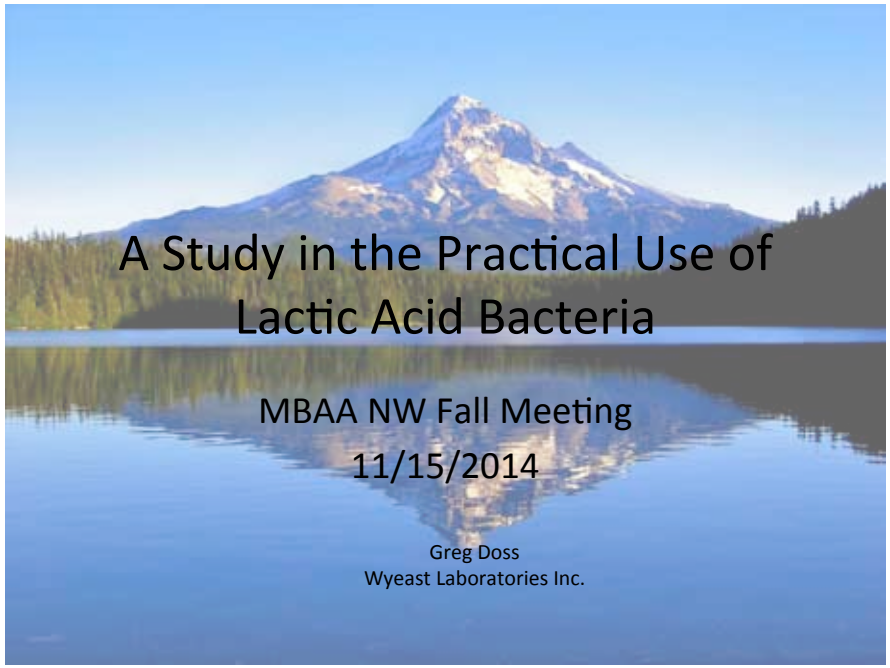


A Study in the Practical Use of Lactic Acid Bacteria

MBAA NW Fall Meeting

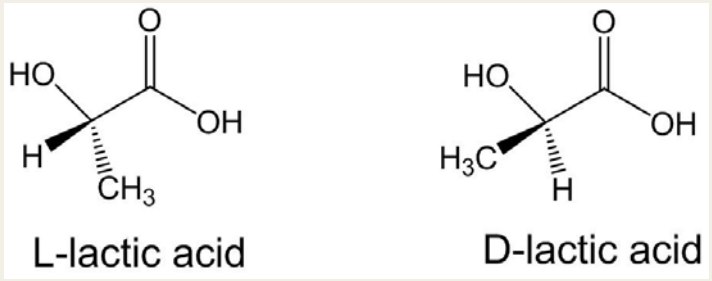
11/15/2014

Greg Doss
Wyeast Laboratories Inc.

Talk Overview

- General Info
  - Lactic Acid
  - LAB Metabolism
    - Heterofermentive vs. Homofermentive
  - Cultures
  - Enumeration
  - Buffering Capacity
- Study

Lactic Acid



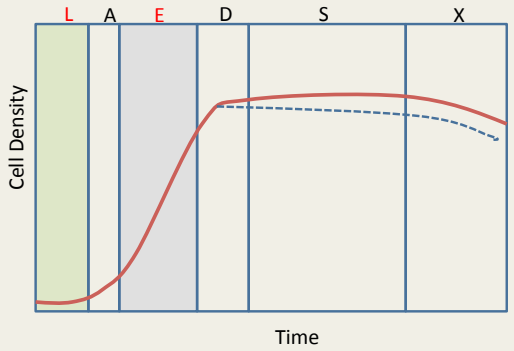
- Isomers
- L(+)-Lactic AKA (S), D(-)-Lactic AKA (R)
- Weak Acid – Only partially dissociates in water
- pKA 3.86
- Do they have different sensory properties???

