

# ASBC Annual Meeting

June 4–7 ■ Fort Myers, Florida

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## Mapping the Microbiome of Malted Barley and Wort Soured Using Malted Barley

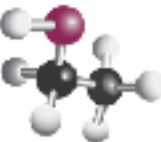
by Jeff Young (Blue Owl Brewing) and Dr. Matthew Bochman (Indiana University)



INDIANA UNIVERSITY

# Background

- Naturally occurring bacteria, wild yeast, and molds (referred to as the microbiome) can be found on grains.
- Grains have been used historically to acidify (sour) wort.



# Questions

- What is the native microbiome on various grains?
- What portion(s) of the microbiome thrive during “wort-souring” conditions?
- What is the resulting organic acid profile from the various grains?



# Experiment: Sample Set

- Grains determined to be best suited for wort-souring tend to be very lightly malted barley.
- Germination of barley produces large increase in resident microbiome.
- Processing, handling, and packaging can alter microbiome.



# Experiment: Sample Set

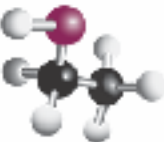
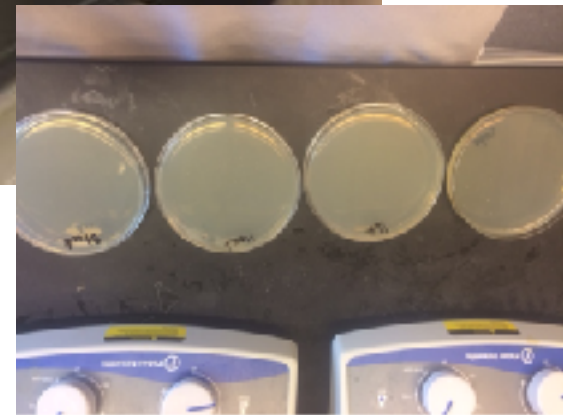
- 2016 vs 2015
- 4 maltster
- 5 regions
- 6 Varietals
- Pale/Pilsner
- Similar vs Unique Malt Houses

Harvest Year	Maltster	Style	Varietal	Region
2015	Briess	Pilsen	Merit 57	Wyoming
2015	Briess	Pilsen	Copeland	Canada
2015	Patagonia	Pale	Sebastian	Chile
2016	Briess	Pilsen	Merit 57	Wyoming
2016	Briess	Pilsen	Synergy	Wyoming
2016	Weyermann	Pilsner	Barke	Germany
2016	Blacklands Malt	Pale	Endeavor	Texas



# Experiment: Sample Preparation (NRBS/Wort Microbiome)

- NRBS (nutrient-rich buffered starter) containing:
  - malt extract,
  - yeast nutrient,
  - $\text{CaCO}_3$ ,
  - 20g malted barley.
- T=0, 24hr
- NOTE: All studies conducted at 109F and stirred 1000RPM



# Experiment: Sample Preparation (NRBS/Wort Microbiome)

- Wort: 10P wort,
- pH = 4.7,
- 25 mL of NRBS.
- T=0, 24, 48hr
- NOTE: All studies conducted at 109F and stirred 1000RPM



# Experiment: Sample Preparation (Wort NMR)

- Wort: 10P wort,
- pH = 4.7,
- 25 mL of NRBS.
- T=0, 24, 48hr
- NOTE: All studies conducted at 109F and stirred 1000RPM



