Evaluating the Core Microbiome of *Manduca sexta*

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Introduction

The animal intestinal microbiome comprises a diverse community of microorganisms, which influence host development, physiology, and response to pathogens. However, the mechanisms underlying these complex interactions remain poorly understood. While we reference the microbiome as a single entity, disruptions of homeostatic conditions in the intestinal environment (i.e. temperature, diet, antibiotics) can lead to dramatic shifts in the relative abundance of each species, affecting the overall community composition and perturbing the equilibrium amongst residents. Using *Manduca sexta* (tobacco hornworm), as an experimentally tractable model, our aim is to develop effective strategies and experimental approaches that will advance our understanding of how these complex microbial ecosystems shape animal health and behavior. Relative to mammalian models, the *M. sexta* gastrointestinal tract is morphologically simple, comprising foregut (FG), midgut (MG), and hindgut (HG) regions (Fig. 1). **The MG epithelium is the primary site of absorption, secretion, and colonization**. Because it is exposed to xenobiotics and entomopathogens that cause damage to apically exposed enterocytes, the MG has the highest turnover rate of any fixed-cell population in the body. Consequently, the growth rate of microorganisms stably colonized in the mucosal layer of the MG must exceed the rate of developmental and damage-mediated tissue turnover. Our objective was use a combination of metagenomics approaches to identify a core group of microorganisms colonized in the *M. sexta* MG mucosal layer of larvae reared on either artificial diet or its preferred host, *Nicotiana attenuata* (wild tobacco).

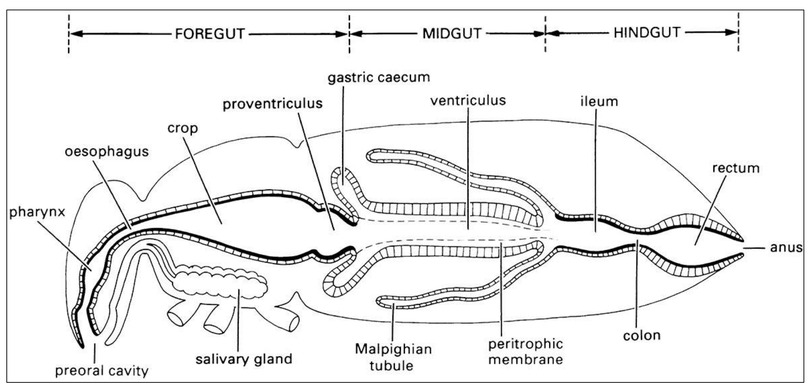

Figure 1: Generalized physiology of the insect gastrointestinal tract

Methods

Insects: *M. sexta* eggs were purchased from Carolina (Burlington, NC). After hatching, larvae were maintained on a wheat germ-based artificial diet under natural light at 24°C or were placed on the base leaves of *N. attenuata* to hatch on the plant and kept under natural light at 24°C.

Plants: *N. attenuata* seeds were planted and grown in Miracle-Gro® potting mix. Plants were initially grown under 200W LED lights and watered as needed. They were then moved to 400W LED lights and fertilized weekly with a complete liquid fertilizer (Miracle-Gro®).

Dissections: Larvae from each instar were dissected by anesthetizing on ice for ~5 min. Midguts were removed by cutting behind the third set of true legs and prolegs, immersed in PBS in a micro-centrifuge tube, and gently vortexed for ~5 min. Midgut tissues were removed and dislodged mucus/bacteria were pelleted by centrifugation followed by aspiration of the supernatant. All pellets were kept at –70°C.

DNA extraction and quantification: Genomic DNA (gDNA) was extracted using the QIAamp PowerFecal® DNA Kit (Qiagen, Carlsbad, CA, USA) to obtain high-purity DNA for more successful polymerase chain reaction (PCR) amplification. DNA obtained was quantified using a Biophotometer (Eppendorf, Hauppauge, NY, USA).

16s rRNA gene amplification: The 16S rRNA gene was amplified by PCR using the following primers: 16S_rRNA_8Fwd (GAGTTTGATCCTGGCTCAG) and 16S_rRNA_1492R (GGTTACCTTGTTACGACTT). The 25-µl PCR reaction mixtures contained 0.4 µM of each forward and reverse primer (final concentration), 1 µl extracted template DNA, and 12.5 µl 2X GoTaq® Hot Start Green Master Mix (Promega). PCR reactions included a denaturation for 5 min at 95°C, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 45 s, and elongation at 72°C for 1.5 min, with a final elongation step at 72°C for 5 min.