LAB Metabolism

- Homofermentive
  - EMP Pathway
  - Byproducts
    - Lactic Acid
    - 2 mol ATP/Glucose
- Heterofermentive
  - Pentose Phosphate Pathway (Lacks enzyme)
  - Byproducts
    - Lactic Acid
    - Ethanol
    - CO2
    - Acetic acid (Minor)
    - 1 mol ATP/Glucose

LAB Metabolism

L - Lag Phase
A - Acceleration Phase
E - Exponential Phase
D - Deceleration Phase
S - Stationary Phase
X – Death Phase

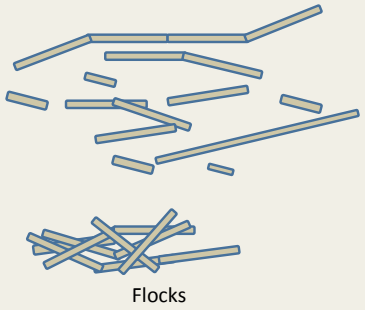


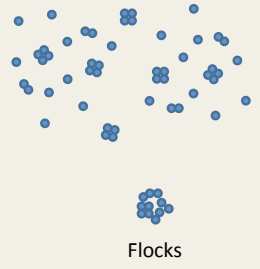
Cell Population
OD_{600}

LAB Cultures

Lactobacillus sp.

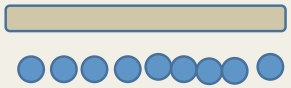
Pediococcus sp.



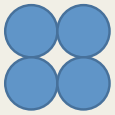


Flocks

Flocks



Size Difference



Pediococcus Tetrad

LAB Cultures

Lactobacillus sp.

- Morphology
  - Rod Shaped
  - Can be short and long
  - Fillamentous chains and clumps (flocks)
- Gram+
- Produces L and D isomers.
- Sensitive to low pH (<3.5)
- Alcohol tolerant (*L.fructivorans* 20%)
- Homofermentive spp.
  - *L. casei, L. plantarum, L. sake, L. delbrueckii*
- Heterofermentive spp.
  - *L. brevis, L. hilgardii, L.fructivorans , L. buchneri, L. fermentum*

LAB Cultures

Pediococcus sp.

- Morphology
  - Cocci (Round)
  - Single, Pairs, Tetrads
  - Tetrads resulting from divisions in two planes
  - Clumps or flocks
- Gram +
- Homofermentive
- Produces D or L isomers
- Sensitive to low pH (<3.5)
- Alcohol tolerance 12-14%.
- Tetrads resulting from divisions in two planes
- P. *parvalus, P.pentosaceus, P. damnosis*
- Diacetyl
  - Yeast reduction?
- Ropiness, Sickness – Extracellular dextrins
  - Yeast reduction?

Enumeration Bacteria

- Microscope & Hemacytometer
- Plate counts
- Optical Density @ 600nm
  - Spectrophotometer
- pH/Turbidity/Density
  - Eyes, pH meter, hydrometer

Enumeration Bacteria

- Microscope & Hemacytometer
  - Pros
    - Counts all cells - flocks
    - Instant data
  - Cons
    - Time consuming process
    - Dilutions - possible errors
    - Viability

Enumeration Bacteria

- Plate Counts
  - Pros
    - Counts all viable CFU
  - Cons
    - Time consuming process
    - Dilutions - possible errors
    - Long incubation times
    - Growth on plate?
    - Flocks counted as single CFU

Enumeration Bacteria

- Optical Density
  - Pros
    - Fast
    - No potential error from dilution
    - Cell count can be extrapolated from curve
  - Cons
    - No viability
    - Flocks can skew reading
    - Have to establish a reference point

Enumeration Bacteria

In Brewhouse - Enumeration Bacteria

- Estimate Turbidity/pH/Density
  - Pros
    - Fast
    - Cheap
    - Contamination check
  - Cons
    - Estimate
    - Sample needs to be agitated – watch for flocculation/ sedimentation
    - No viability
    - No cell count

