



The Science of Beer



The Art & Science of Sour Beer Production

Andy Parker-Avery Brewing Company

Lauren Woods Salazar, Kelly Tretter and Rob Christiansen-
New Belgium Brewing

World Brewing Congress August 16th, 2016



New Belgium and Avery Sour Program History



Avery Brewing Company

2004: First barrel experimentation. 12 oak barrels total.

2007: Barrel Aged Series

2012: Annual Barrel Series

2015: Barrels & Botanicals Series

2016: Total barrel-aged beer roundup. 3500 total oak barrels.

Total capacity volume: 13K hL/yr



New Belgium Brewing Company

1998: first seven wine barrels, first and only inoculation.

1999: La Folie Sour Brown first release. 2000- La Folie wins gold at GABF.

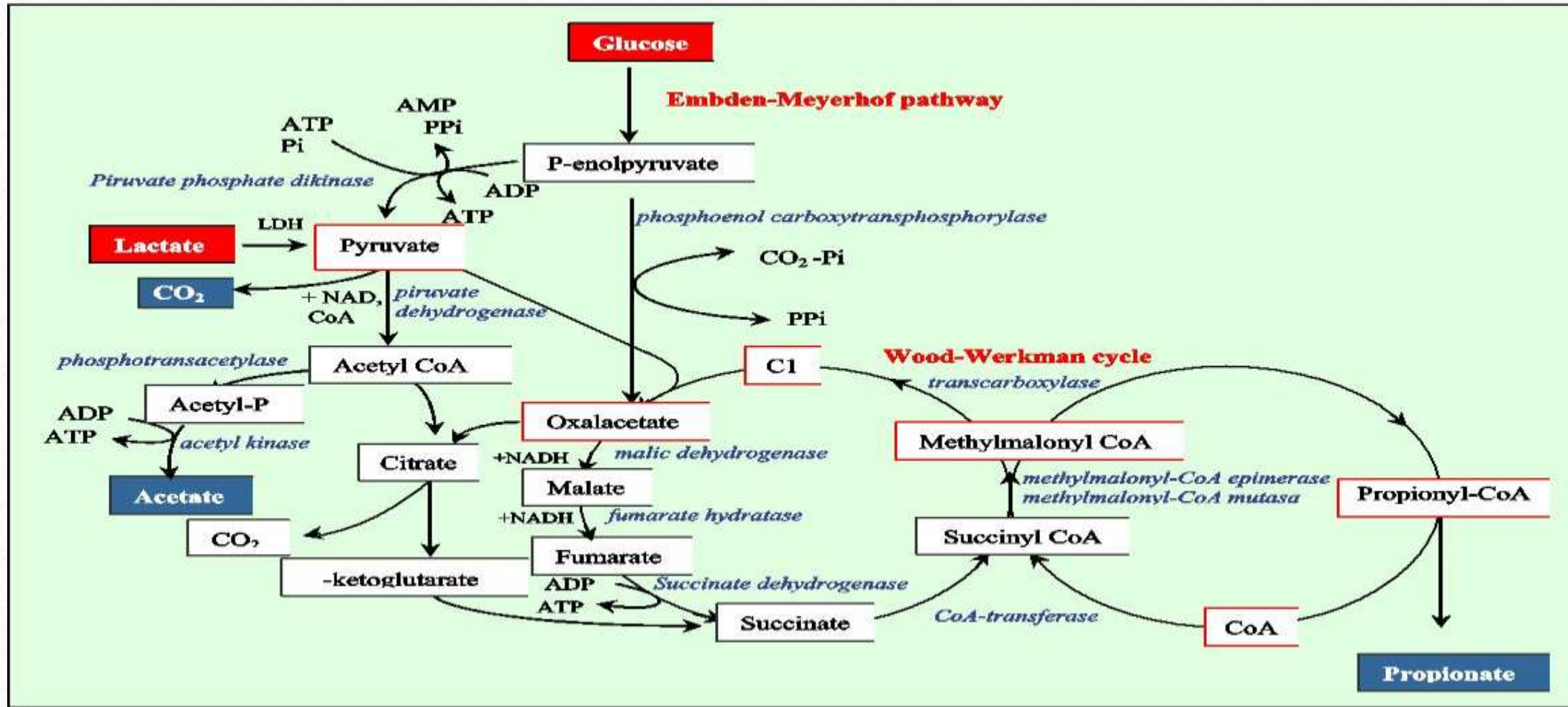
2001: first four 60hl foeders, inoc from barrels. 2nd base beer introduced.

