



WORLD BREWING CONGRESS

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#ElevateBeer



Investigation of
Lachancea thermotolerans
as a novel, single-strain brewing yeast

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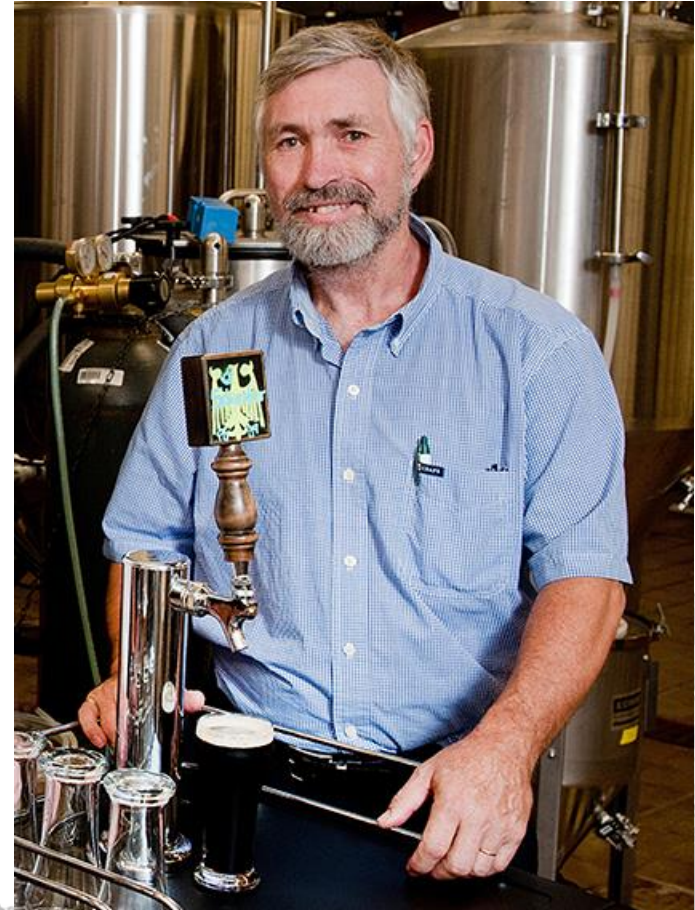
Raleigh, NC





Investigating Wild Yeast for Brewing Beer

- Collaborative project creating local beer
- Dr. Rob Dunn
 - NCSU Biology
 - Isolate wild yeasts
- Dr. John Sheppard
 - NCSU Food Science
 - NCSU Brewery
 - Ferment wild yeasts





Lachancea thermotolerans

- Isolated from a bumble bee
- Identified by PCR and phenotypic analysis
- Confirmed GRAS status





Experimental Goals

- 1) Differentiate *L. thermotolerans* NCSU from **type strain**:
 - *L. thermotolerans* NRRL Y-8284 (ATCC® #56472)
- 2) Demonstrate *L. thermotolerans* NCSU can be used as a **pure culture** brewing strain





Lachancea thermotolerans

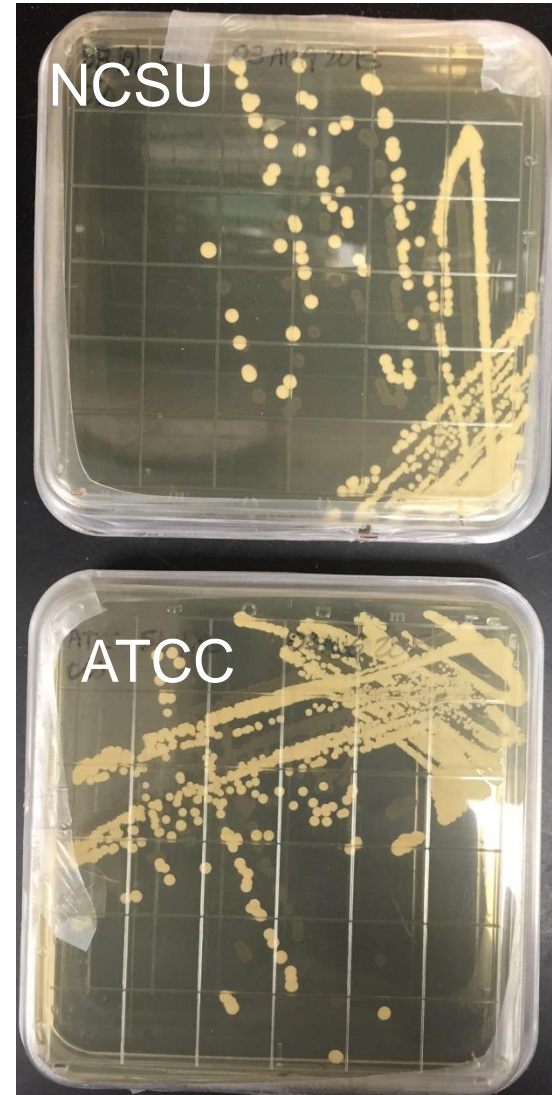
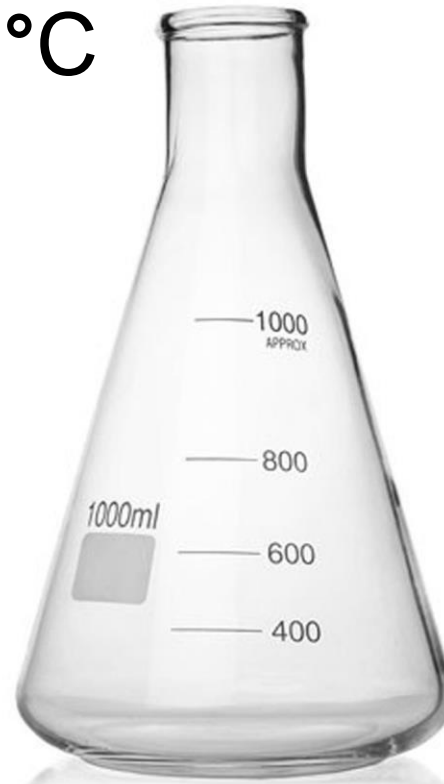
- Previously *Kluyveromyces thermotolerans*
- Mixed evaluations as as a wine yeast:
 - Useful for bioacidification and aroma enhancement
 - Poor fermenter, needs to be co-fermented with *Saccharomyces* for wine production



