The Microbial Community Colonizing Different Varieties of Fresh and Dried Hop (*Humulus lupulus*) Cones as Determined by Next Generation DNA Sequencing

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Introduction

Female cones from the *Humulus lupulus* plant, commonly known as hops, have been used in brewing for centuries. The presence of antimicrobial resins in hops led to a belief amongst the brewing community that the cones do not support a natural community of microorganisms. This was contradicted by the discovery of a microbiome consistent across freshly harvested Cascade variety cones (Britton et al. 2016; Benziger et al. 2015). **Our continued investigation into the microbiome aimed to determine whether the resident microbial community differs with variety of hop plant and whether commercial drying processes change community composition.**

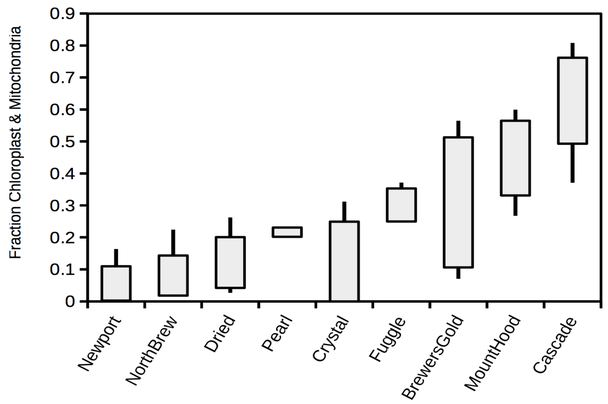
Microbial residents on plants may contribute to plant health and potentially even plant nutrition (Vorholt 2012). Within a plant species, it has been found that variations in genotype can alter the resident microbial community (Whipps et al. 2008), which may influence plant defenses or may cause certain individuals to exhibit different traits, potentially affecting the end product of brewing.

Brewers add hops that are dried and/or fresh at various stages of the brewing process. Craft brewing includes more frequent use of fresh hops and addition after the boiling stage (fresh hopping and back-hopping), which could introduce microbes to beer that could potentially benefit or hinder aroma and flavor in ways currently unrecognized.

Identification of the microorganisms that colonize fresh hop cones and of those that persist following cone drying will make possible downstream investigations into the impacts microbes have on the final beer product.

Methods

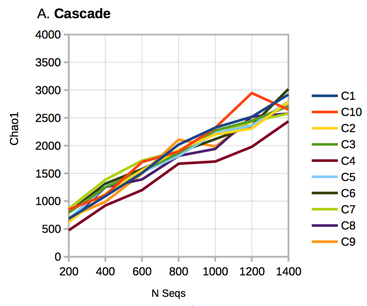
- Hop cones from 8 varieties (Cascade, Pearl, Mt. Hood, Fuggle, Crystal, Newport, Brewer's Gold and Northern Brewer) were collected from Brewery Ommegang, NY.
- Subsamples were dried using commercial processes and equipment (not aseptically).
- Microorganisms were removed from the surfaces of all hop cones with sonication and their DNA was extracted and used for Illumina sequencing of the V3 and V4 regions of the 16S rRNA genes.
- Sequence data were analyzed with Quantitative Insights Into Microbial Ecology (QIIME).

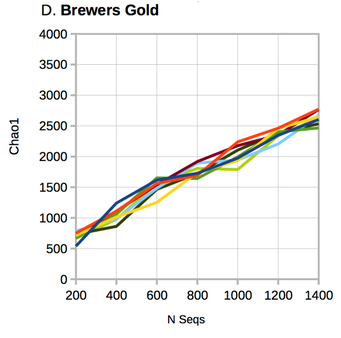


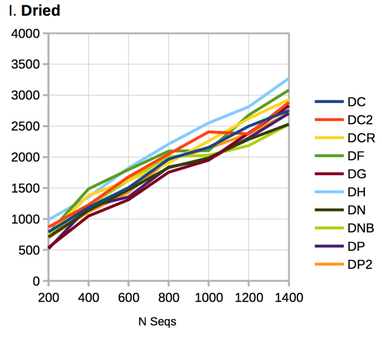
- Chloroplast and mitochondria sequences were removed prior to additional downstream analysis.