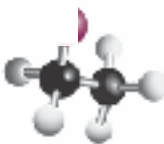
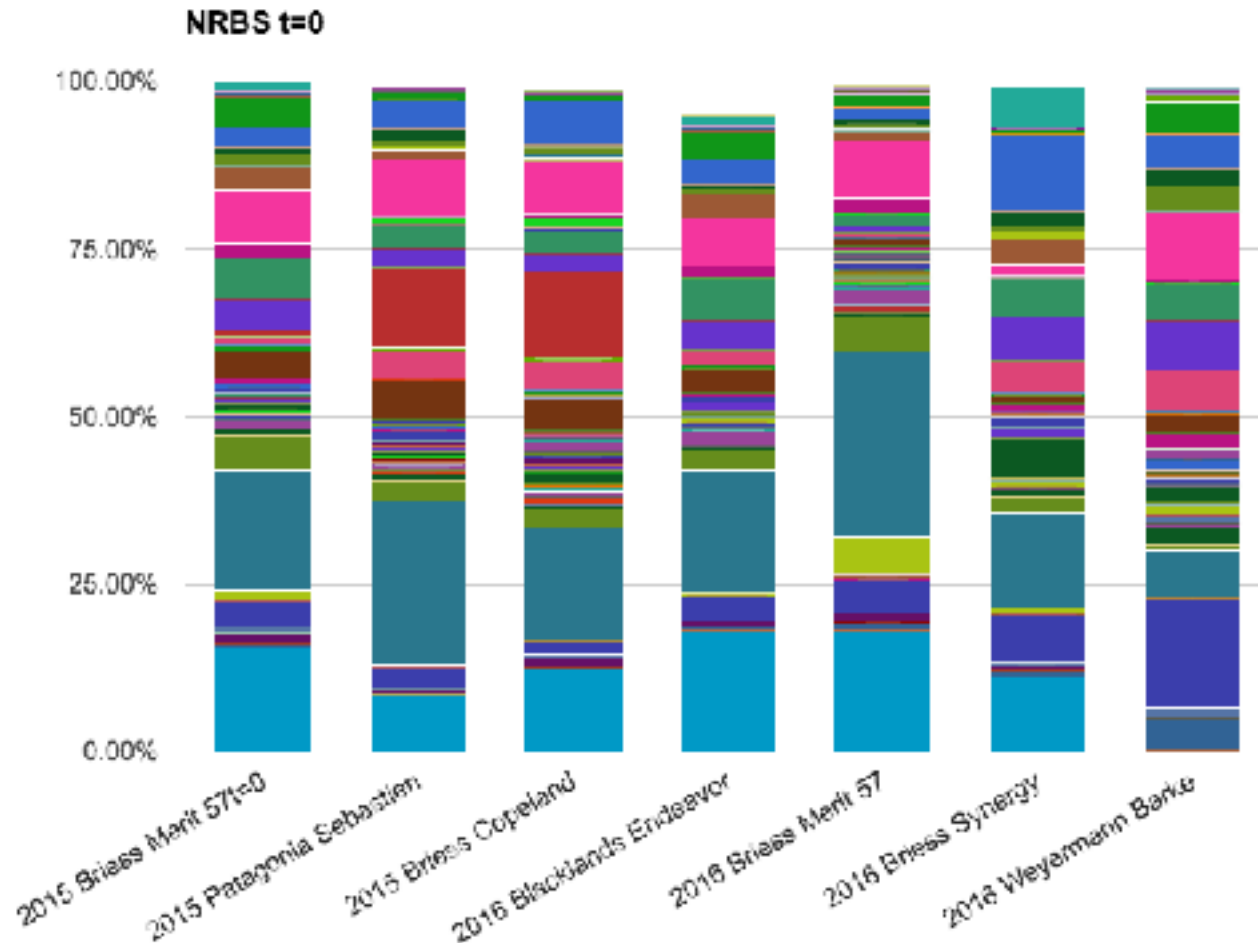


# Experiment: Method

- Malt/Wort Microbiome: Bacterial 16S ribosomal RNA gene targeted sequencing.
- NMR: qNMR Spectroscopy.

