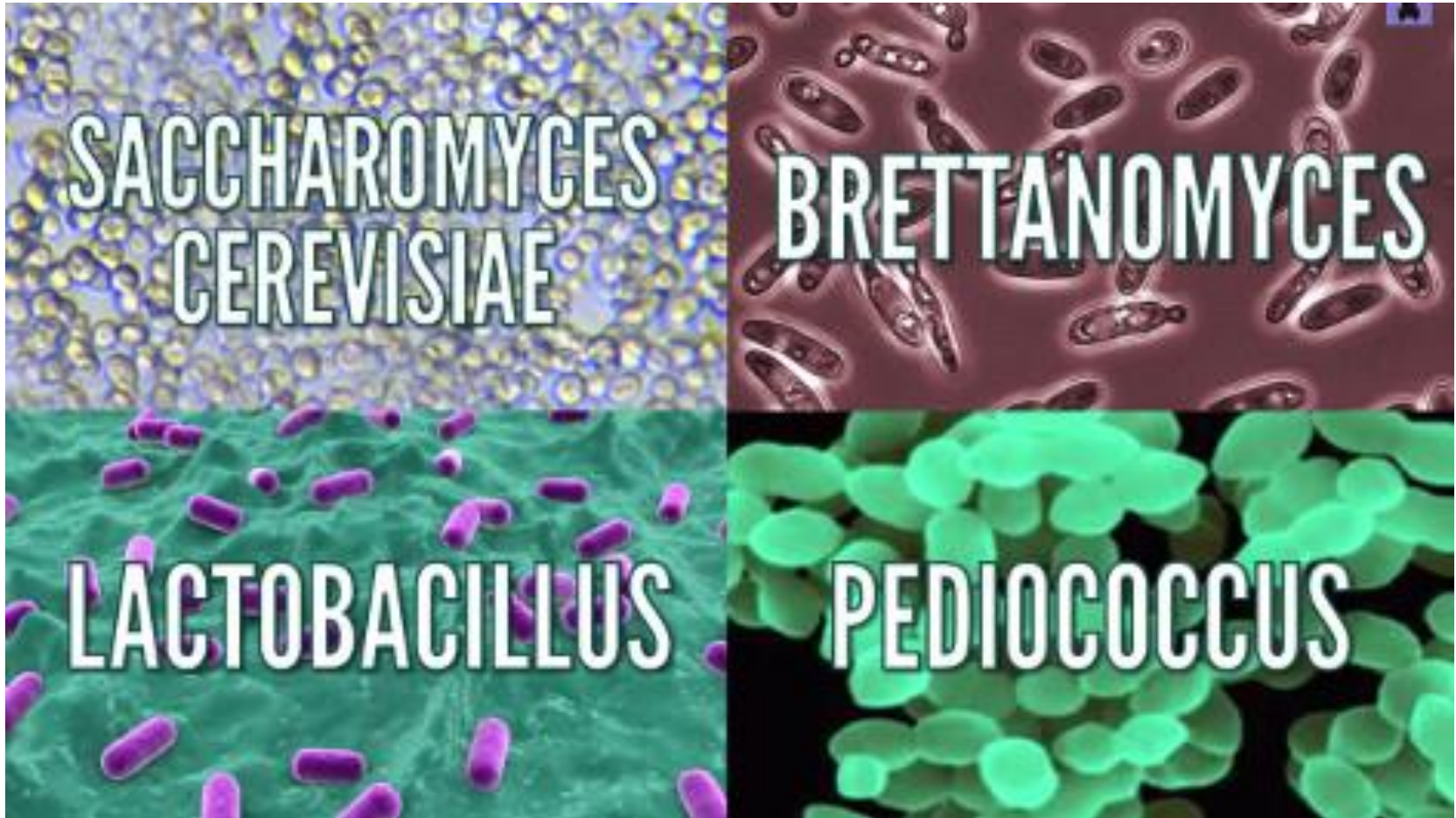




# Souring Organisms

Brittney Berg  
Lallemand Brewing  
[bberg@Lallemand.com](mailto:bberg@Lallemand.com)

# Overview



# History and styles of sour beer

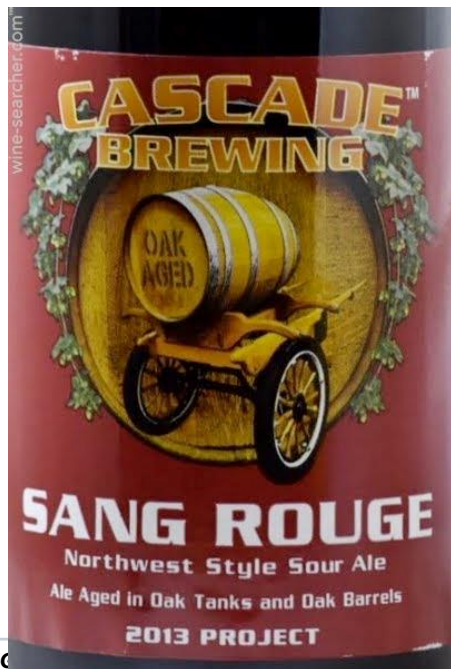
- Sour beer styles have existed for centuries
- What do we mean by Sour beer?
- History and heritage of sour beers have been based in Europe, including:

- Berliner Weisse
- Gose
- Lambic
- Flanders Red Ale



# Modern sours

- Growth in Belgian sour imports in 90's influence US
- Based on historic styles
- Huge diversity in sour styles, flavors and creativity



# Souring applications and techniques

## ➤ Mash Souring

- Liquor, grain adjustment
- Bacteria from grain or inoculated
- 2 – 3 days

## ➤ Kettle Souring

- Wort inoculated with LAB
- 2 -3 days

## ➤ Co-fermentation

- Mixed sacc, LAB & Brett
- Typical fermentation time

## ➤ Barrel/Foeder/spontaneous ageing

- Often in wood (or Keolschip)
- Mixed spectrum of microflora
- Greater complexity



# Key microorganisms

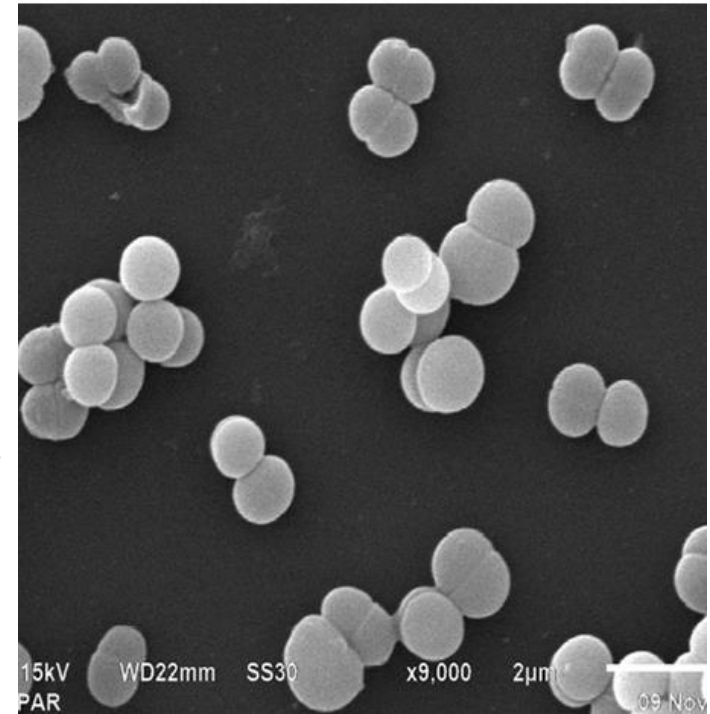
- **Bacteria**
  - Lactic acid producing bacteria
    - Lactobacillus
    - Pediococcus
- **Non-Saccharomyces Wild Yeast**
  - Brettanomyces
  - Lachancea
- **GMO souring yeast**
  - Sourvisiae