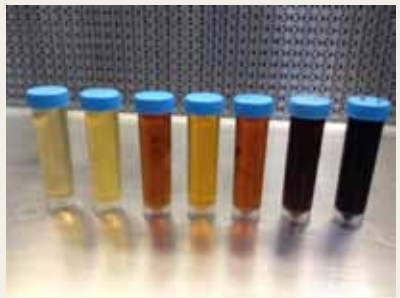
Buffering Capacity

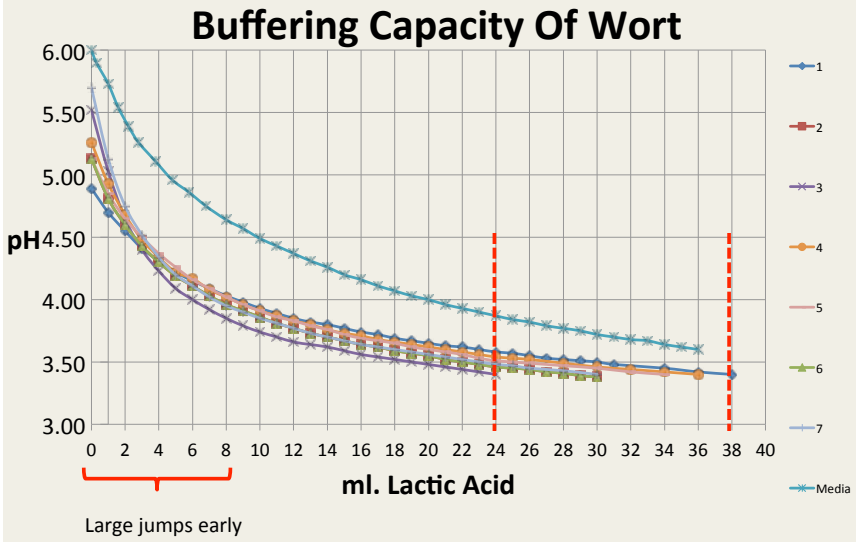
- The ability of the wort to withstand changes in pH
- The same amount of acid will affect worts differently
- Compounds affecting buffering
  - Amino Acids
  - Phosphates
  - Salts
    - Ca+,Mg+, Bicarbonate, etc
  - Fruit additions
    - Malic and Citric Acids
  - Inoculum!
    - Lactic Acid

Buffering Capacity

Test

- Collected random samples from Hood River Breweries
- Titrated to pH 3.4 with 1% Lactic Acid
- Plotted pH vs. ml acid





Large jumps early

Buffering Capacity

Sample	pH	P	ml. Lactic Acid
1	4.89	16.8	38
2	5.13	15.4	30
3	5.52	13.2	24
4	5.26	14.5	36
5	5.13	21.7	34
6	5.04	16.9	28
7	5.70	11.2	30

*Data will be useful when studying sensory.

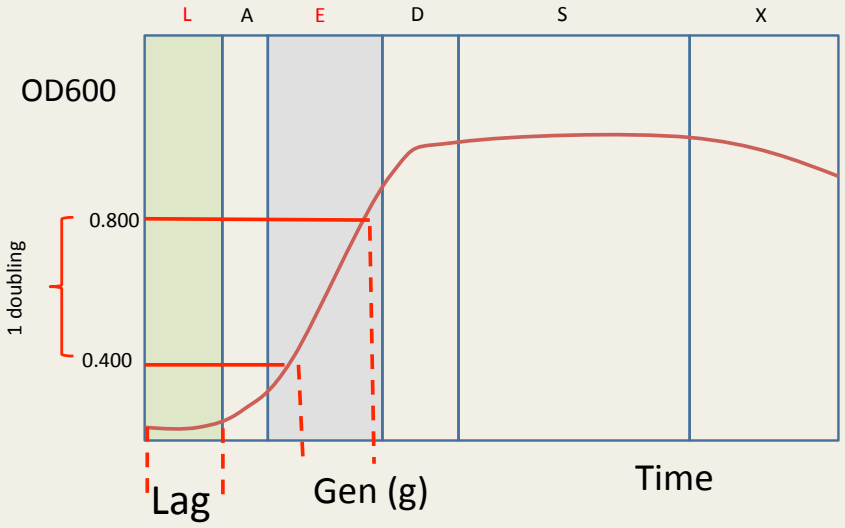
Study

- Pitch Rate
- Temp
- pH
- Alcohol
- Bitterness Units
- Strains
- VDK reduction
- Hop Tolerance/Alc Tolerance
- Nutrients
- Carbon Sources
- Oxygen
- Proteolytic enzyme Activity (Head retention)- Temp/pH
- Sensory
- Exogenous polysaccharide (Ropiness)

Study

- Optimal media
- Study inhibition of various parameters
- Measure growth using optical density
- Also monitor pH and density
- Quantify/compare by looking at
  - Lag time
  - Generation time – Time it take to double population
- Combined inhibition by multiple parameters?