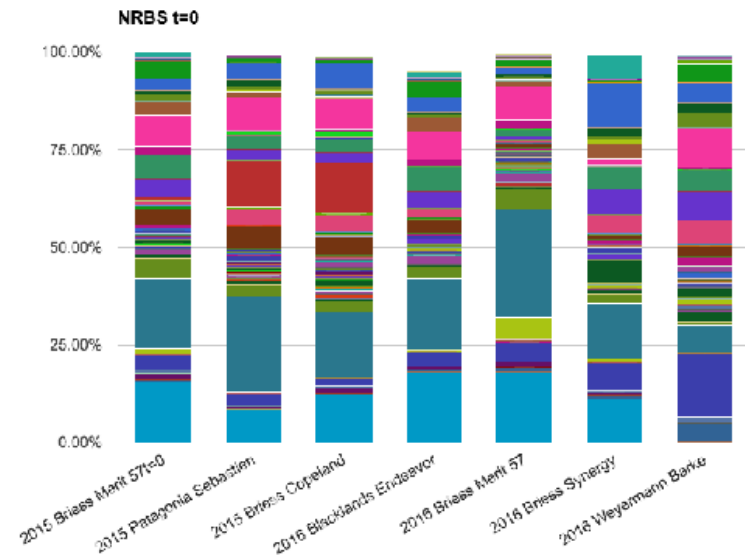


# Results: Malted Barley Microbiome

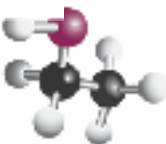
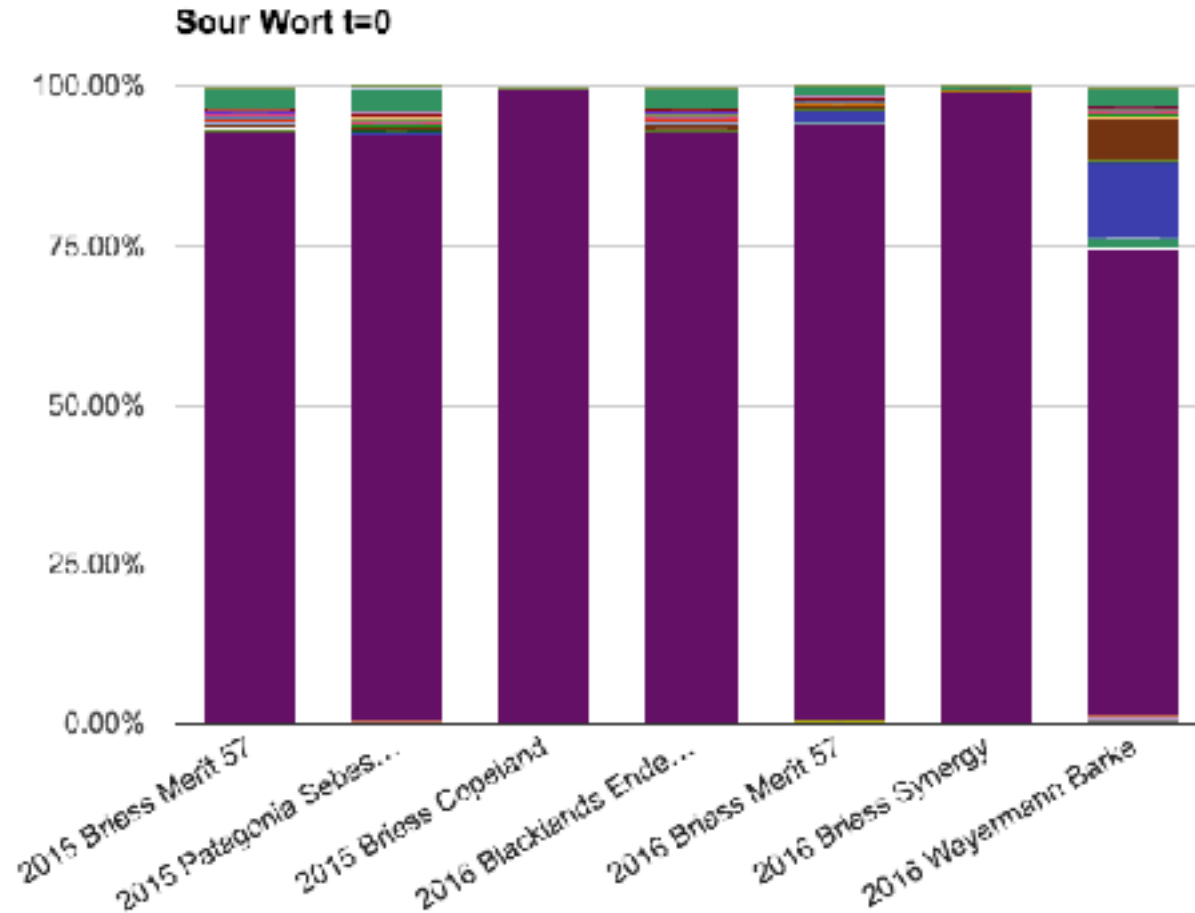


# Results: Malted Barley Microbiome

- *Acinetobacter johnsonii* (not much information)
- *Corynebacterium glutamicum* (typical in soil and plants, helps decompose)
- *Proteobacterium symbiont* (g=arthrobacter, found in soil)
- *Clavibacter michiganensis* (tomato rot!)