2010: 32 foeders ~ 4000hl.

2013: 64 foeders, 44 wine barrels, 80 whiskey ~8000hl. 20-1800hl batch size.

Who does it? Bugs in barrels

Alcohol Fermentation, Lactic Fermentation and TCA Cycle

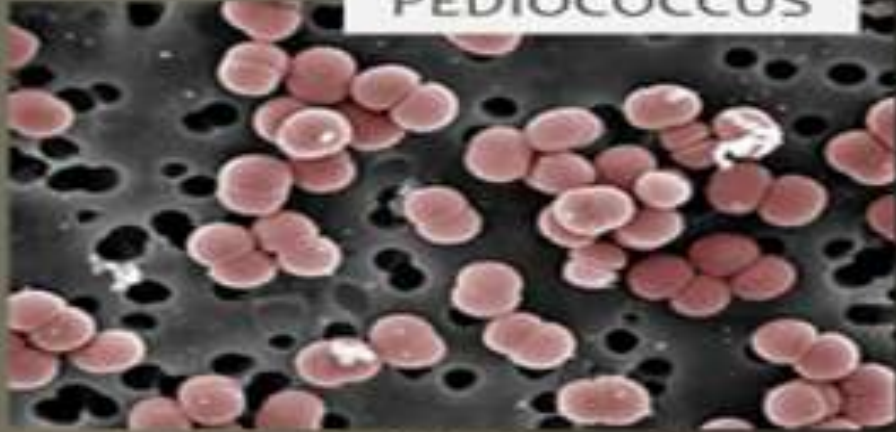


SOURING BACTERIA



PEDIO & LACTO

PEDIOCOCCUS



LACTOBACILLUS



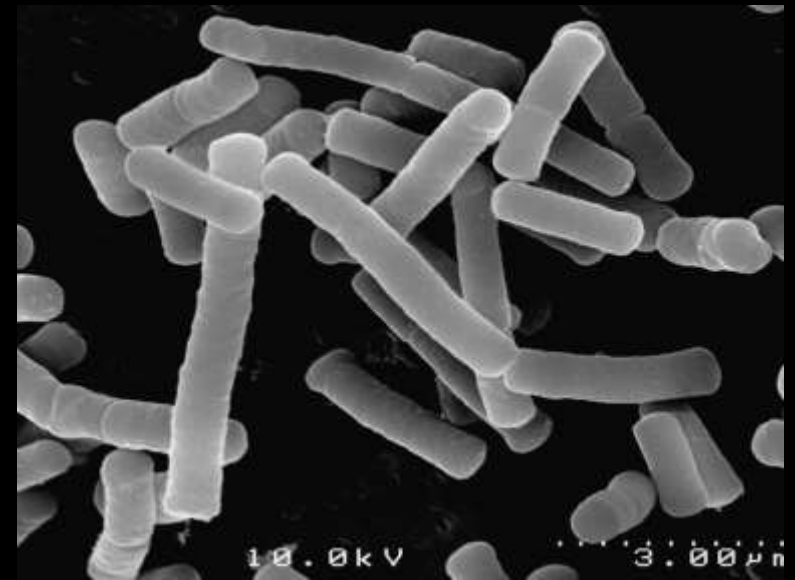
Pediococcus spp.