# Lactic Acid Producing bacteria

Pediococcus & Lactobacillus

# Pediococcus

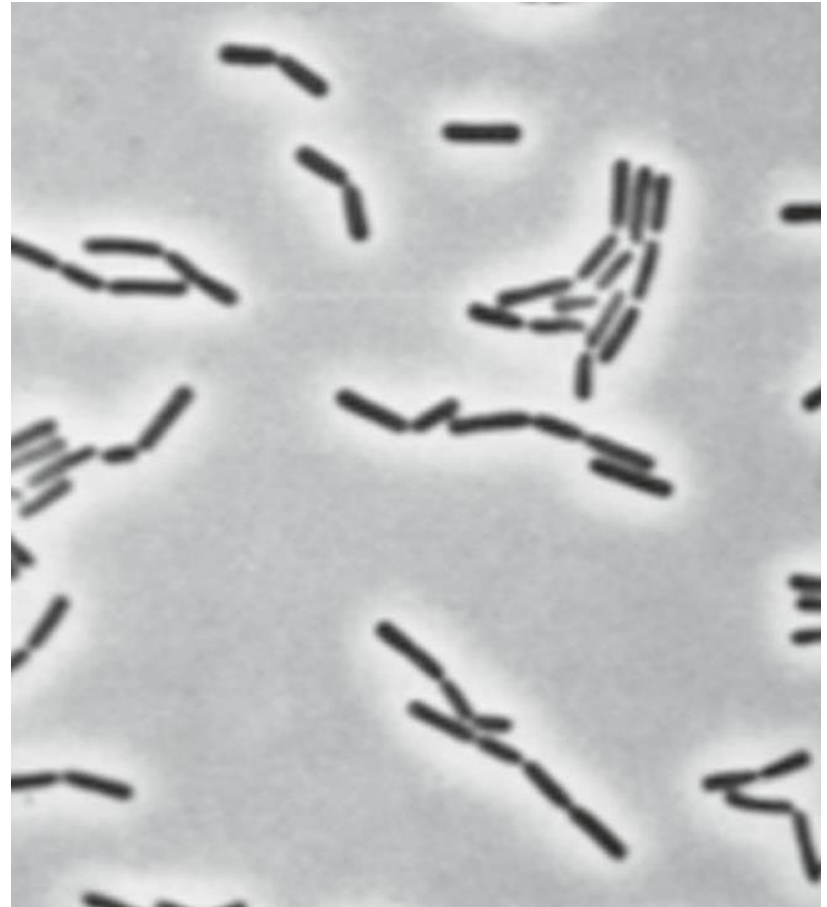
- Lactic acid producing bacteria, can be  $<3.0$  pH
- Slow acting
- Can grow in CO<sub>2</sub> rich environments and in O<sub>2</sub> environments
- Found naturally on most fruits
- Hop tolerant
- A "ropey" diacetyl producer
- Help from Brettanomyces!





# Lactobacillus

- Primary souring bacteria
- Diverse range of sub species
- Temperature sensitive
- Softer and tangier lactic acid
- Sugar Utilization



# Sources of LaB

- Laboratory (pure or mixed culture)
- Bottle cultures
- Nature
- Yogurt
- Un-mashed grain