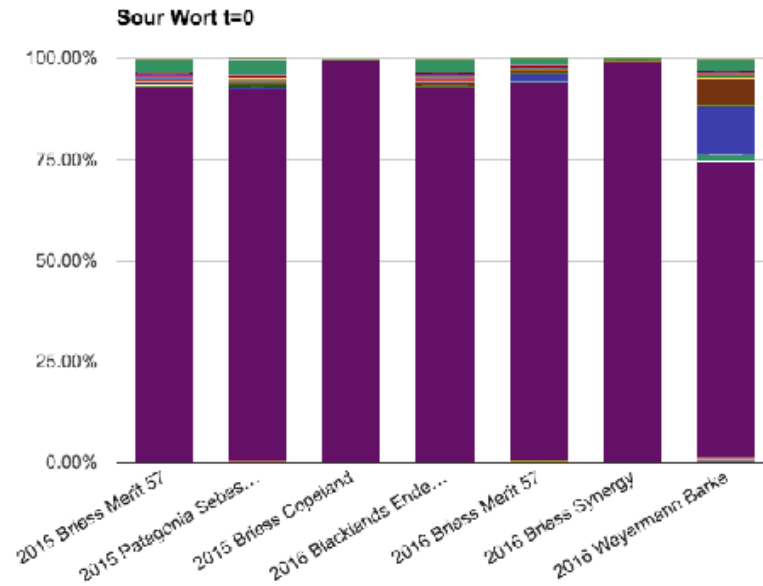


# Results: Soured-Wort Microbiome (t=0)

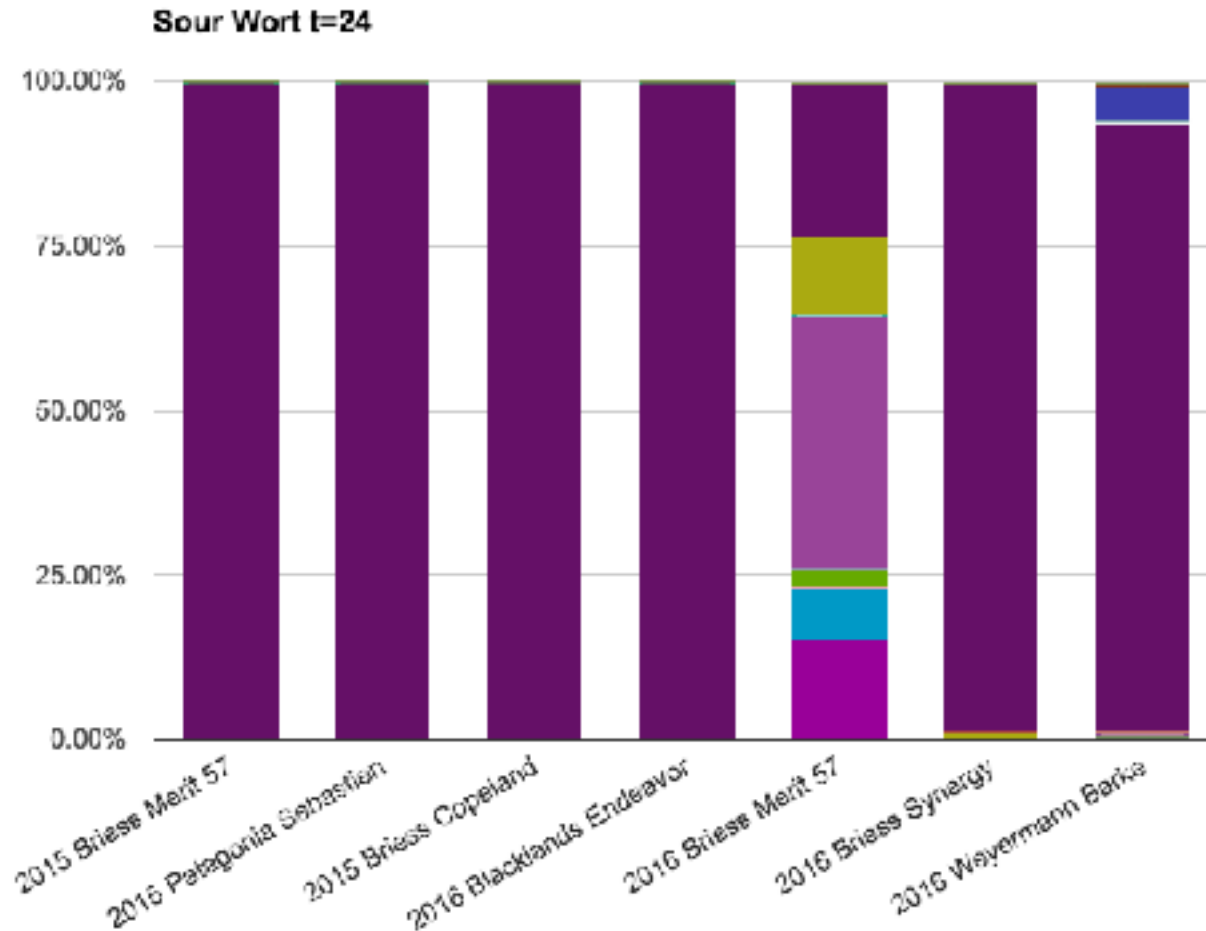


## Results: Soured-Wort Microbiome (t=0)

- *Weisella cibaria*
- *Lactococcus lactis* (used in dairy)
- *Enterobacter aerogenes* (found in GI of humans, generally safe)

