“Longitudinal Analysis of the Vaginal Microbiota Prior to Incident Bacterial Vaginosis Using Shotgun Metagenomic”

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Introduction

Bacterial Vaginosis (BV) is the most common cause of vaginal discharge, characterized by the displacement of lactobacilli with an overgrowth of facultative and strict anaerobic bacteria. BV is associated with multiple adverse health outcomes, including preterm birth, pelvic inflammatory disease, and increased risk for HIV and other sexually transmitted infections. In cases where antibiotics are needed to treat symptomatic BV, recurrence commonly occurs within 3 months to a year. Despite over 60 years of research, the specific etiology of BV remains unclear. In this pilot study, we utilized shotgun metagenomics to identify the discrete changes occurring in the vaginal microbiome prior to the onset of iBV to inform future BV pathogenesis research such as the role of lactobacillus phage in iBV.

Background

BV is characterized by a shift in the vaginal microbiome with a depletion of lactobacillus species and an overgrowth of anaerobes such as *G. vaginalis*, *A. vaginae*, and *P. bivia* verified in our previous study using 16s sequencing. Different species of lactobacillus have a varying degree of resistance to community shifts. Multiple models provide an explanation for the shift in vaginal microbiome during BV. The model that is of greatest interest to our aims is the lactobacillus depletion model. This model hypothesizes that lactobacillus depletion could be caused by the introduction of BVAB from sexual contact or by lactobacillus phage activity.

In previous metagenomic analysis, the presence of lactobacillus phage was detected in multiple subjects and increased in two of the subjects on the days leading up to iBV diagnosis. Further investigation into the identity and function of these phages could better determine the source of their activation. Additionally, metagenomic functional analysis could provide insight into the genetic components contributing lactobacillus species that better resist community alterations such as *L. crispatus.*

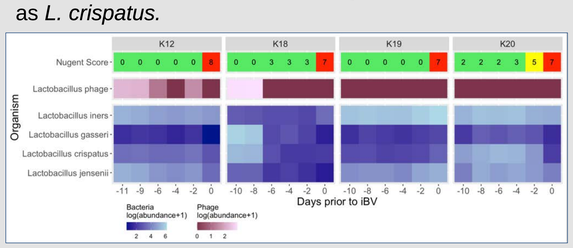
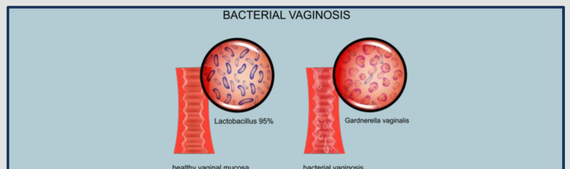


Figure 1. Abundance of Lactobacillus phage and *Lactobacillus* spp.

Methods



Vaginal samples were self collected daily over a 90 day time period in women with normal baseline vaginal microbiota (no Amsel criteria, negative Nugent score); daily Nugent scores were obtained. iBV was defined as a Nugent score of 7-10 on at least 2-3 consecutive days.

Samples from enrolled women following iBV diagnosis were sequenced using 16s sequencing. Due to the expensive nature of shotgun sequencing, four women were selected due to their unique 16s profile for shotgun metagenomic sequencing. Sequencing was carried out on 6 samples collected on the days leading up to iBV. Samples were selected beginning 10-11 days prior to iBV.

Megahit
Metagenomic assembly, short reads are assembled into large contiguous sequences of DNA

- **Data Cleaning**
- **Genome Binning**
- **Downstream Phage Analysis with Vibrant and Propagate**

Biobakery 3
Metagenomic Analysis through read mapping to known databases

- **Data Cleaning**
- **Taxonomic Classification with MetaPhlAn**
- **Functional Analysis with MetaPhlAn**

Results

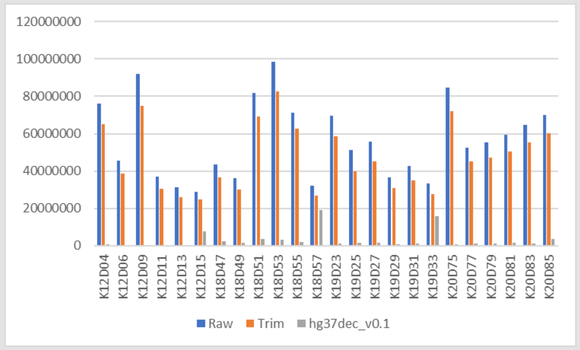


Figure 2. Quality Control and Read Mapping Summary from the Biobakery Workflow.

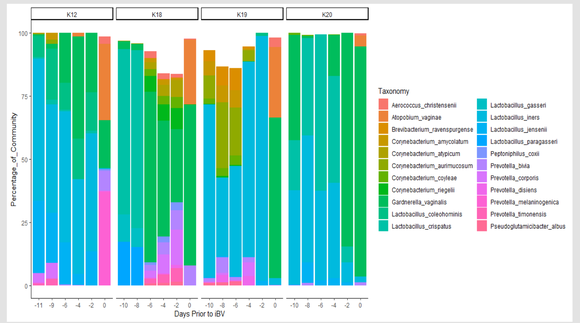


Figure 3. Taxonomic Classification Bar Chart. Highlights the transition that occurs leading up to iBV

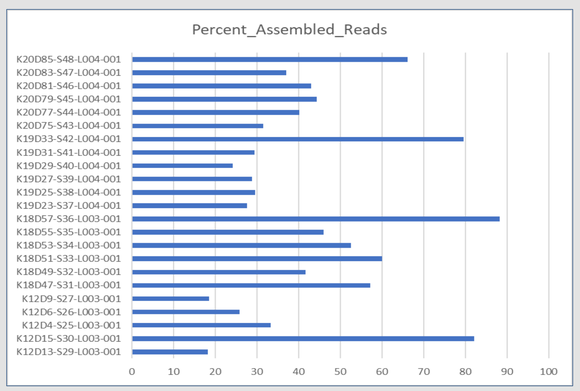


Figure 4. Metagenomic Assembly Percentage of Reads Assembled.

Results Continued

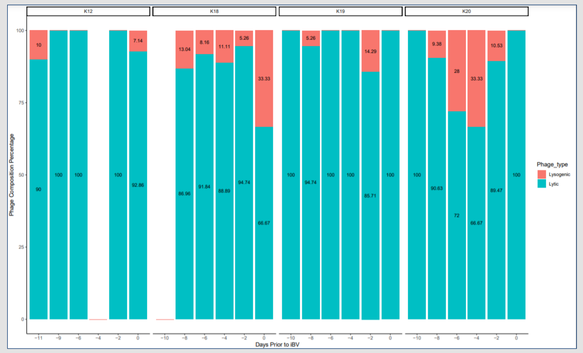


Figure 5. Bacteriophage Classification: Displays the percentage of lytic phage contigs compared to lysogenic phage contigs.

Conclusions and Future Directions

Previous results from our lab indicated lactobacillus phage abundance increased leading up to iBV. These findings highlight the potential role of lactobacillus phage in the development of iBV. We followed up on this finding by investigating the predominance of phages in the lytic vs lysogenic phase for samples on days leading up to iBV. Activity analysis of identified high-quality lysogenic phage DNA found these phages to be dormant. This finding suggests bacteriophage was introduced to the community rather than activated.

Future aims are to verify the hosts of the bacteriophages identified following assembly analysis along with detailed viromics to better assess the potential role of bacteriophage in iBV development. Lastly we plan to increase the number of patients included in the analysis from this limited pilot study of four patients.

Citations

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