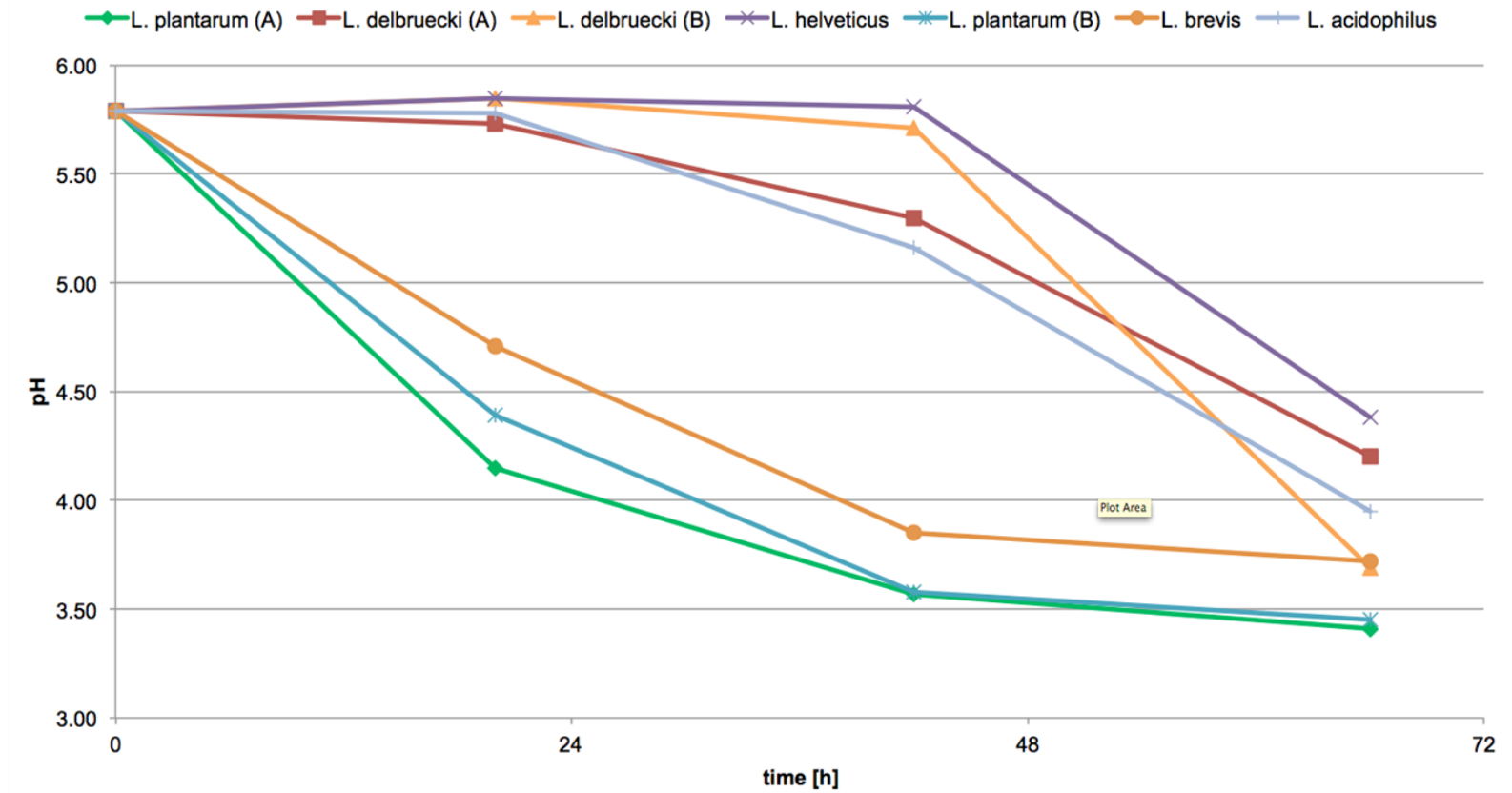


# R&D Trials

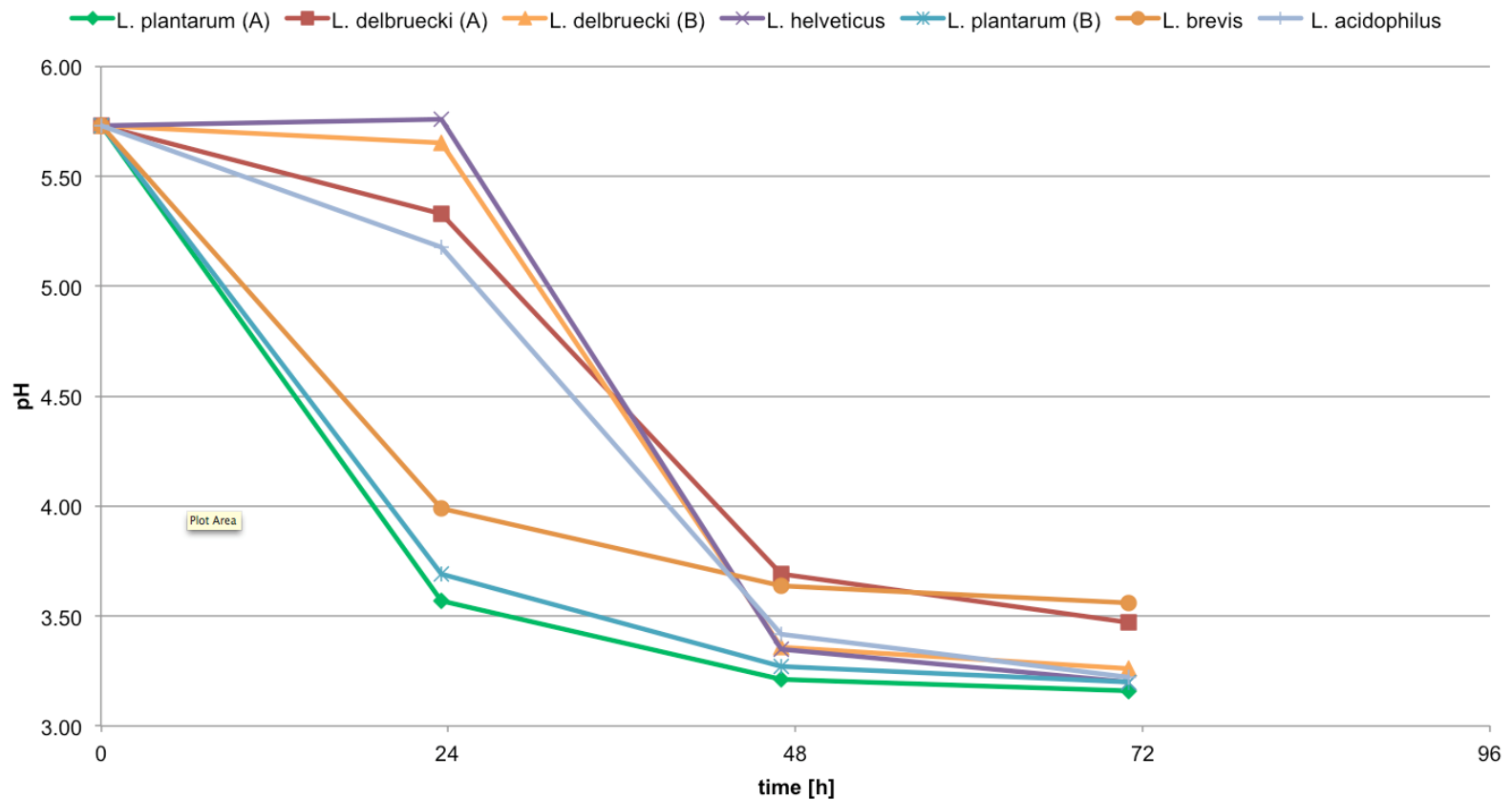
- Achieve pH 3.5 or lower in <48 hours
- Achieve high lactic acid vs low acetic concentration
- Fermentations at 4 different temperatures
- Gravity and pH measurement daily
- Acid and glycerol analysis by HPLC
- Sensory assessment of samples by panel

Strains
<i>L. plantarum</i> (A)
<i>L. delbrueckii</i> (A)
<i>L. delbrueckii</i> (B)
<i>L. helveticus</i>
<i>L. plantarum</i> (B)
<i>L. brevis</i>
<i>L. acidophilus</i>

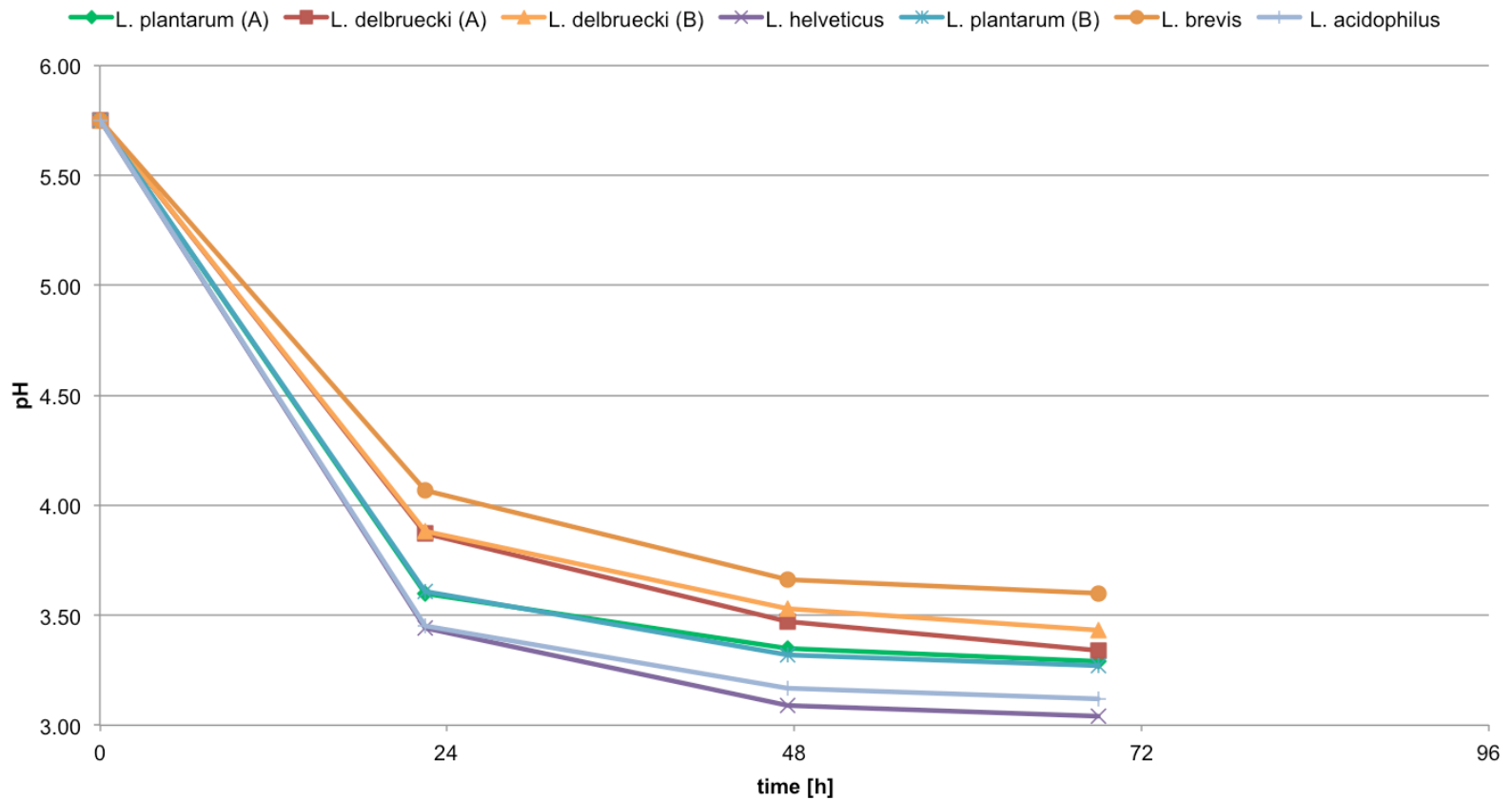
# R&d trials: Fermentations (20C) (..F)