- Acidification of sauerkraut and dried sausages
- Slower than *Lacto* to sour
- More hop tolerant
- Reduce the pH in beer to <3.0
- Sharper and more harsh lactic acid
- Goes through its' 'sick' phase
- *Pedio* can't take up diacetyl so it's nice to have some brett in your "soup"

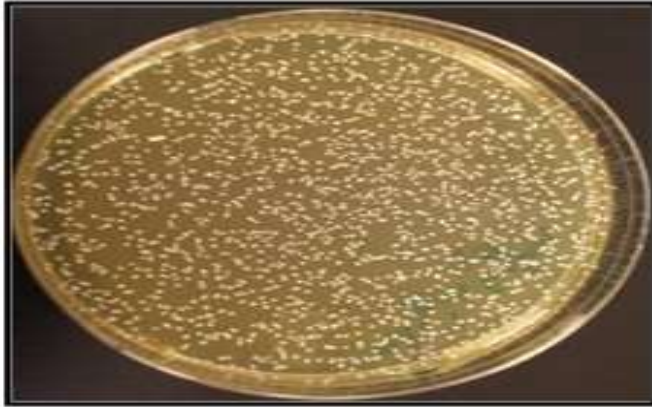


Lactobacillus spp.

- Primary souring bacteria
- Thrives between 38-49C
- Softer and tangier lactic acid
- Lowers pH to 3.3-3.4



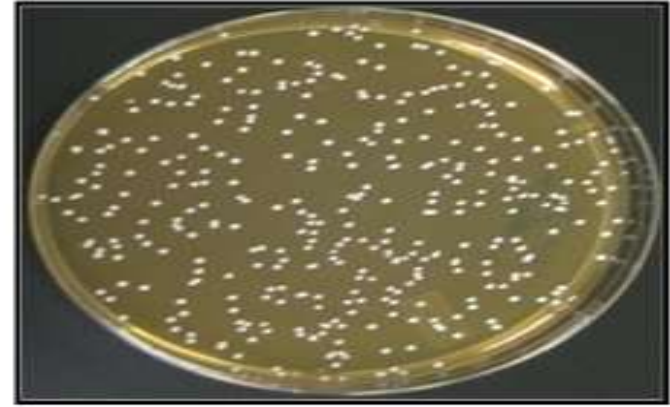
Pediococcus



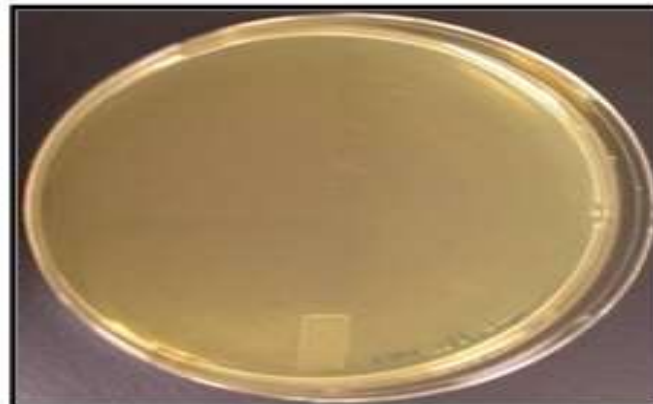
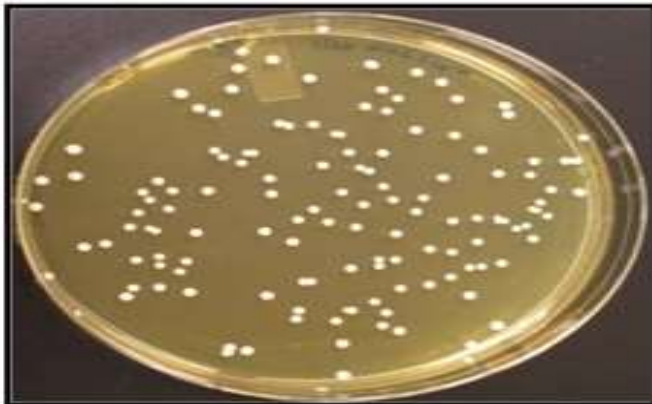
pH 6.2



