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DIVISION OF AGRICULTURE
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Pylogenetic Diversity of the Bacterial Communities in Craft Beer

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Introduction

The craft brewing industry is increasing in popularity in the state of Arkansas and the United States. Beer has several parameters that keep the product safe from pathogens: low pH (3.8-4.7), low oxygen content (<0.1ppm), high CO_2 concentration (0.5% w/w), presence of ethanol (4-5%), competitive fermentation yeast, and the addition of hops (17-55ppm). However, bacterial spoilage can still occur in beer. The craft brewing process typically does not include a pasteurization or filtration step, therefore the boiling process is the primary method of inhibiting bacterial growth. Any microorganisms introduced after boiling, or those that survive boiling and mashing, are likely to participate in fermentation and persist in the final product. It is suggested that the microbiological status of beer depends on the microbiota of the raw materials, the nature of the process and product, and the sanitary conditions of beer contact surfaces. Previous culture-based studies have isolated bacteria and yeast from craft beers at specific points in the process, but little research has been done on the process as a whole. Also, there has been limited research on the microbiota of malted grain and hops and the sanitary conditions of the brewery environment.

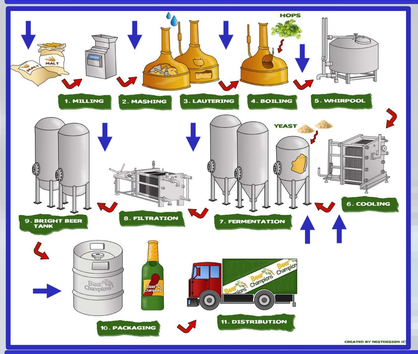
Objectives

1. Track bacteria dynamics through the brewing process
2. Compare beer microbiota to environmental brewery swabs

Significance

- Provide the first extensive microbiota research of craft beers
- Allow craft brewers to have a better understanding of the microbiology of their product
- Initiate further research about the role that microorganisms play on the quality and organoleptic properties of beer

Materials & Methods



DNA Extraction

PCR (Accuprime mix) with dual indexed primers

Normalization (Invitrogen, SequPrepal Kit)

Library Pooling

Library Quantification

Library Concentration mixed with PhiX

DNA 16S Sequencing (Illumina, MiSeq)

Results

Sample	Bacteria (+)		Fungi (+)	
	#	%	#	%
Malted Grain	43/51	84.3%	44/51	86.3%
Hops	5/28	17.9%	3/28	10.7%
Environmental Swabs	9/60	15%	4/60	6.7%
From Mash Tun	10/10	100%	10/10	100%
Pre-Boil	6/10	60%	5/10	50%
Post-Boil	9/10	90%	4/10	40%
After Cooling	10/10	100%	6/10	60%
Fermentation Day 0	8/10	80%	7/10	70%
Fermentation Day 2	2/10	20%	2/10	20%
End of Fermentation	2/2	100%	2/2	100%
Pre-Filter	4/5	80%	4/5	80%
Post-Filter	7/7	100%	5/7	71.4%
Final Product	9/10	90%	9/10	90%

Table 1. Positive Samples for Bacteria and Fungi; Bacteria is more prevalent than fungi

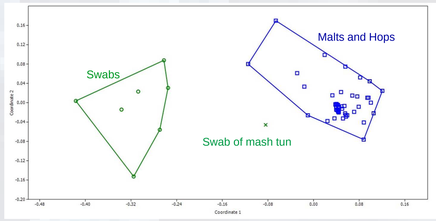


Figure 1. NMDS plot with Bray Curtis similarity index; ANOSIM: P<0.05; Vessels have a distinct bacterial composition compared to raw materials

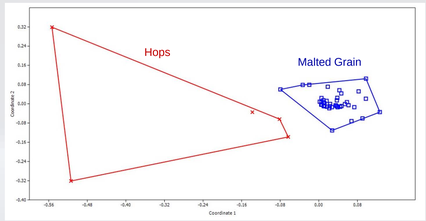


Figure 2. NMDS plot with Bray Curtis similarity index. ANOSIM: P<0.05; Hops and malted grain have a separate, distinct bacterial composition

Results

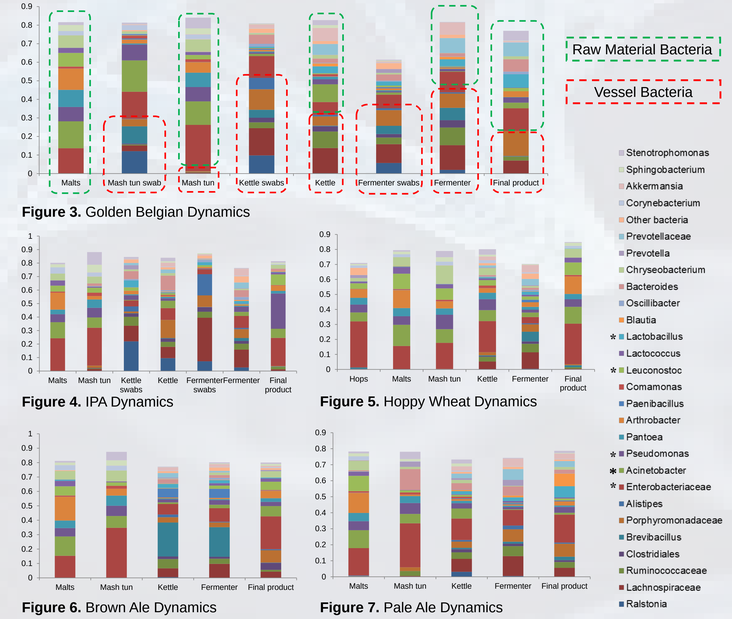


Figure 3. Golden Belgian Dynamics

Figure 4. IPA Dynamics

Figure 5. Hoppy Wheat Dynamics

Figure 6. Brown Ale Dynamics

Figure 7. Pale Ale Dynamics

***Note:** *Lactobacillus, Leuconostoc* and *Enterobacteriaceae* are common beer spoilers; *Acinetobacter* and *Pseudomonas* are known to spoil other food products

Conclusions

- Plant and soil borne genera are present in raw material and mash samples
- Microorganisms from raw materials can withstand mash and boil process
- Vessel associated bacteria are more prominent in the early stages than malted grain associated bacteria
- Final sample microbiota is a composite of swab, raw materials, and process samples
- Final samples contain diverse bacterial community