Quantify



Cultures Used

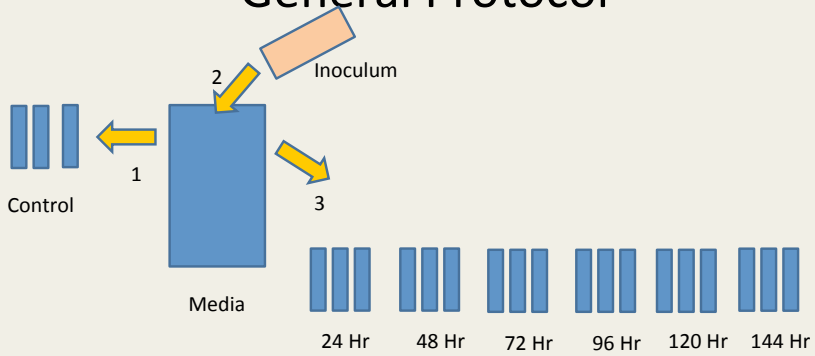
- Wyeast 5223
  - *Lactobacillus brevis*
  - Isolated from sour beer sample
- Wyeast 5335
  - *Lactobacillus buchneri*
  - Isolated from Schultheiss Berliner Weisse
- Wyeast 5733
  - *Pediococcus damnosis*
  - Isolated from British beer

General Protocol

4-6 Day test

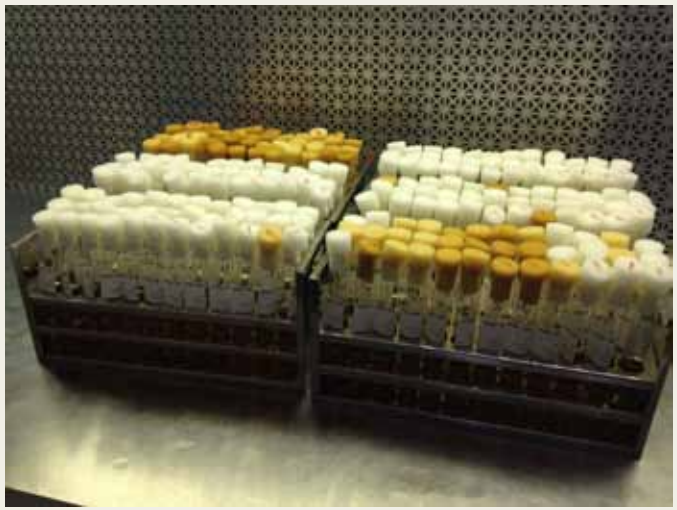
- Sterilize and clarify media
- Tempered for 48 hour in CO_2 incubator
- Dispense 3 x 10 ml. tubes of control media
- Inoculate large volume of media at specific pitchrate
- Dispense 4-5 sets of 3 x 10 ml. tubes
- Incubate
- Remove one set of tubes every 24 hours and test
  - OD (Turbidity)
  - pH
  - Density

General Protocol



- 18 tubes for each variable tested
  - 3 strains
  - 4 pitch rates
  - 3 Control
  - 3 x 4 x 18 + 9 = 225 Tubes