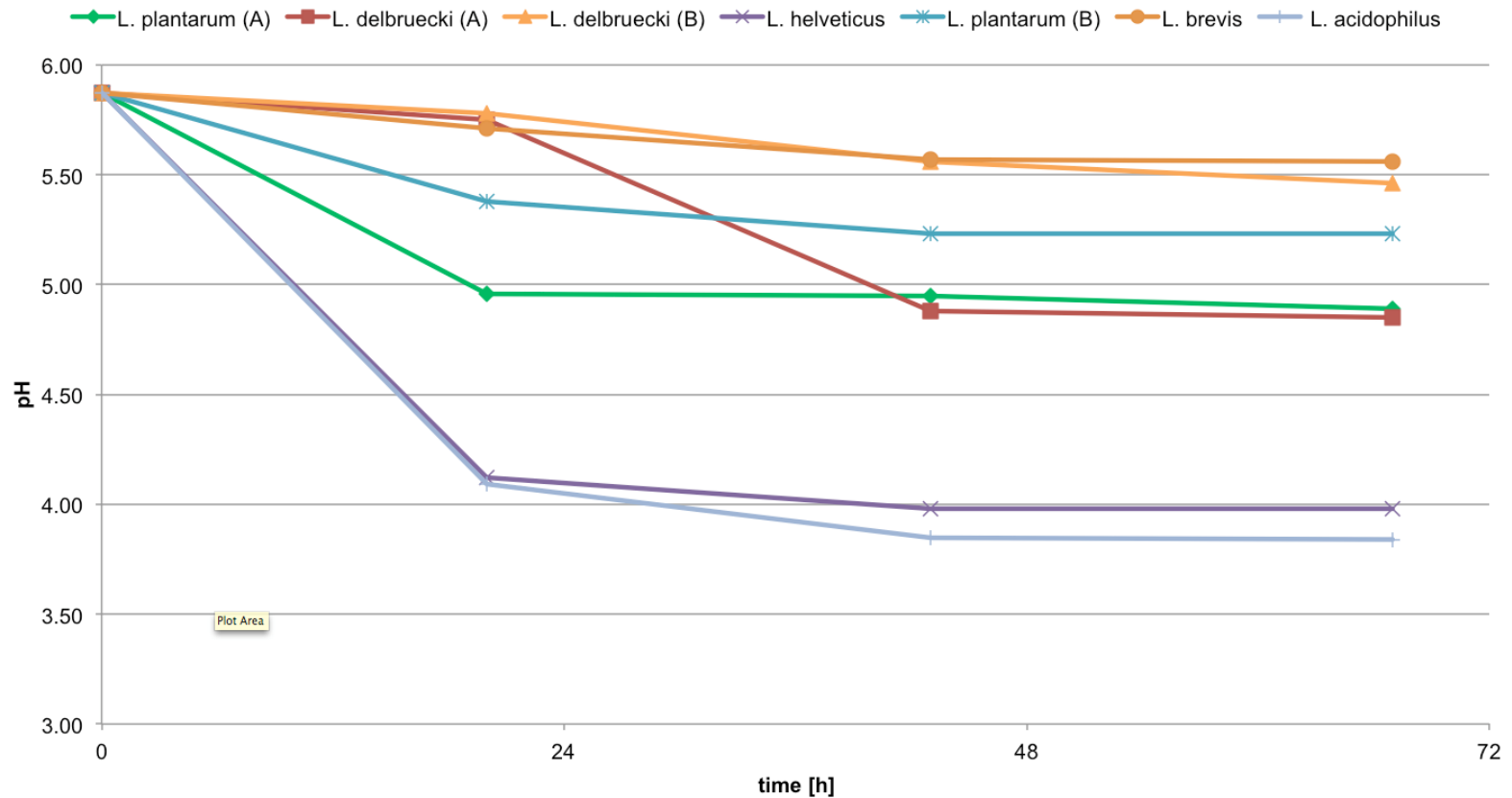
# R&D trials: Fermentations (30C)



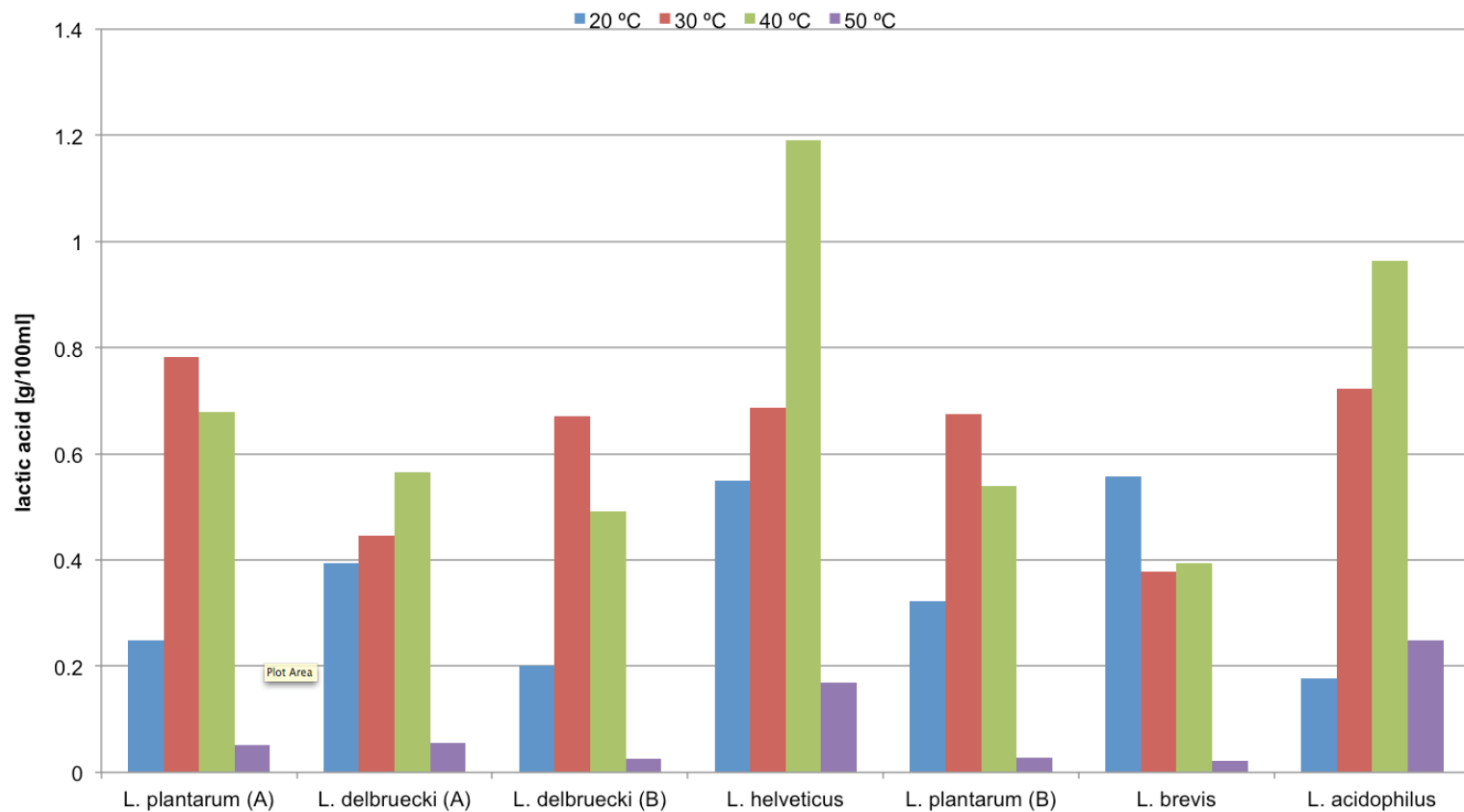
# R&D trials: fermentations (40C)