Results and Conclusions

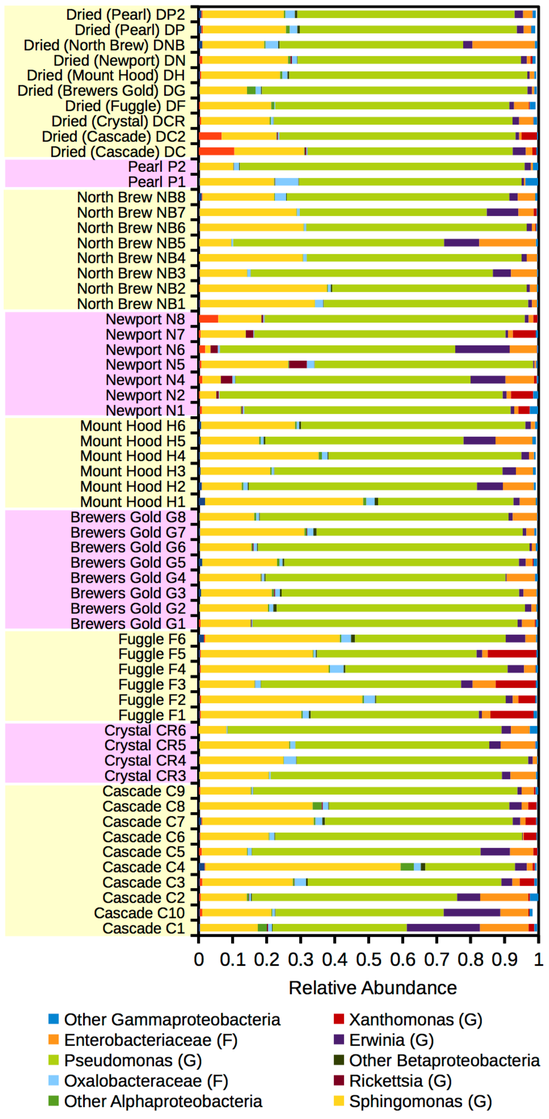
Rarefaction curves indicate that in all samples there were many rare taxa. Examples are shown here for Cascade and Brewers Gold varieties and Dried samples.



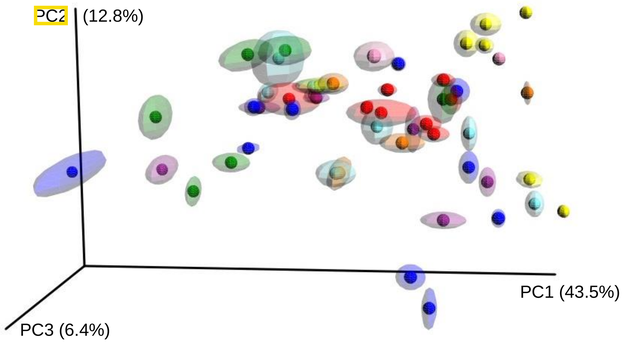




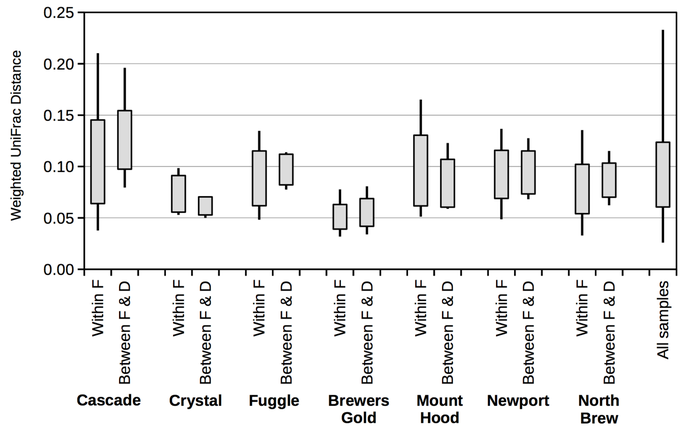
Pseudomonas and *Sphingomonas* were the most abundant taxa on fresh and dried hops of every variety, accounting for at least 60% of the sequences.



Weighted Unifrac analysis shows similar diversity of the microbiomes of all eight varieties. Only Newport and Fuggle appeared to have microbiomes distinct from each other.



Weighted Unifrac analysis also showed that drying does not appear to greatly reduce diversity; compared to one another dried (D) and fresh (F) hops microbomes were not distinct.



Most studies of the phyllosphere that include flowers describe microbial communities associated with individual flower parts (e.g. nectar, petals, pollen, etc) and very few include next generation DNA sequencing (Aleklett et al. 2014). So the data presented here represent a rare, in depth analysis of the microbial community of whole flowers. Hops cone microbiomes are dominated by *Pseudomonas*, a group also common in tomato flowers (Ottesen et al. 2013) but not in apple blossoms (Shade et al. 2013). The high amount of similarity amongst the microbiomes of all eight hop varieties suggests that for hop cones any differences in microbial communities are less attributable to genotype than to geography (Knief et al. 2010).

Acknowledgements: Financial support for this project was provided by an ASM-URF awarded to Nate Cole, a Hartwick College Faculty Research Grant awarded to Dr. M.E. Allen, and from Duvel-Moortgat and the Hartwick College Center for Craft Food and Beverage. Thank you to Dr. Jeff Werner, SUNY Cortland, for his assistance with the data analysis and bioinformatics and to the Union College Chemistry Department for use of the Illumina MiSeq.

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