Electrophoresis: Electrophoresis of PCR products was performed on 1% agarose (Fisher) using Tris-Acetate-EDTA (TAE) buffer at 100V.

Preliminary Results

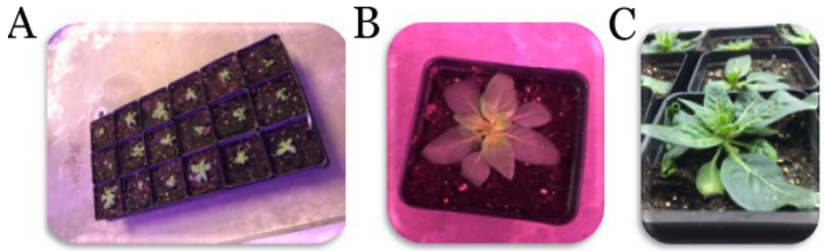

Figure 2: Overview of plant growth and maturation. *N. attenuata* after (A) 3 weeks (B) 5 weeks (C) 8 weeks growth

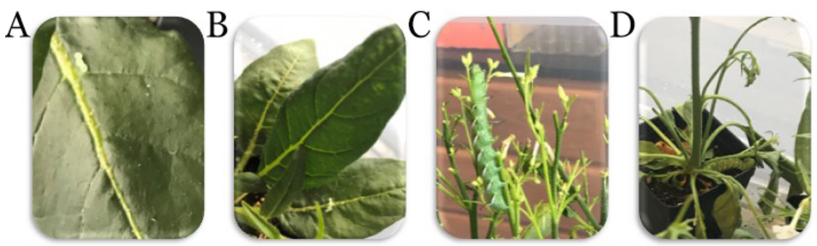

Figure 3: Stages of growth of *M. sexta* on *N. attenuata*. (A) *M. sexta* eggs on base leaves of *N. attenuata (B)* 1st instar *M. sexta* larvae (C) 5th instar *M. sexta* larvae (D) Destroyed plant as result of *M. sexta* feeding

Conclusions

- We have successfully extracted small quantities of gDNA from mucus obtained from the MG epithelium of *M. sexta* larvae
- No significant differences in growth rates were observed in *M. sexta* larvae maintained on artificial diet or *N. attenuata*. Relative to larvae fed artificial diet, larvae maintained on N. attenuata appeared bright green.

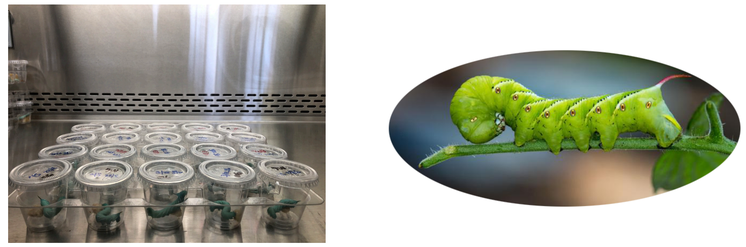

Figure 4: Hornworms. (*Left*) Maintained on artificial diet in the laboratory or (*Right*) on plants

Future Work

- Sequence 16s rRNA DNA and RNA from bacteria colonized in *M. sexta* MG epithelium.
- Develop a *M. sexta* for studying *Escherichia coli* physiology in the midguts on larvae provided different food sources.

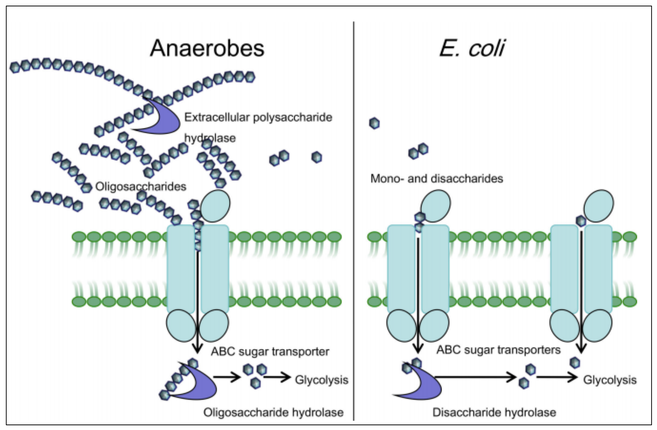

Figure 5: Carbohydrate metabolism in the mammalian intestine. Anaerobes in the intestine secrete hydrolases that digest polysaccharides in the intestinal mucosa. *E. coli* lacks these enzymes, but is able to utilize liberated mono- and disaccharides as carbon sources to make ATP.

Acknowledgements

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References

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