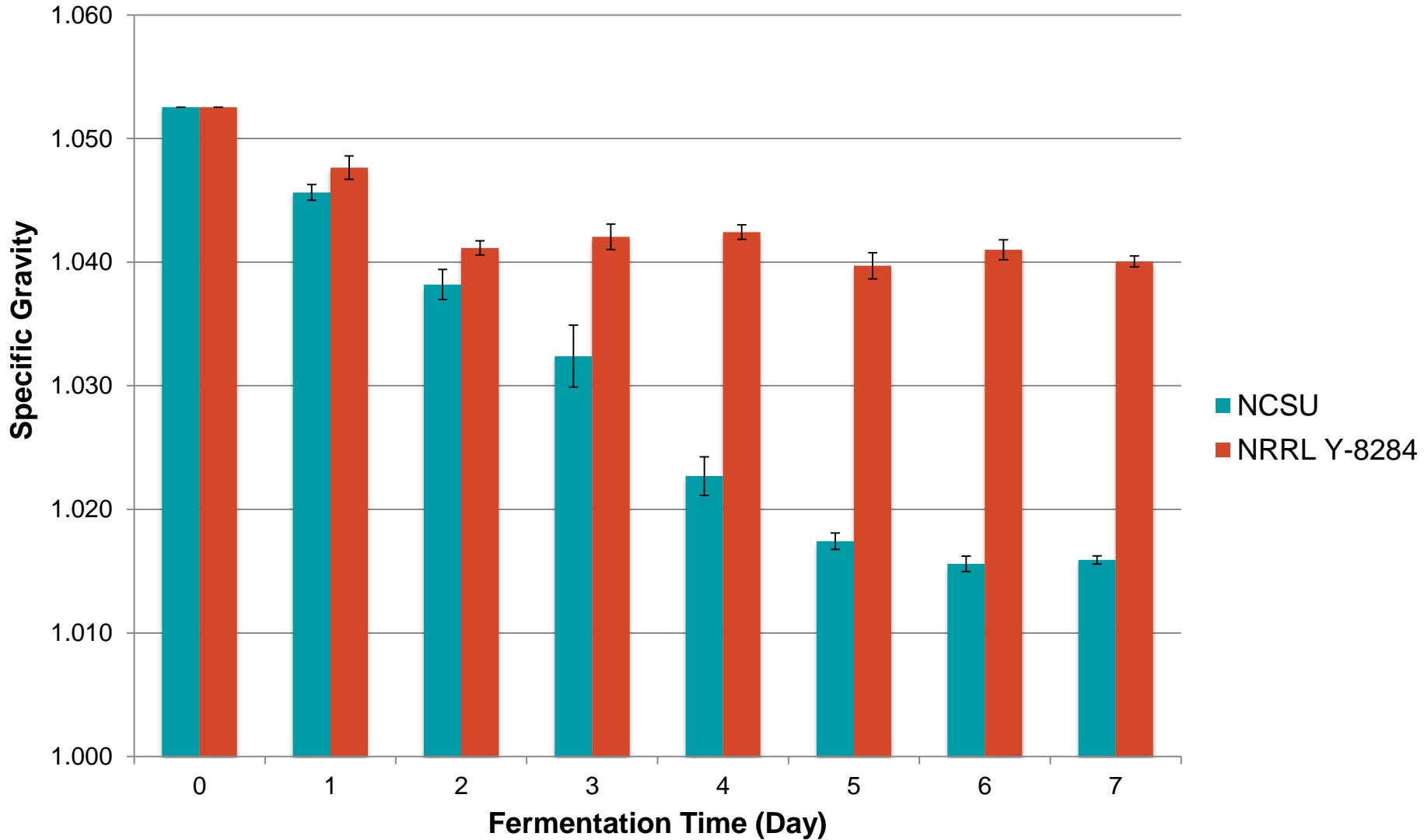


Fermentation Conditions

- Laboratory scale
 - Fermented in triplicate
- Pilsner wort at 18 °C
- OG: 13 °P
- Sampled daily

