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Characterization of *Humulus lupulus* microbial communities by Illumina 16S rRNA Gene Amplicon Sequencing

Scott J. Britton*[1], Mary E. Allen[2], Andrew J. Piefer[3], Peter T. Benziger[2], Joseph M. Balnis[3]

Duvel Moortgat USA, Quality Department[1]; Hartwick College, Department of Biology[2]; Hartwick College, Department of Chemistry[3]

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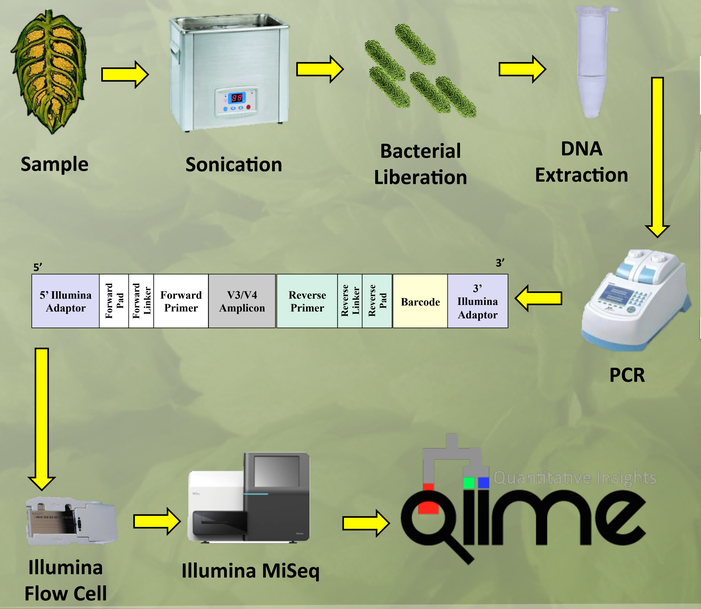
INTRODUCTION

The female inflorescence of the common hop (*Humulus lupulus)* is a necessitous ingredient that provides distinctive aromatics, bitterness, and antiseptic properties to beer. Although substantial research has been conducted on the antiseptic properties of hops, almost no research exists on the natural microbial communities that populate this perennial herbaceous vine. High-throughput DNA sequencing was conducted on samples from hop plants to characterize the microbial community of cones, leaves, and from the soils located at the base of mature plants. Samples were obtained aseptically from hops vines in September 2014 and stored at -80°C until DNA extraction.

METHODS



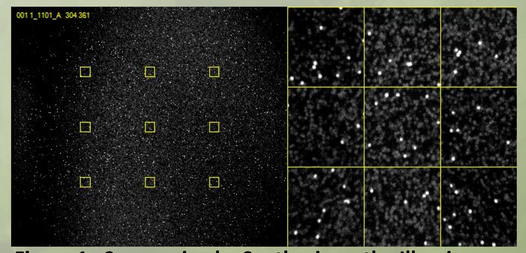


Figure 1. Sequencing by Synthesis on the Illumina Flow Cell utilizes fluorescently-labeled nucleotides to sequence amplicon clusters bound to the flow cell surface. Following the incorporation the fluorescent dye is imaged to identify the base and then cleaved to allow for the next incorporation.

Figure 3. Bacterial 16S rRNA gene

Figure 2. Upstate New York Hop Yard

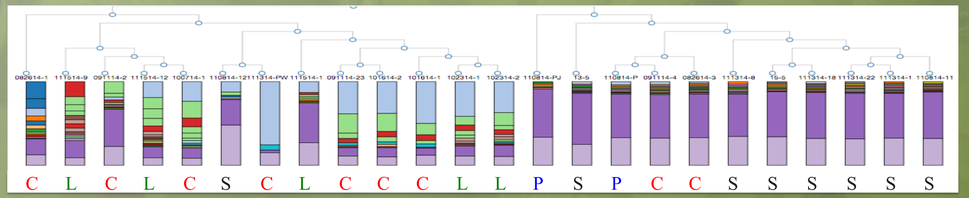


Figure 4. Clustered Samples According to Similarity. *Preliminary data* from Illumina Basespace prior to cleanup through the QIIME pipeline. Most notable is that Cone (C) and Leaf (L) samples cluster together and away from Soils (S) and Pellets (P).

Table 1. Results from QIIME Pipeline

Source	Total Number of Sequences	Operational Taxonomic Units (OTUs)
Humulus lupulus - Cones	1,754,262	8,730
Humulus lupulus - Leaves	1,059,433	3,371
Soil	1,526,238	14,349

PRELIMINARY CONCLUSIONS

This study represents the first in depth analysis of the diverse microbial community colonizing the common hop (*Humulus lupulus), present* despite antiseptic properties of the cone, conferred by hoponoic acids. The community on cones is distinct from soil at the base of the plants and more diverse than the leaf microbial community (Figure 4; Table 1). Bacteria found to be residing on cones include the beer spoilage bacteria *Lactobacillus* and *Acetobacter,* and many species previously unassociated with hops. One of the latter is *Planctomyces* – these microbes have a cell wall lacking murein and include species that inhabit acidic environments, which may explain their tolerance to hopanoic acids. *Planctomyces,* and other bacteria in the hop residential flora, may protect these reproductive structures from disease and contribute to properties of hops including bitterness. Knowledge of this diverse community has the potential to contribute much to our understanding of hops and their contribution to beer.

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