# R&D trials: fermentations (50C)

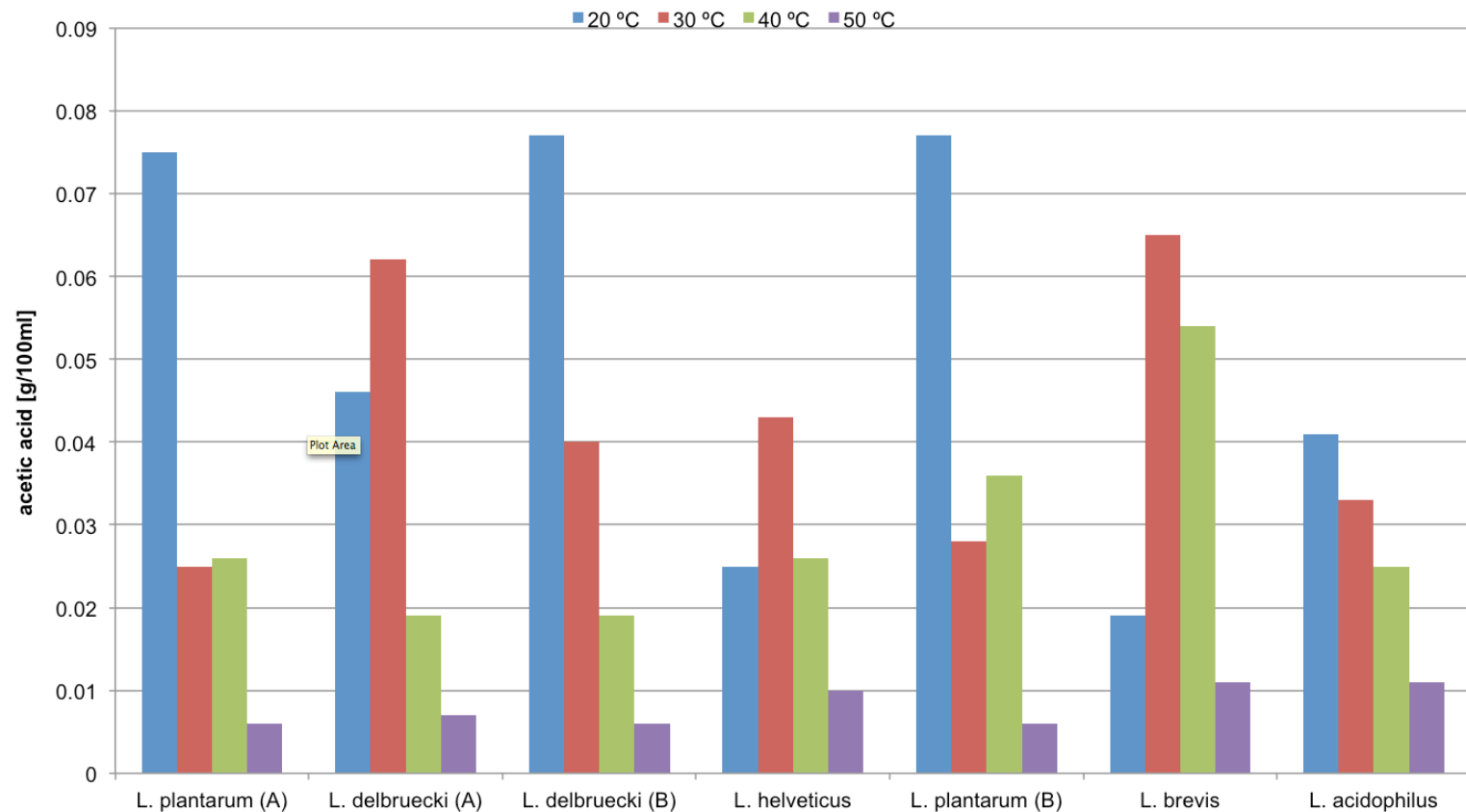


# R&D trials: lactic acid production





# R&D trials: acetic acid production



# R&D trials: Sensory

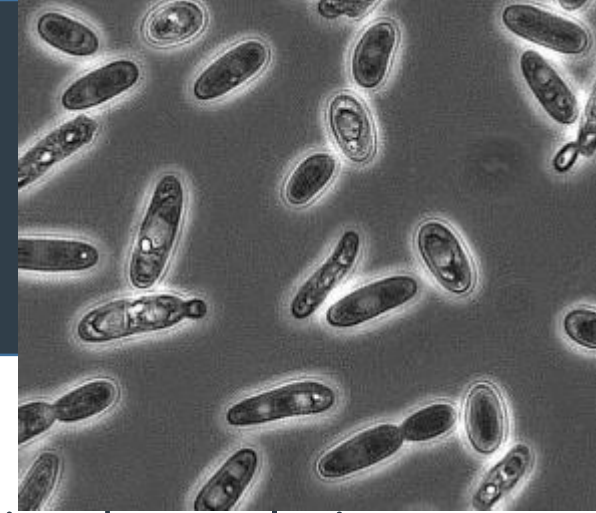
## Characteristics

	20 °C	30 °C	40 °C	50 °C
<i>L. plantarum</i> (A)	Sweet, sour, apple	Fruity smell, sour light tart taste	little sour, wort taste and smell	Wort flavour and smell, not sour
<i>L. delbrueckii</i> (A)	Burnt rubber smell, sweet taste	Sweet, thin, not very sour	Fruity, citrusy sour taste	Wort flavour and smell, not sour
<i>L. delbrueckii</i> (B)	“burnt fruit”, apricot smell, sour taste	Sour light tart taste	Fruity, citrusy sour taste	Wort flavour and smell, not sour
<i>L. helveticus</i>	Fruity smell, sweet taste	Smells like Resins, sour taste	Very sour taste and smell	Light sour taste
<i>L. plantarum</i> (B)	Sweet smell, sour taste	Sweet sour smell, sour taste	Malty, bread smell, sour taste	Wort flavour and smell, not sour
<i>L. brevis</i>	Sweet smell, light burnt taste	Sweet, thin, not very sour	Sweet malt smell, not very sour taste	Wort flavour and smell, not sour
<i>L. acidophilus</i>	Smells like mango, tastes like green mango	Sour smell, tastes sour, green apple	Very sour taste and smell but better balanced vs. HA-128	Light sour taste

# Wild non-saccharomyces strains

Brettanomyces & Lachancea

# brettanomyces



- A wild yeast; large genetic diversity
- Was commonly seen as spoilage yeast, gained popularity
- Barnyard, horsey, band-aid & fruity esters
- Tolerant to harsh conditions
- Sugar utilization
- **Attenuation**
  - Good attenuators due to production of a range of alpha & beta-glucosidases
- **Secondary Metabolites**
  - Varies based on the composition of the wort, available oxygen, and time.
  - Good at producing acetic acid even low levels of O<sub>2</sub>

# Common *Brettanomyces* strains

## 1. *Brettanomyces bruxellensis*

- Most popular strain – widely used
- Medium intensity Brett character
- Commonly found in Belgian pales and Lambics
- Typically starts as 'horse blanket' / leathery and become more light & earthy as time goes on

## 2. *Brettanomyces lambicus*

- Most intense out of the bunch – very horsey, spicy/smokey flavor
- Found in Lambics
- Keep O<sub>2</sub> low to avoid "nail polish" – ethyl acetate