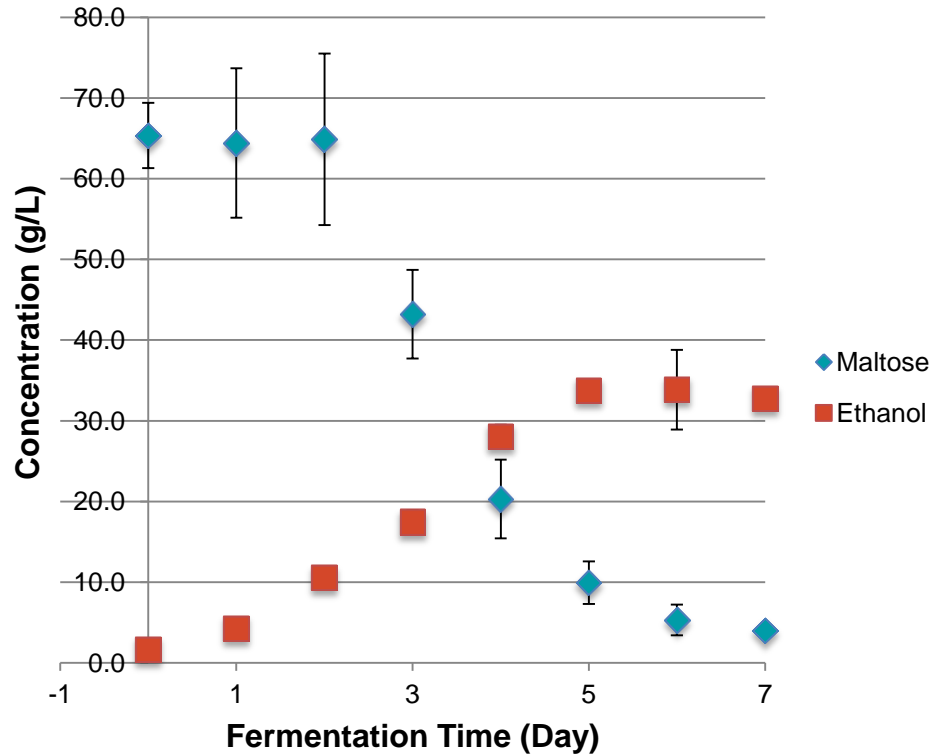


Wort Attenuation

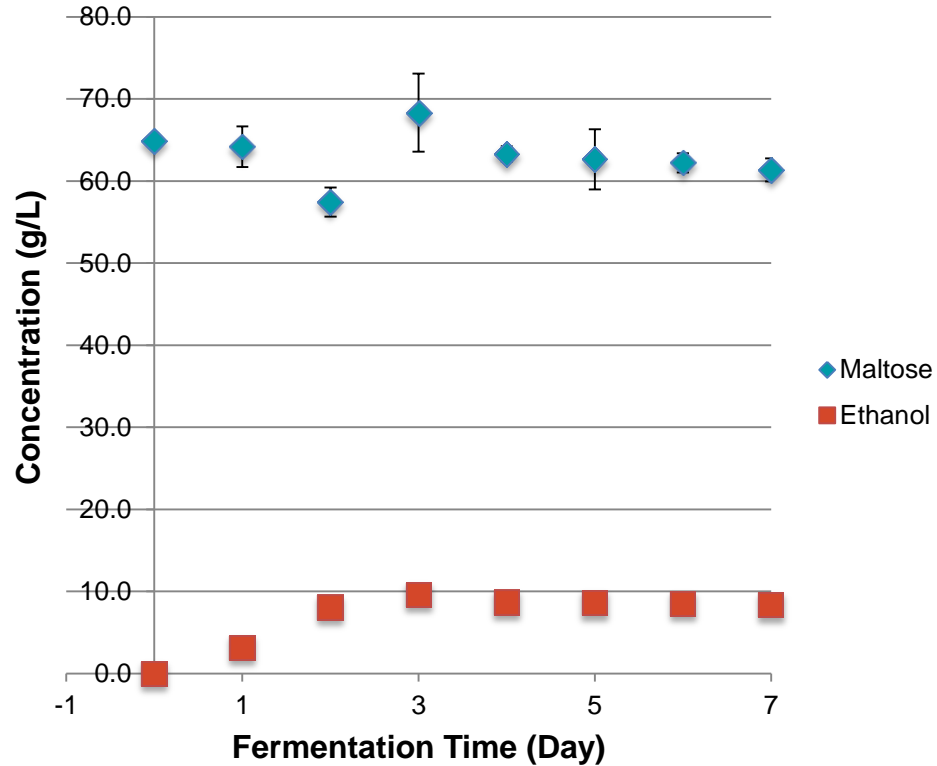


Maltose Utilization and Ethanol Production

NCSU

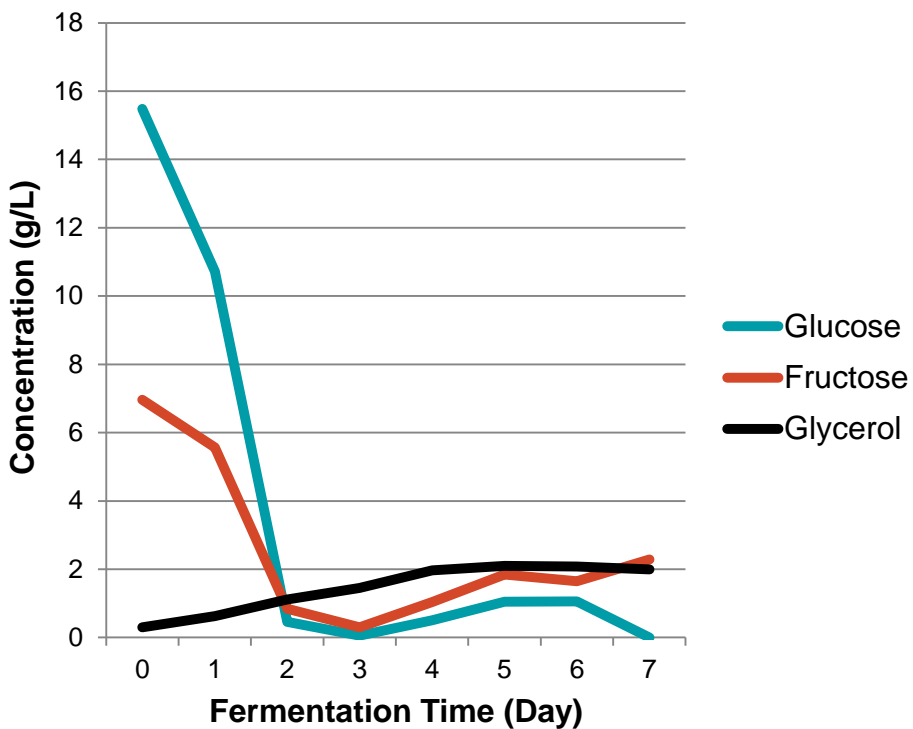


