callahan, Benjamin J., et al. "DADA2: high-resolution sample inference from Illumina amplicon data." Nature methods 13.7 (2016): 581-583.