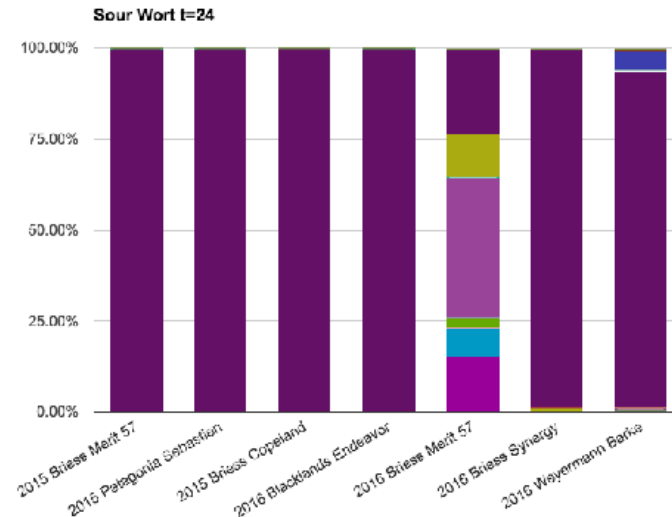


# Results: Soured-Wort Microbiome (t=24)

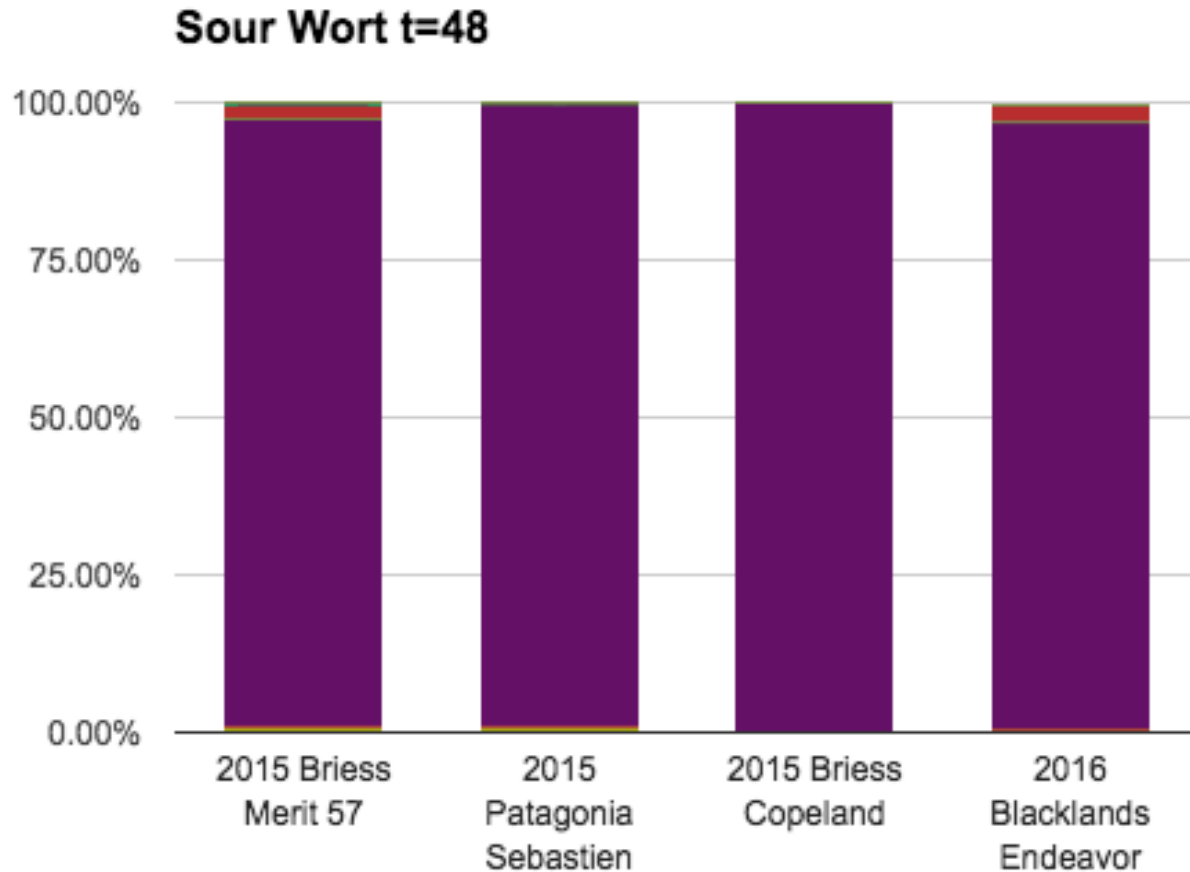


## Results: Soured-Wort Microbiome (t=24)

- *Weisella cibaria*
- *Lactococcus lactis* (used in dairy)