NRRL Y-8284

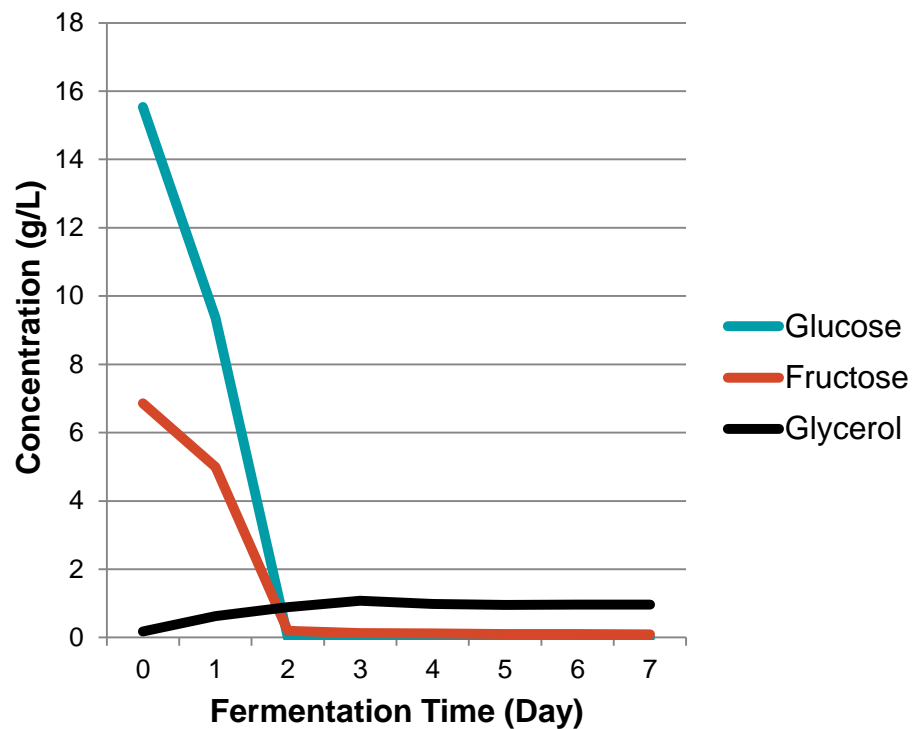


Monosaccharide Utilization and Glycerol Production

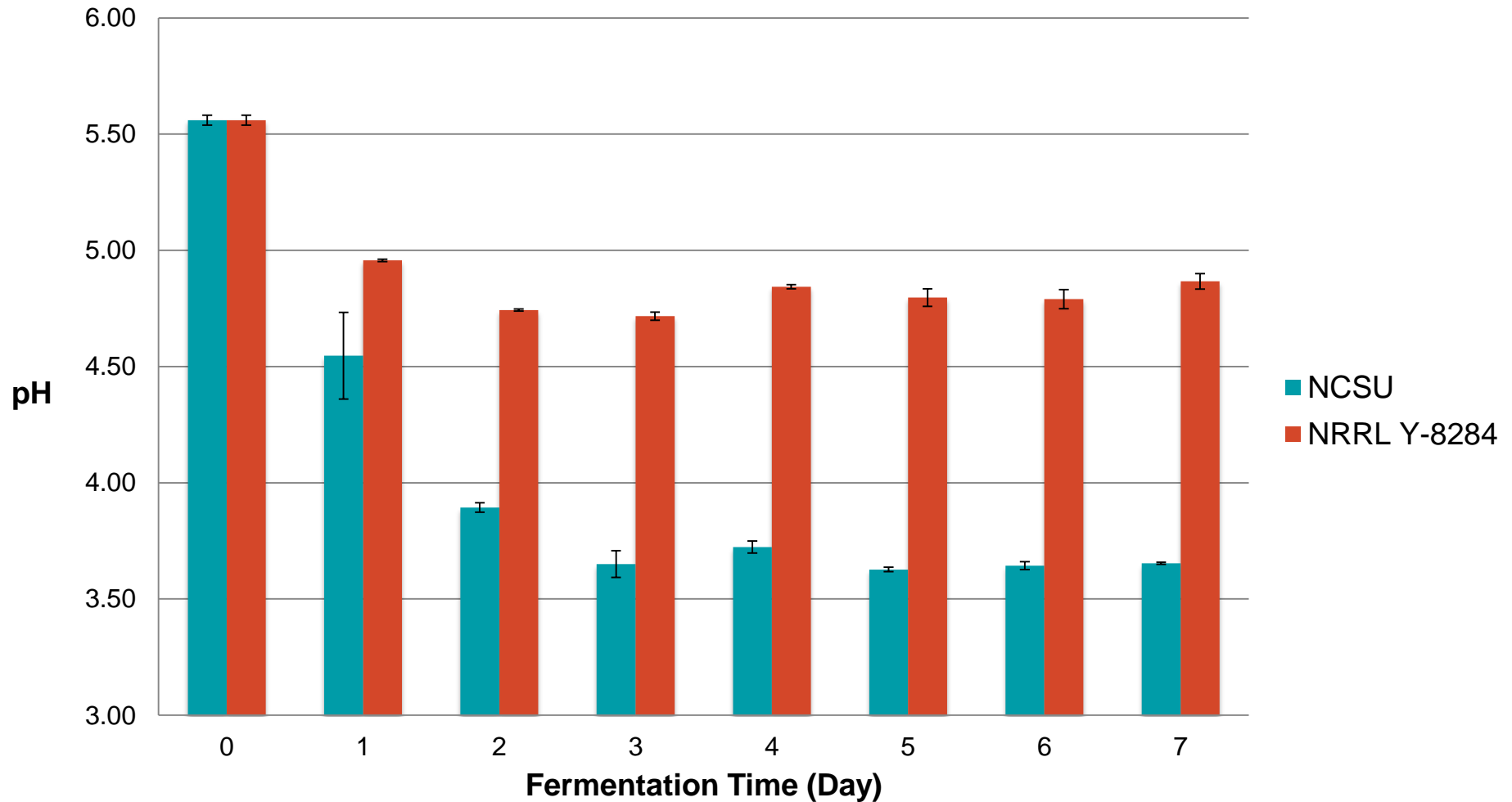
NCSU



NRRL Y-8284



Wort Acidification





L. thermotolerans Comparison

- Strain **NCSU** compared to **NRRL Y-8284**
 - Increased wort attenuation
 - Increased wort acidification
 - Increased ethanol production
 - Increased glycerol production