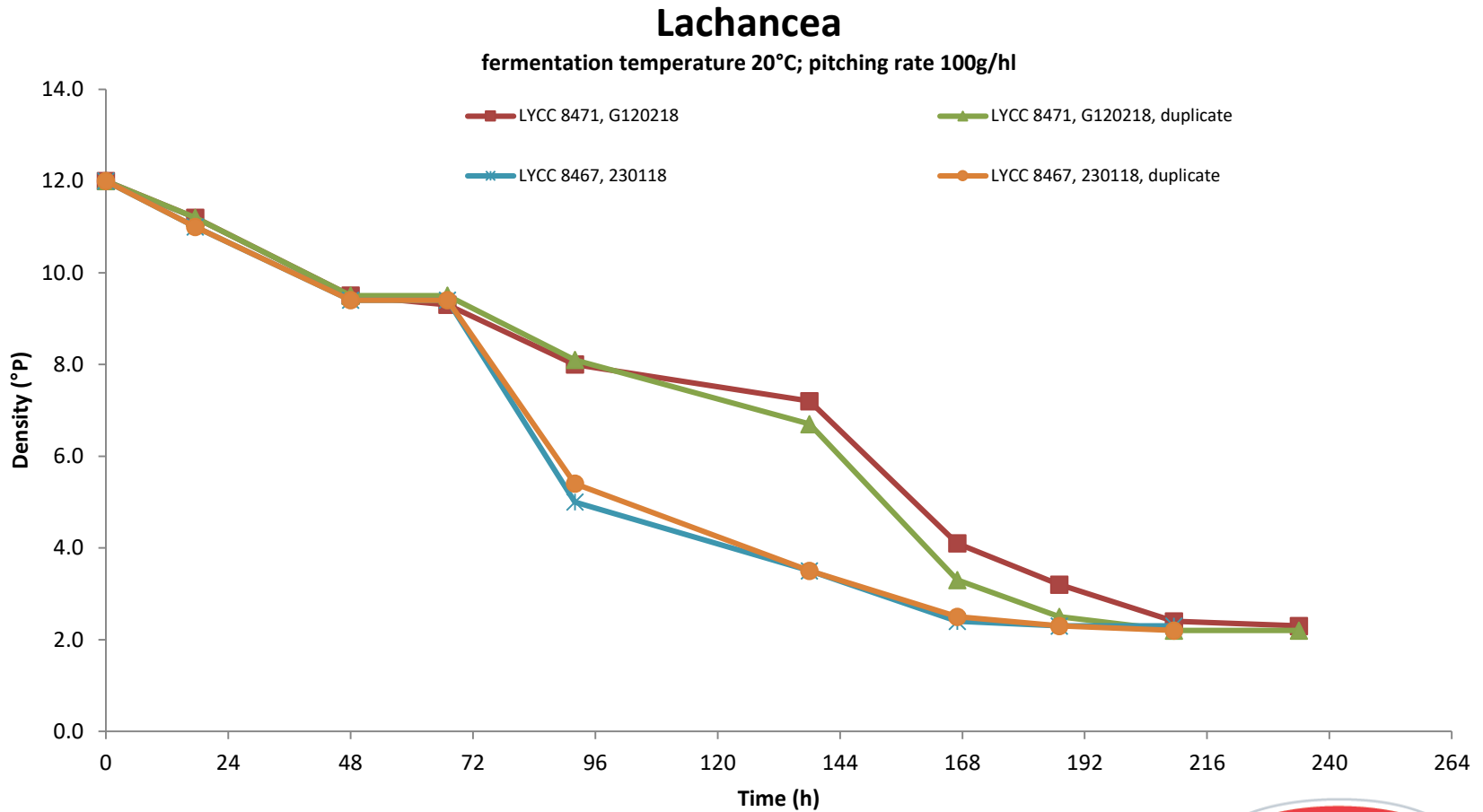
## 3. *Brettanomyces claussenii*

- *Light intensity Brett, can take many months to fully develop noticeable characteristics*
- *Often associated with fruity aromas – pineapple & mango*

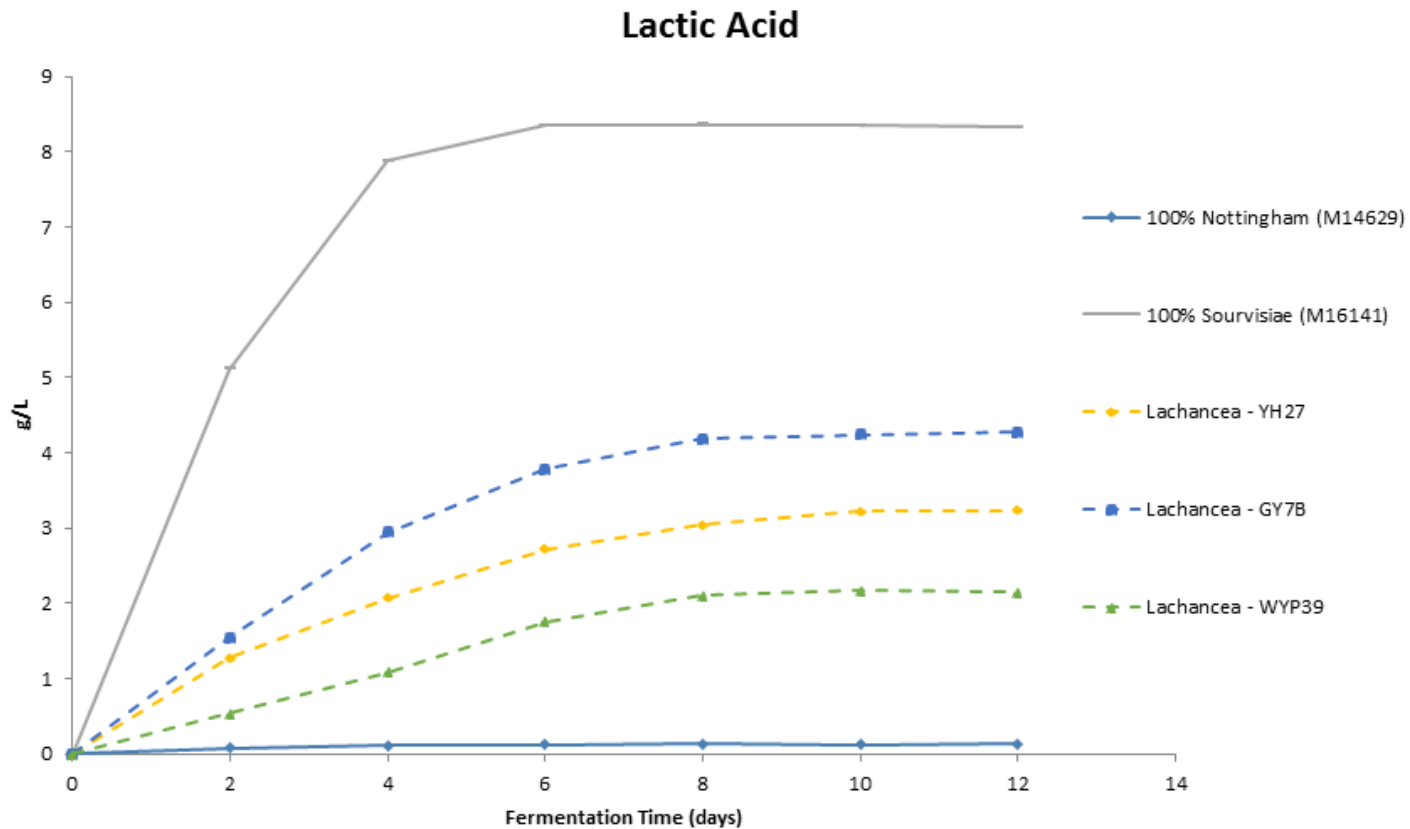
# Lachancea

- a lactic acid producing non-sachh yeast
- Like most wild strains, it is a very slow fermenter
- Can be used in primary or secondary ferms
- Not as much know with this strain and not used as often in commercial breweries
- Known strains: *L. fermentati* and *L. thermotolerans*
- New unknown Lachancea strain has been discovered and is believed to be genetically different from that of *L.fermentati* and *L.thermotolerans*
-