- *Pediococcus pentosaceus*
- *Lactobacillus reuteri, fermentum, helveticus, delbruekii*
- *Enterobacter aerogenes* (0.3%)



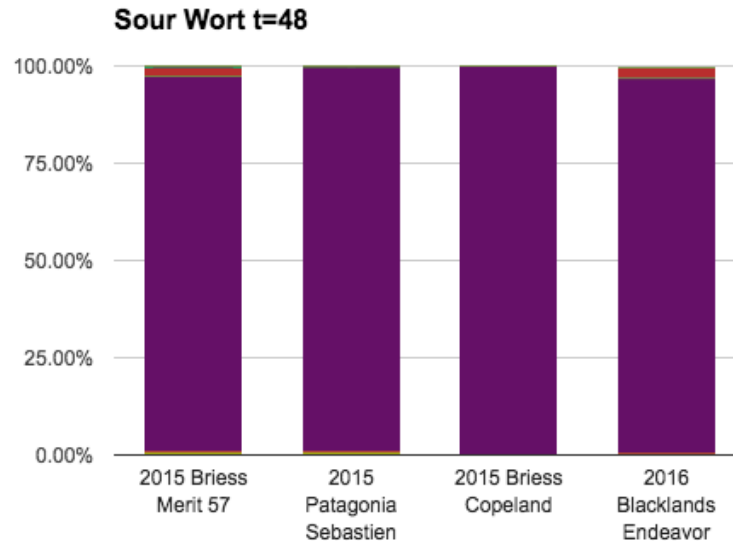
# Results: Soured-Wort Microbiome (t=48)