General Protocol



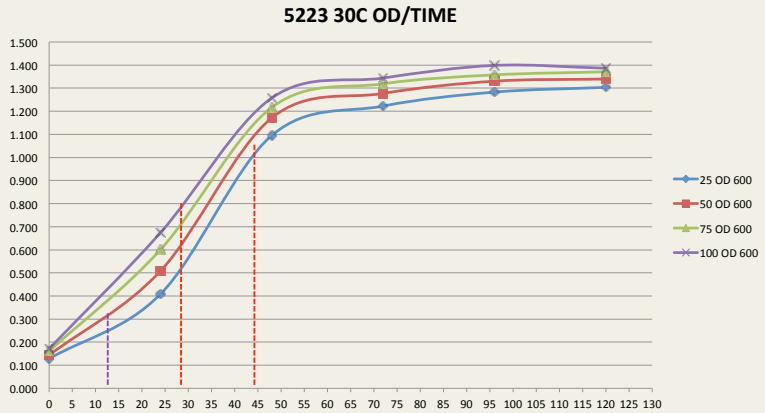
Temperature/Pitch Rates

- Determine parameters for experiments
  - Not too fast or too slow
- 4 Pitch Rates

		Wyeast Inoculum	
– 5.0E6	100%	6L/Bbl	5.0%
– 3.75E6	75%	4.5L/Bbl	3.75%
– 2.5E6	50%	3L/Bbl	2.5%
– 1.25E6	25%	2L/Bbl	1.25%

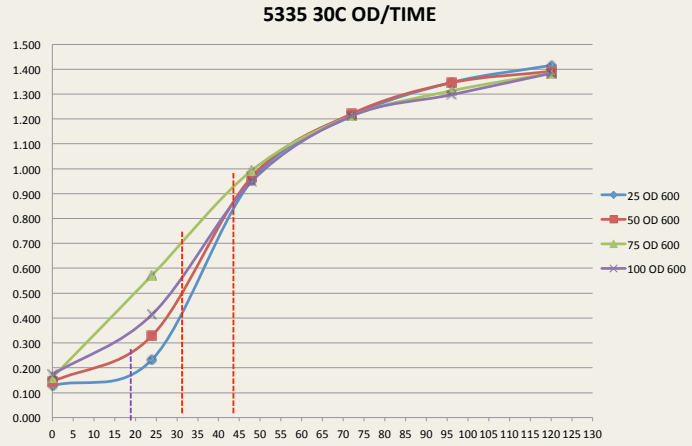
- 3 Temperatures
  - 38C
  - 30C
  - 22C