pH 2.0

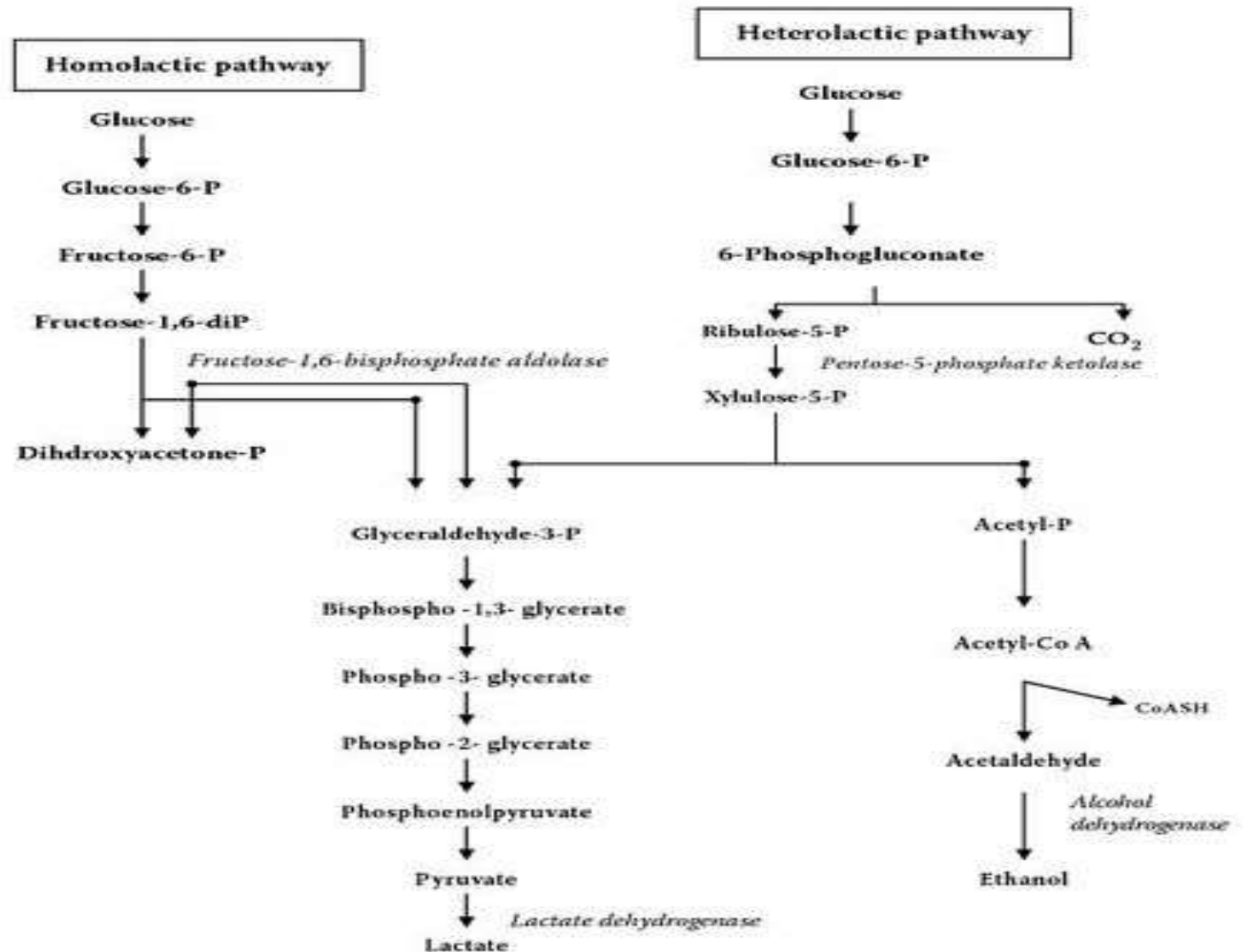


pH 1.5



Lactobacillus