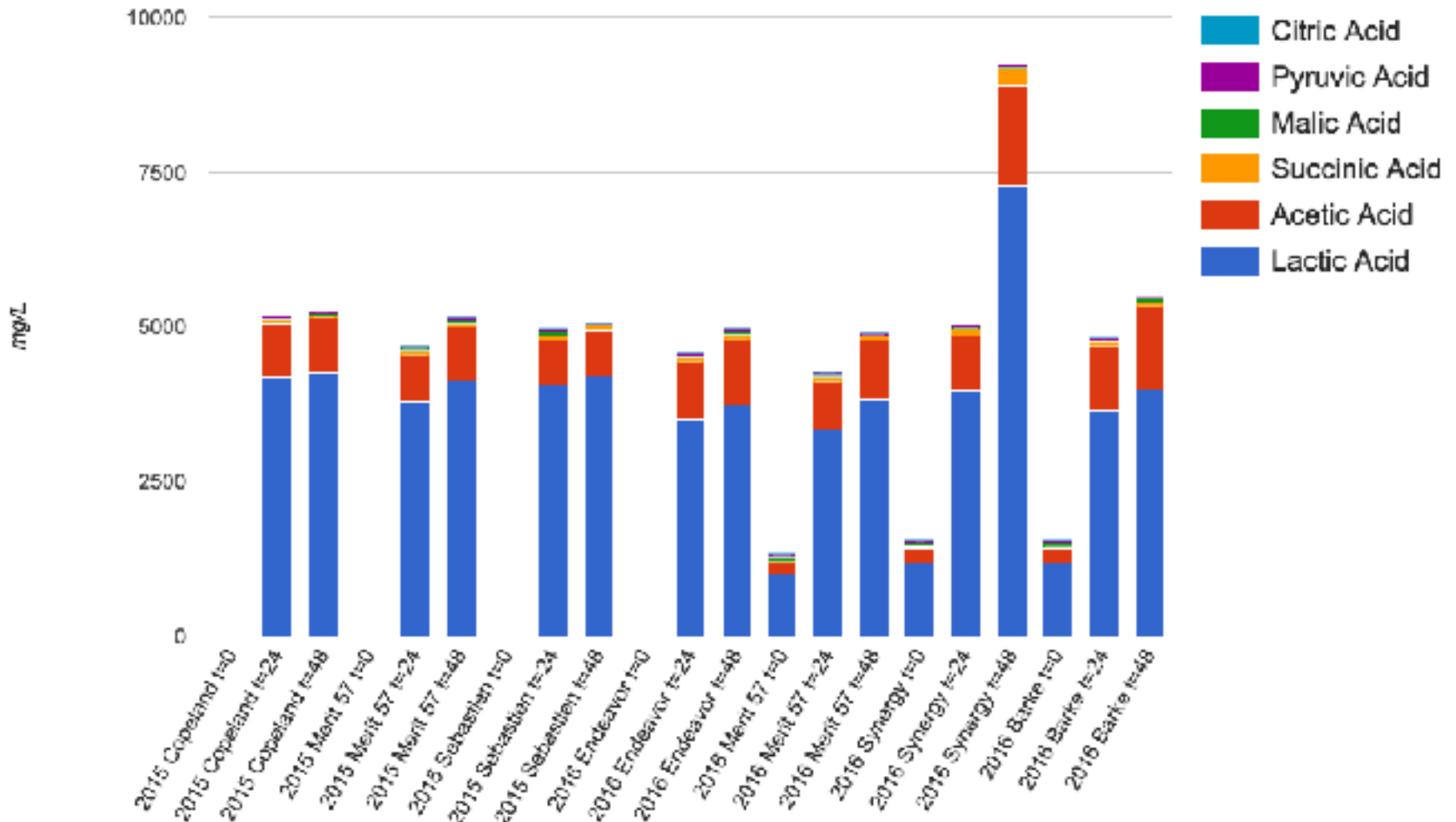


## Results: Soured-Wort Microbiome (t=48)

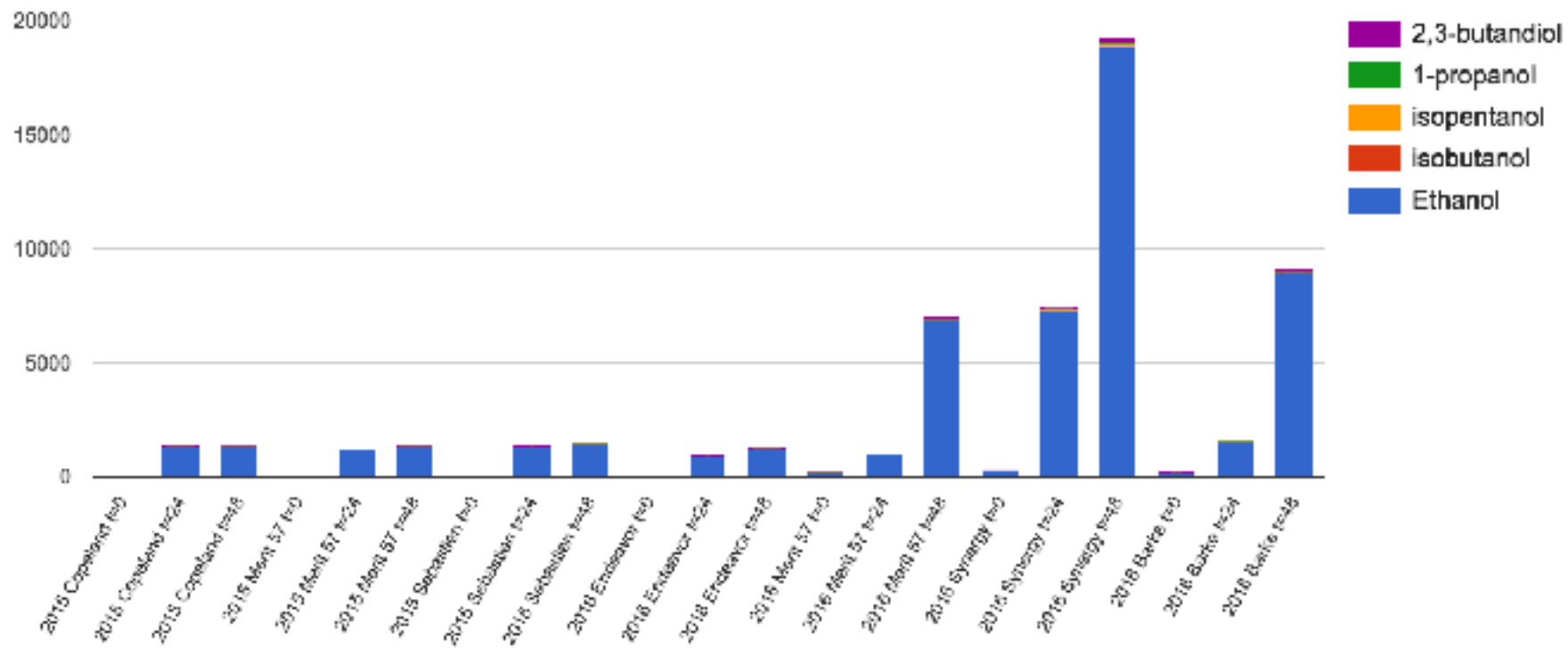
- *Weisella cibaria*
- *Pediococcus pentosaceus* (0.7%)
- *Enterobacter* (2.5%)



# Results: Soured-Wort NMR (organic acids)



# Results: Soured-Wort NMR (alcohols)



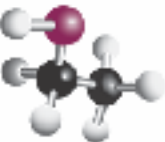
# Discussion

- *Weissella cibaria*: first described in 2002 originating from Thai fermented foods (originally called *W. kimchii*) and European sourdough, closely related to *W. confusa*.
- Gram-positive, catalase-negative, non-endospore forming cells with coccoid or rod-shaped morphology. Family *Lueconostocaceae*.
- Obligately heterofermentative
- 59F-113F
- Hydrolyse Arginine
- Produces D and L lactic acid, acetic acid, CO<sub>2</sub>,
- Does not metabolize lactose, galactose
- Dextran (non-digestible polysaccharide) produced from sucrose
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4362408/>



# Further Experiments

- Different conditions: Temperature!
- Nutrition: Amino acids?



# Thanks and Contact Information

A huge thanks to:

–Bob Hansen, Technical Services Manager, Briess Malting

–Zymo Research Corp.

–Dr. John C. Edwards, Process NMR Associates

–Scott Britton, R&D Scientist, Brouwerij Moortgat (Duvel Moortgat, NV)

and of course,

–Dr Matthew Bochman (Doc Boc), Indiana University

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2017 ASBC Meeting