Growth 5223 30C



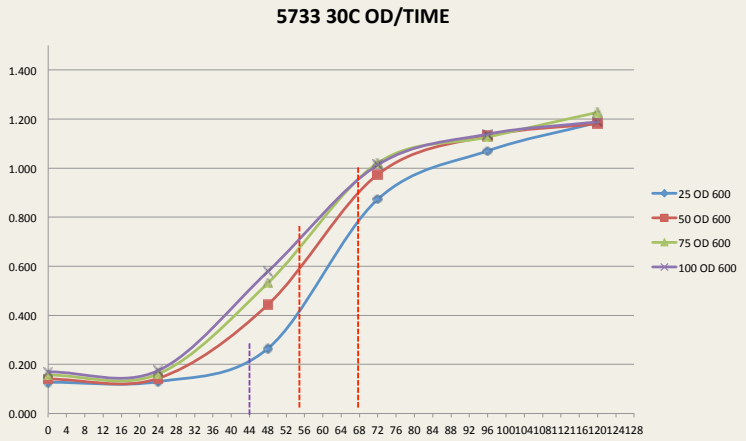
Growth rate = 18 hr Lag = 11 hr

Growth 5335 30C



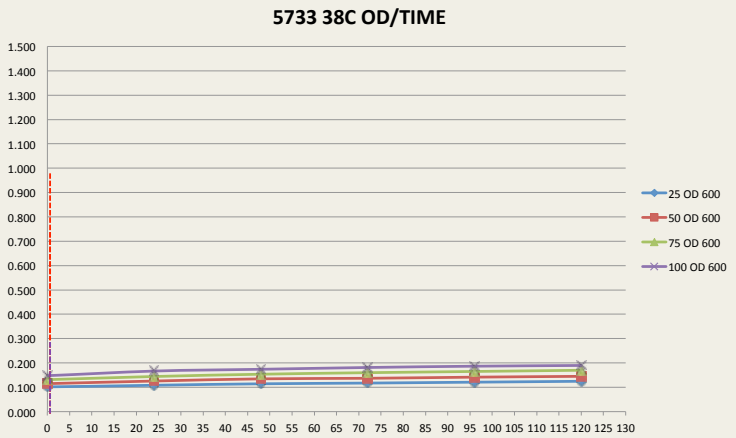
Growth rate = 18 hr Lag = 19 hr

Growth 5733 30C



Growth Rate (g) = 13 hr Lag = 44 hr

Growth 5733 38C



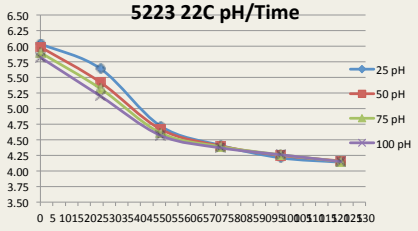
Growth Rate (g) = 0 hr Lag = None

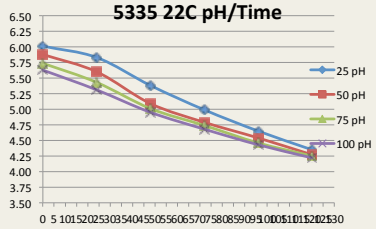
Summary Growth Rates vs. Temp

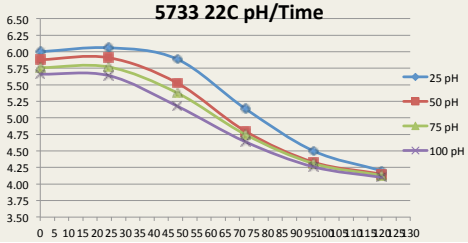
Strain	22C		30C		38C	
	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)
5223	20	18	18	11	?	?
5335	44	39	19	18	20	15
5733	50	24	44	13	No Growth	No Growth

- 5223 short lags but similar growth rate
- 5733 long lags. Paul's "Pedio bloom?"
- No pedio @ 38C

All pH vs Temp & Pitch Rate







Summary pH vs Temp & Pitch Rate

- Under optimal conditions
  - Pitch rate less important
  - Temp very important

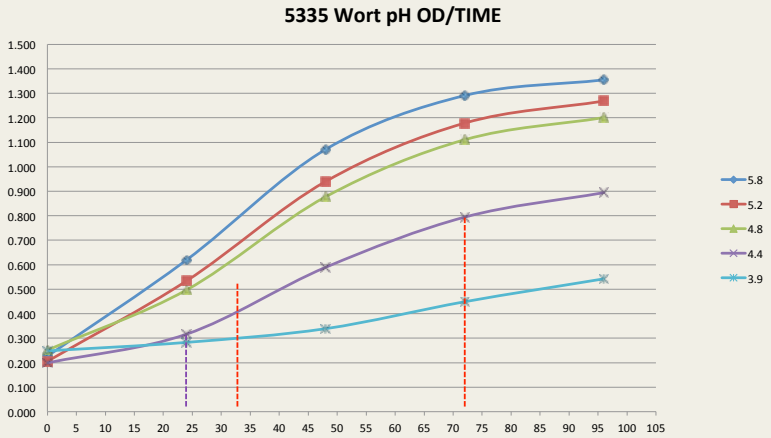
Summary Density vs Temp & Pitch Rate

- Heterofermentive strains produce alcohol
  - Drop apparent extract
  - Not sure about sugar consumption
- Finished fermentation for each strain sent for analysis
  - Apparent Degree Fermentation
    - Heterofermentive 27-29% (0.45% ABV)
    - Homofermentive 5%(0.12% ABV)

Different starting pH

- Why do cultures sometimes fail with co-inoc?
- Optimal Media
- Pitch Rate 5E6
- 30C
- Different starting pH
  - 6.2 (5.8) Growth media
  - 5.2 (5.2) Wort
  - 4.7 (4.8) Mid Fermentation
  - 4.2 (4.4) Beer
  - 3.7 (3.9) Sour beer