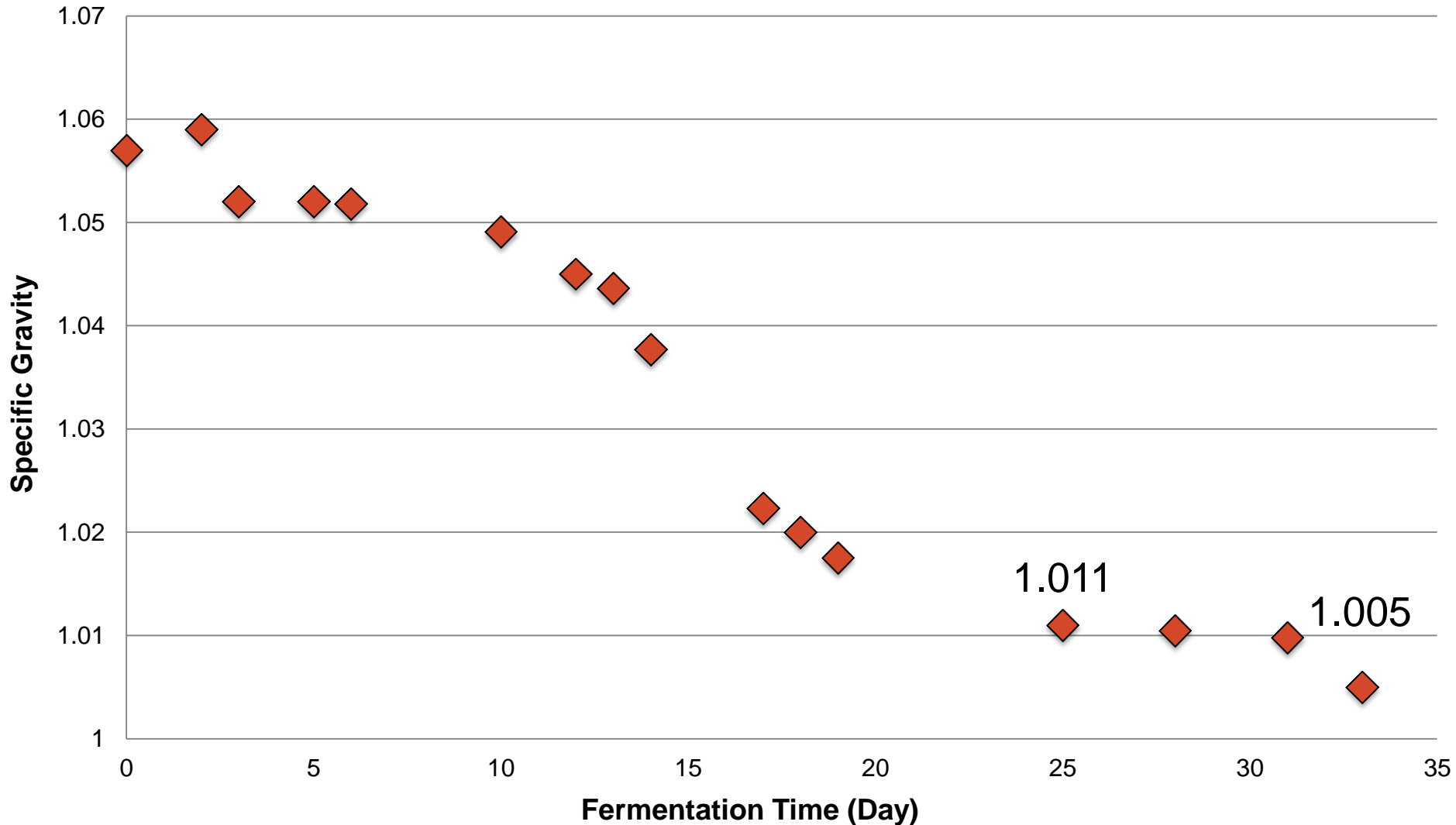


“Lambic Style” Fermentation

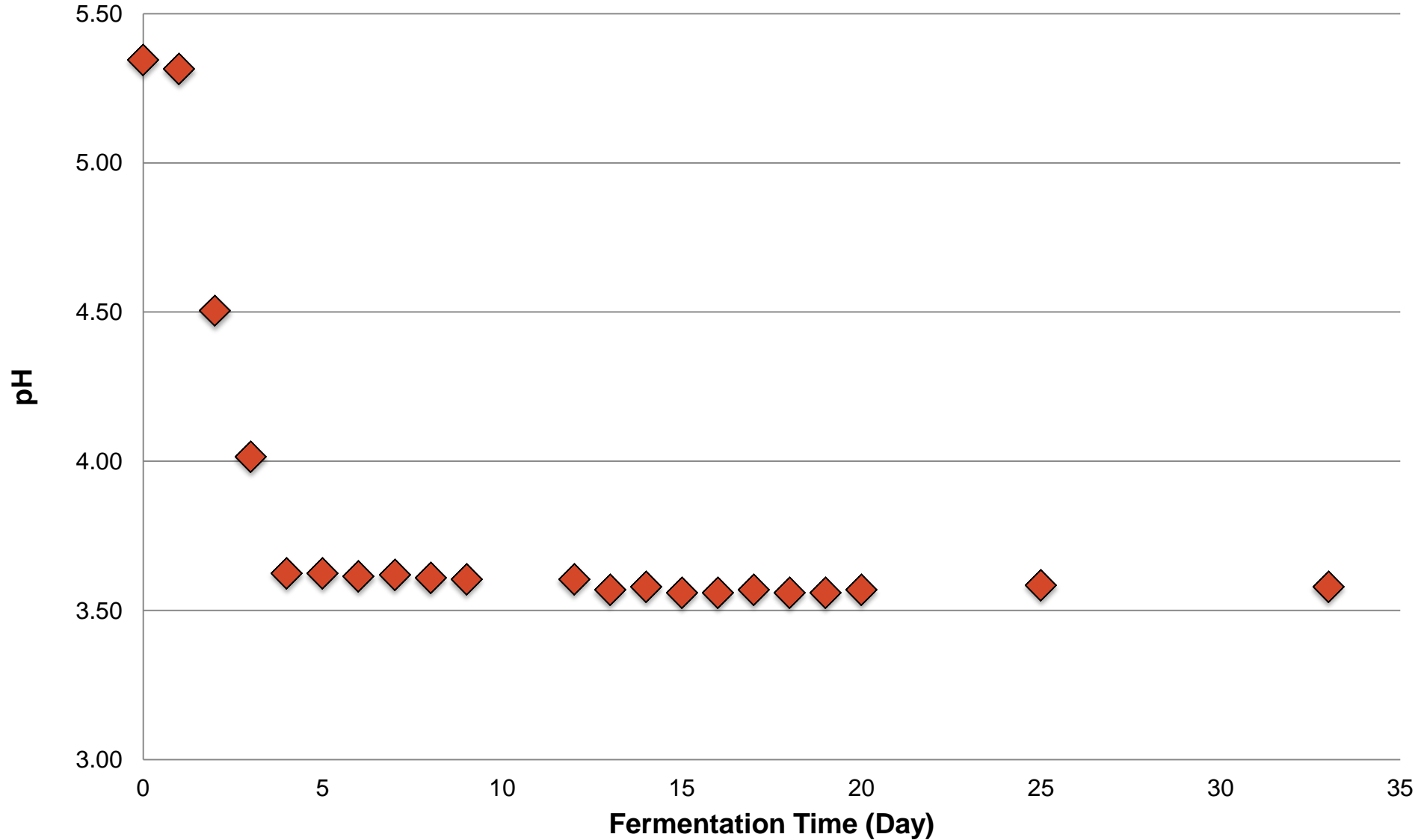
- Lambic wort (barley and wheat, lightly hopped)
- Pure culture of *L. thermotolerans* NCSU
- Pilot-plant scale
(300 L) at 18 °C
- Pitched 4 L
of active yeast