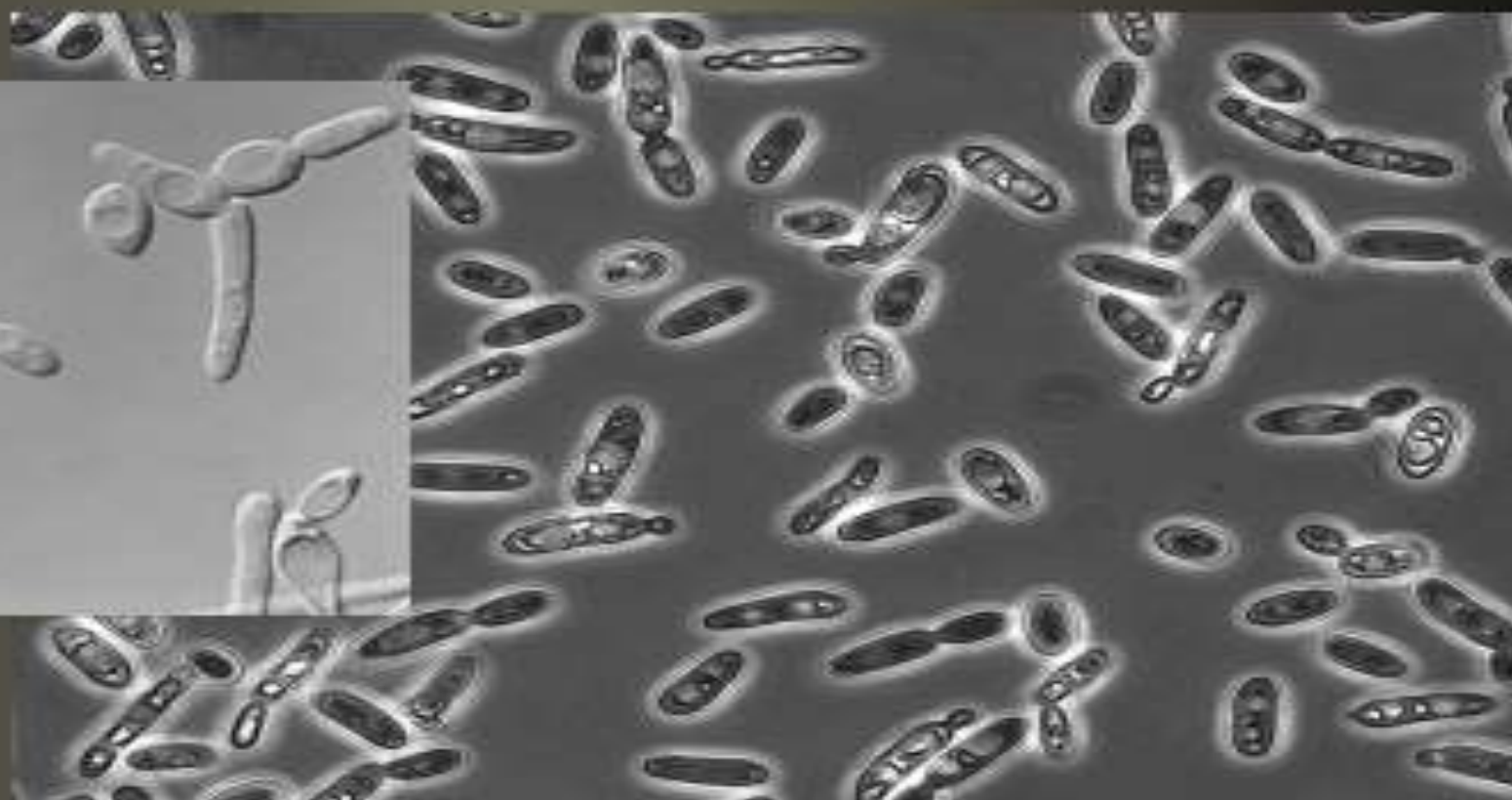
Heterolactic vs. Homolactic Fermentation