Different starting pH



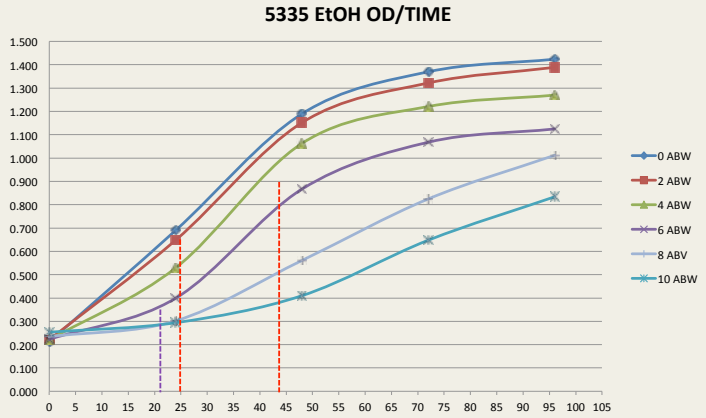
Summary Different starting pH

Strain	5.8		5.2		4.8		4.4		3.9	
	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)
5223	x	20	x	12	x	20	x	30	50	160
5335	x	25	x	28	x	30	25	40	40	70
5733	25	30	25	30	25	40	40	45	Min Growth	Min Growth

Different % Alcohol

- Optimal Media
- Pitch Rate 5E6
- 30C
- Different starting Alcohol (%/vol)
  - 10% (8.8)
  - 8% (7.3)
  - 6% (5.5)
  - 4% (3.6)
  - 2% (1.8)
  - 0% (0)

Different % Alcohol



Alcohol inhibition pH dependent ?
When culture reaches critical pH, growth stops

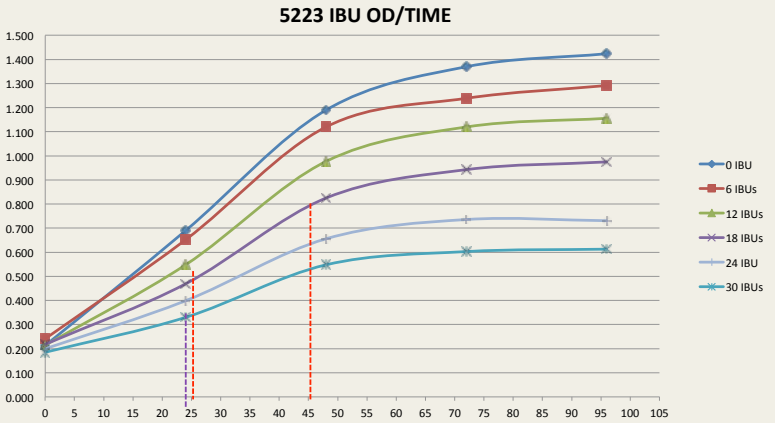
Summary of Different % Alcohol

Strain	0		1.8		3.6		5.5		7.3		8.8	
	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)
5223	x	20	x	20	15	20	25	20	30	40	30	65
5335	x	25	x	25	15	23	25	23	30	46	40	50
5733	25	25	25	25	25	25	45	25	60	35	x	x

Different IBU

- Optimal Media
- Pitch Rate 5E6
- 30C
- Different starting IBU (IsoHop)
  - 30 (24.2)
  - 24 (20.6)
  - 18 (16.9)
  - 12 (9.6)
  - 6 (5.7)
  - 0 (0)
- Did not look at hop tolerance

Different IBU



Hop inhibition is pH dependent
When culture reaches critical pH, growth stops

Summary of Different IBU

Strain	0		5.7		9.6		16.9		20.6		24.2	
	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)	Lag (Hr)	g (Hr)
5223	x	22	x	25	10	27	25	30	35	35	30	35
5335	10	20	25	35	25	55	NG	NG	NG	NG	NG	NG
5733	30	20	40	27	55	55	NG	NG	NG	NG	NG	NG

That's all for now

- What's next
  - More data analysis
  - Different strains
- VDK reduction
- Hop Tolerance/Alc Tolerance
- Nutrients
- Carbon Sources
- Oxygen
- Proteolytic enzyme Activity (Head retention)
- Sensory
- Exogenous polysaccharide (Ropiness)

Thanks For the Help

- Jess Caudill – Wyeast Laboratories
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- Darek Smith – Bighorse
- Scott Bruslind – Analysis Laboratories
- Scott Garden - Barth-Haas Group

Thank you

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