Wort Attenuation in Lambic Fermentation



Wort Acidification in Lambic Fermentation



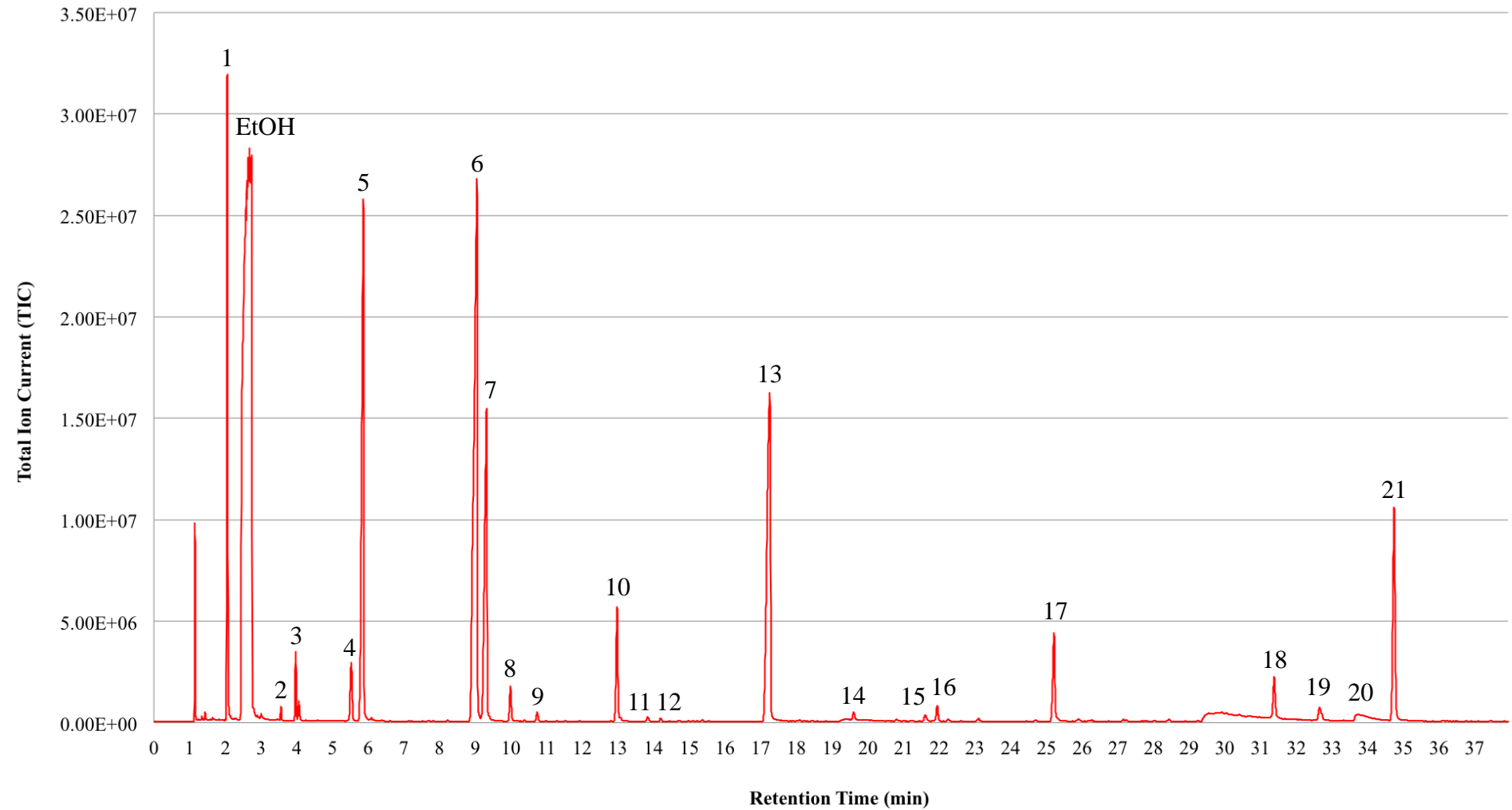
“Lambic” Sugar Utilization

Fermentation Time (Day)	Maltotriose (g/L)	Maltose (g/L)	Glucose (g/L)	Fructose (g/L)
0	29.0	67.0	18.7	6.8
25	4.8	3.5	0.8	n.d.

Metabolite Production

Fermentation Time (Day)	Lactic Acid (g/L)	Glycerol (g/L)	Ethanol (g/L)	ABV%
0	n.d.	n.d.	n.d.	n.d.
25	7.3	2.4	54	6.8%

Aromatic Analysis





Detected Esters

Peak #	Compound	Peak Ratio	Flavor Descriptor
1	Ethyl acetate	3.56	fruity, sweet
2	Isobutyl acetate	0.08	fruity, banana
3	Ethyl butyrate	0.41	fruity, papaya
5	Isoamyl acetate	6.57	fruity, banana
7	Ethyl hexanoate	4.28	fruity, sour apple
9	Acetic acid, hexyl ester	0.08	fruity, green apple
10*	2-Heptanol	1.00	n.a.
11	Ethyl lactate	0.04	fruity, raspberry
13	Ethyl octanoate	6.67	fruity, apple
17	Ethyl decanoate	1.04	fruity, apple
18	Phenylethyl acetate	0.47	roses, honey
19	Ethyl laurate	0.17	caprylic, estery



Other Detected Aromatics

Peak #	Compound	Peak Ratio	Flavor Descriptor
4	Isobutanol	0.58	alcoholic, solvent-like
6	Isoamyl alcohol	9.77	alcohol, sweet
8	Styrene	0.35	sweet, floral
10*	2-Heptanol	1.00	n.a.
12	1-Hexanol	0.04	coconut, unpleasant
14	2-Ethylhexanol	0.07	citrus, floral
15	2,3-Butanediol	0.06	warming, butterscotch
16	Linalool	0.15	fruity, flowerly
20	Caproic acid	0.38	sour, fatty



“Lambic” Fermentation

- Successful fermentation in brewhouse environment
- Reduces pH through lactic acid production
- Final ABV of 6.8%





Conclusions for *L. thermotolerans*

- *L. thermotolerans* NCSU is:
 - Metabolically different from the type strain, *L. thermotolerans* NRRL Y-8284
 - A yeast brewers may use for pure culture, sour beer fermentations
 - Patent pending for fermenting various beverages (beer, cider, etc.)



Questions?



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**FOOD, BIOPROCESSING
& NUTRITION SCIENCES**



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