# Lachancea fermentation data

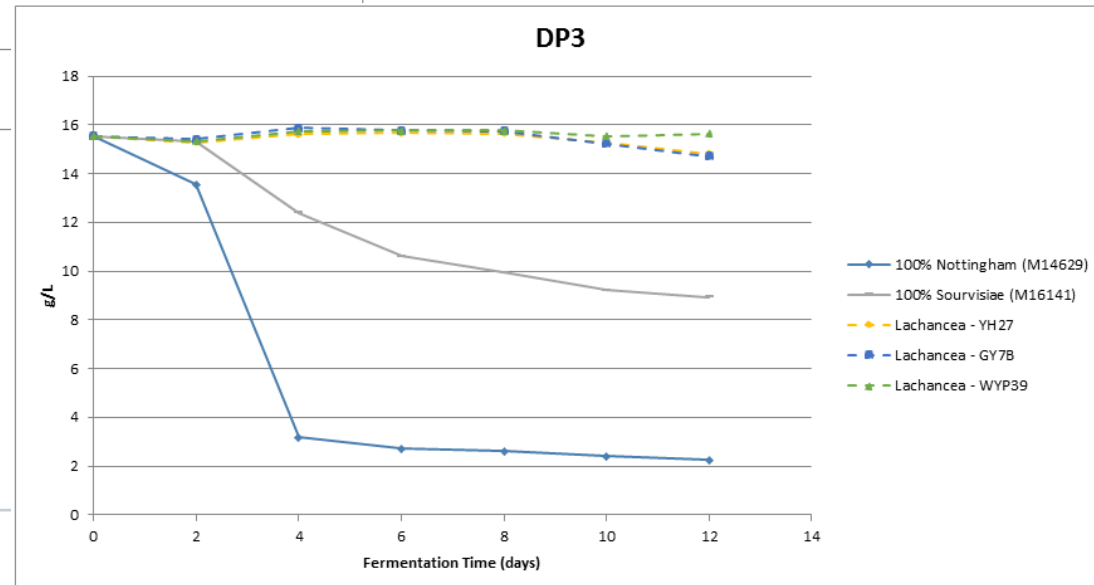
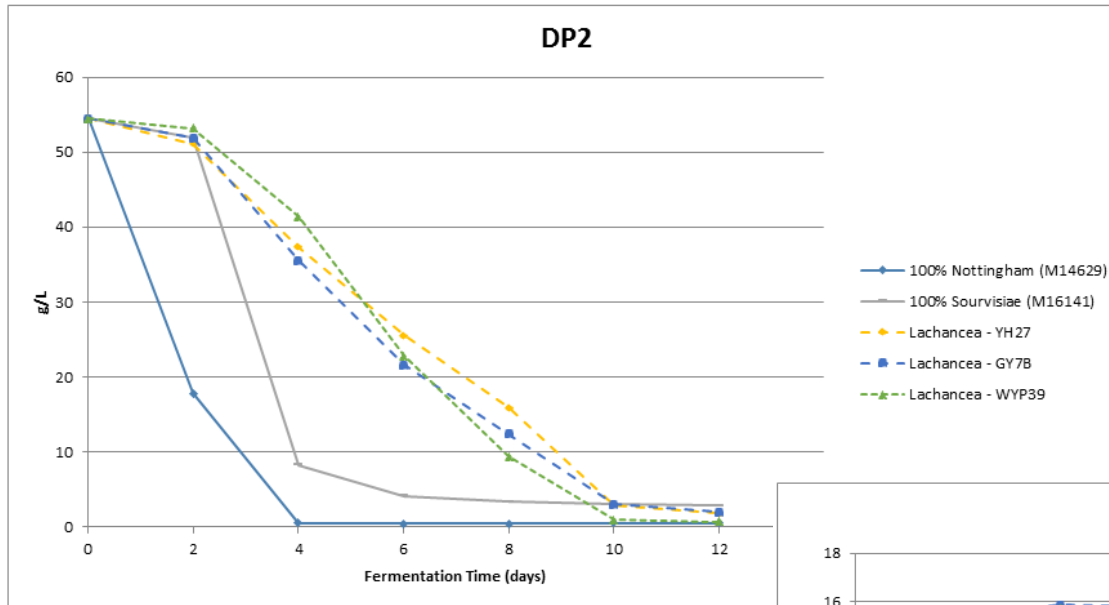


# Lachancea lactic acid production





# Lachancea sugar metabolism



# New Technology

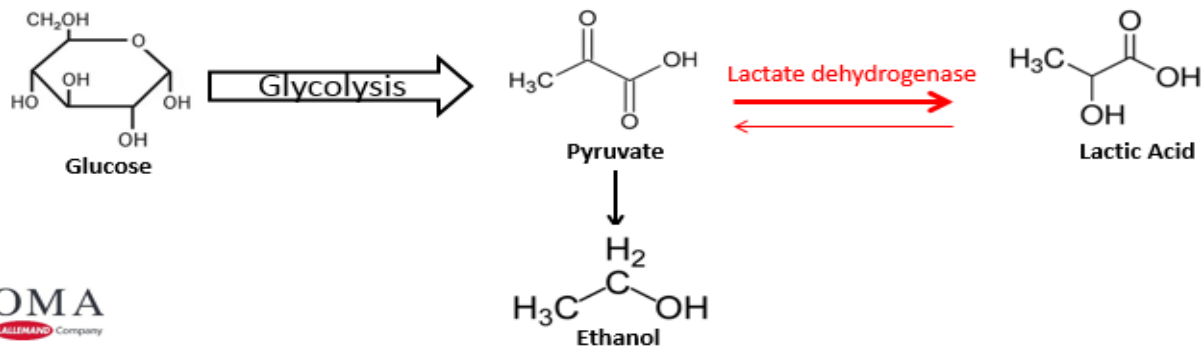
GMO Strain – Sourvisiae™ from Mascoma

# New technology: bioengineered strain

Sourvisiae™ is a bioengineered ale yeast (*Saccharomyces cerevisiae*) capable of producing lactic acid during primary fermentation:

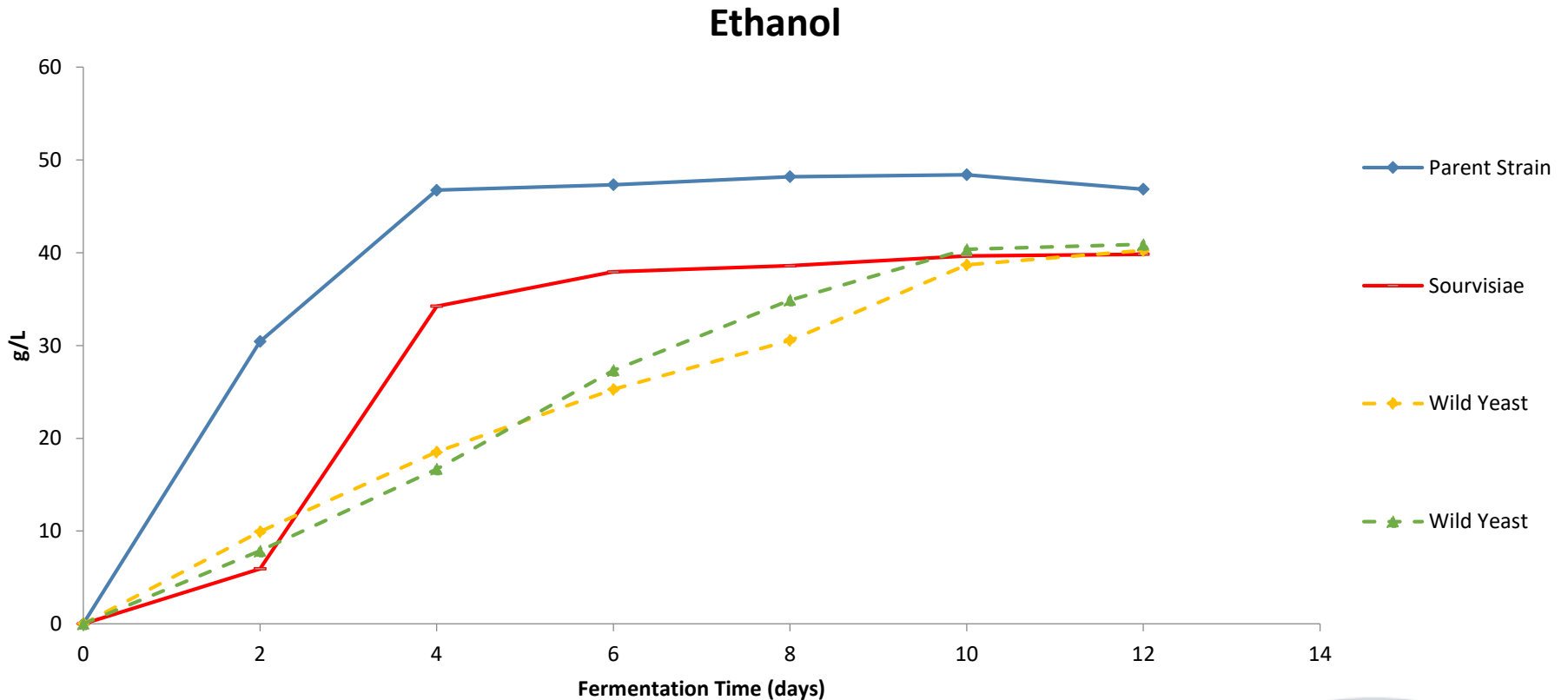
- Reproducible
- Mono-culture
- No other flavor compounds produced
- Generally Regarded as Safe (GRAS)

Pathway for lactic production in Sourvisiae™:



# Sourvisiae™

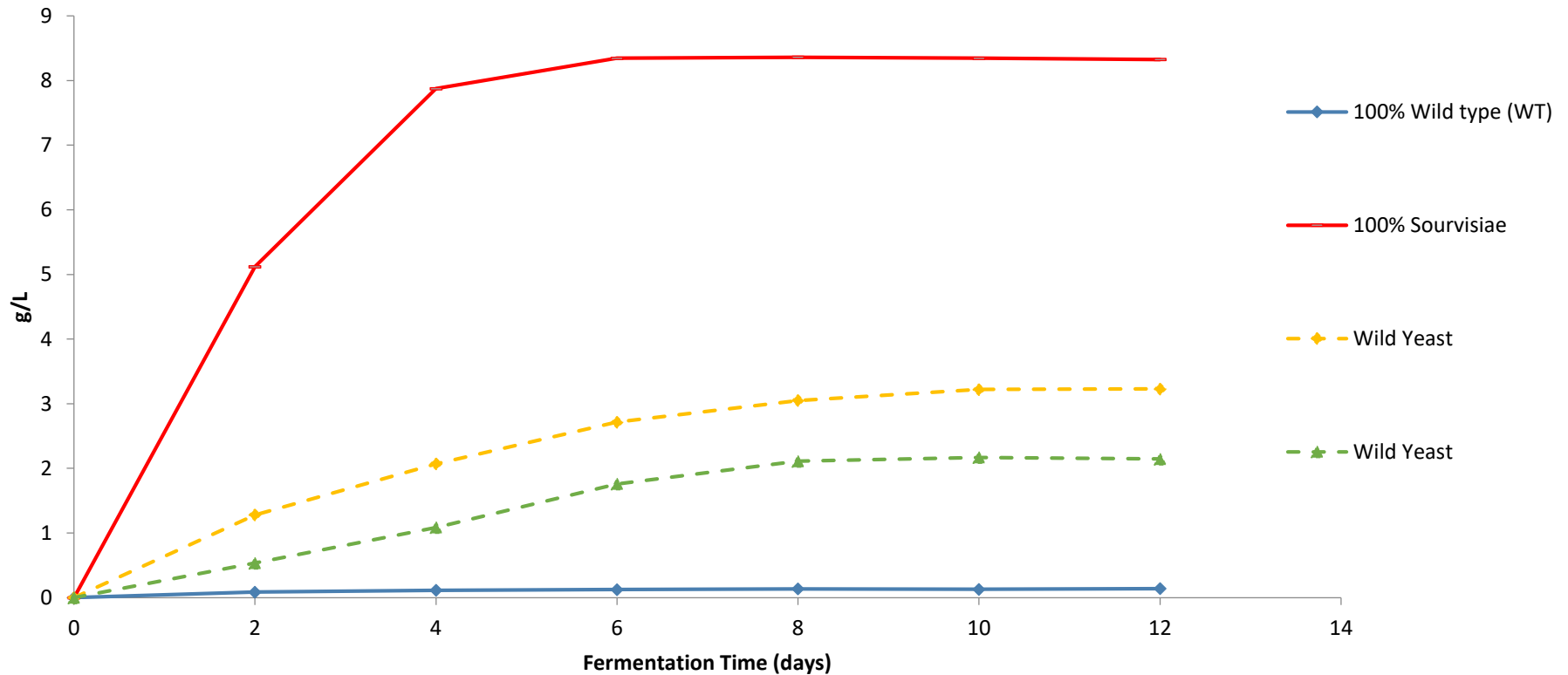
Ethanol production



# Sourvisiae™

Lactic acid production

## Lactic Acid



# Sourvisiae Pros/Cons

## Pros

- Will not hold up your kettle; saves time and money
- So far no THP discovered through sensory

## Cons

- GMO may not be for everyone – and that's ok!
- Difficulty predicting initial sourness, relying on blending in the end

# Final notes

- Know your final flavor/aroma wants
- SOPs – stay clean!
- Strive for continuous improvement and experimentation
- If you're going to brew sour beer, be prepared to dump it



# Questions?

Brittney Berg – Lallemand Brewing  
[bberg@lallemand.com](mailto:bberg@lallemand.com)





# CBA Trials

## Control vs Sourvisiae



**CRAFT BREW**  
**ALLIANCE**

### INGREDIENTS AND PROFILE

Malts	90% Great Western Two-Row, 10% Briess Caramel C60
Hops	Bittering - Alchemy Aroma - Cascade
Yeast	Sourvisiae

#### **Wort Analysis:**

OG: 12.76

pH:5.52

BU:41

SRM: 8.7

#### **Pitch rate:**

100g/bbl into 5 bbls of wort

Pitch Temperature: 65F

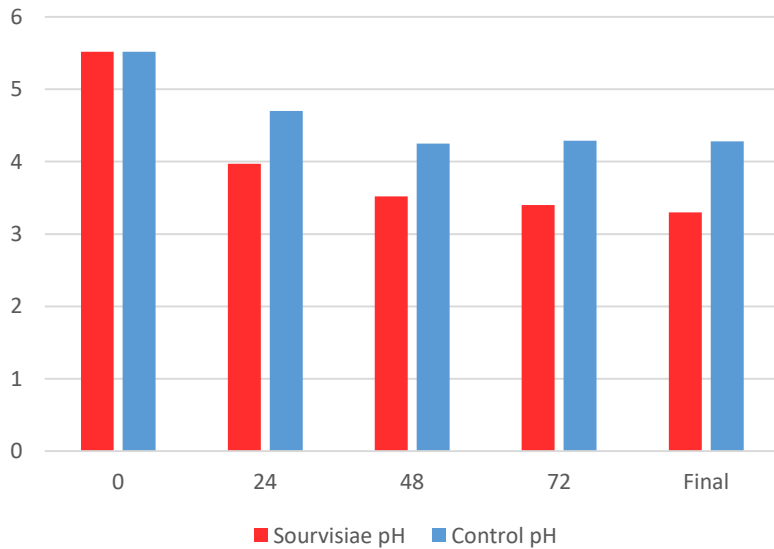
Primary Fermentation Setpoint: 66F

Aeration Type: Filtered Plant Air, ~12g/L over 10 minutes of cooling

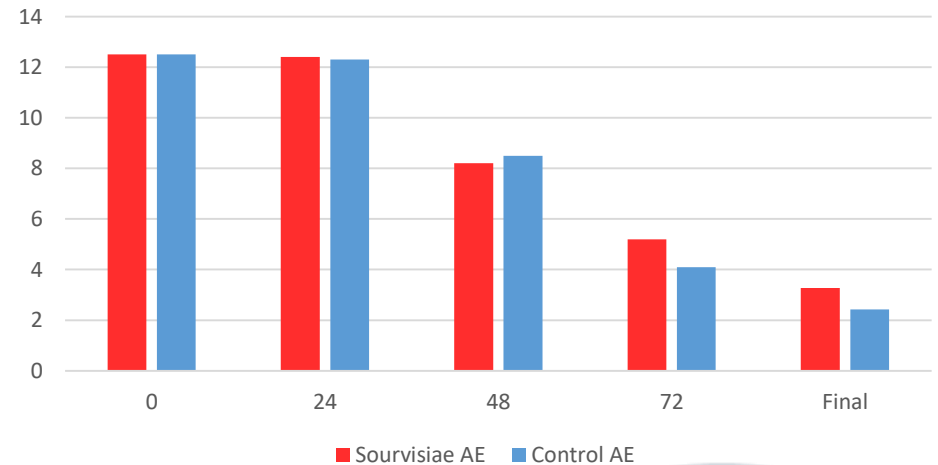
# Trials - Sourvisiae

CBA/Widmer Control vs Sourvisiae

### Trial pH Comparison



### Trial AE Comparison



# Introduction to Genetic Modification

Direct manipulation of genome with biotechnology:

- Specifically tailored function
- Use of familiar, single template strain
- Yeast are great at accepting other yeasts' DNA
- Finer tuning than classical approaches