Homofermentative and Heterofermentative Organisms

Obligatory Homofermentative [62] ⬅	Obligatory Heterofermentative ➡	Facultatively Heterofermentative ➡
<i>L. acidophilus</i>	<i>L. brevis</i>	<i>L. casei</i>
<i>L. delbruekii</i>	<i>L. buchneri</i>	<i>L. curvatus</i>
<i>L. helveticus</i>	<i>L. fermentum</i>	<i>L. plantarum</i>
<i>L. salivarius</i>	<i>L. reuteri</i>	<i>L. sakei</i>
<i>L. rhamnosus</i> [63]	<i>L. pontis</i>	<i>L. bavaricus</i> [64]
<i>L. lactis</i> [64]	<i>L. cellobiosus</i> [64]	<i>L. coryniformis</i> [64]
<i>L. leichmannii</i> [64]	<i>L. confusus</i> [64]	
<i>Streptococcus bovis</i> [64]	<i>L. coprophilus</i> [64]	
<i>Streptococcus thermophilus</i> [64]	<i>L. fermentatum</i> [64]	
<i>Pediococcus acidilactici</i> [64]	<i>L. sanfrancisco</i> [64]	
<i>Pediococcus damnosus</i> [64]	<i>Leuconostoc dextranicum</i> [64]	
<i>Pediococcus pentococcus</i> [64]	<i>Leuconostoc mesenteroides</i> [64]	
<i>Enterococcus faecium</i> [64]	<i>Leuconostoc paramesenteroides</i> [64]	
<i>Enterococcus faecalis</i> [64]		

WILD YEAST



Brettanomyces spp.

- Alpha-glucosidase breaks down dextrans
- Doesn't contribute a lot of acidity unless there is a lot of O₂ present=acetic acid!
- Lactic acid + Brett= ethyl lactate



- A LOT of lactic acid can be bad, though

How we measure- Sensory Analysis



Old World Blending



Evaluation, Analysis & Interpretation



Avery's Sensory Evaluation Process

- Too many barrels for full lab tests on every one. Trained tasters can detect flaws right away
- All barrel tasters are trained in common flavors, off-flavors, and palate gaps
- A brave new world of flavors and off-flavors
- Each barrel is sampled by at least two trained tasters in process, then again on the day of debarreling
- Barrels with notable off-flavors are dumped

LaFolie 2001

Barrels for blending = 20hL

lactic / acetic pH's		sweet	pH's
x	72 (1554)	3.52	54 3.69
x	32 (1554)	3.64	2 (1554) 3.57
✓	9 (1554)	3.39	41 3.71
x	43	3.46	70 3.55
x	47 (aleic)?	3.63	→ NO USE 5 or 2
x	50 aleic	3.47	→ NO USE F1 or 30 instead
others			
x	42	3.74	
✓	46	3.55	
x	51	3.77	
x	52	3.53	
x	58	3.76	
x	62	3.57	
✓	69	3.73	
	Innoc 2	3.62	
	NBB pH2	3.63	
	74	3.55	

avg pH = 3.614

10
 13

avg pH = 3.614

HO

13 NACH

3/17/01

TEST BOTTLE CONDITIONING #2

of bottles = 4 cell count of yeast = 102.75 c/ml
amount of sugar = 9.6g/l

Procedure: Blend 150 ml of ex sample in holding vessel, add sugar. Pipet yeast into bottles, siphon mixed beer from vessel. Cap bottles.

La Folie 2001 & 2016

FINAL	WHAT W/ ELSE??	REFERENCE 2017/2018	21
FINAL	FINAL LaFolie + Symposium	NOV 30 th 2015	55
U3	1 nice dark fruits - not over the top sour but great start		55
U3	2 Real sour - kinda rapid - simple, tangy but great - sour/apple		→K
W3	5 nice sour start, keg run Fo' SHO		→K
B3	7 Nice tart almost smiling		→K
B3	8 man, I almost wanna - love the grape skin - but I'll be on		→K
B3	9 getting there, lovely chewing cola-frizzy - patience glass		→K
B3	10 pretty herbal tart - actually waiter		→K
B3	11 for Ilmo it's not that crazy, still bear notes - I'll use it.		120
B3	14 some pithy coza but love the sour - yeah		120
B3	15 I'm sad that after Ilmo it's still not there - get rid of the lacquer!		→K
B3	16 really?? Ilmo?? no! maybe some zing but ok beer too.		→K
W3	17 totally almost turning		KP
U3	18 weird aroma. Amazing sour!		80
W3	19 turning beer - no 'drink me' → taste again soon.		→K
U3	20 little corker but nice dark fruit & sour. SHORT ROUND		60 70
W3	22 start o' twang - straight up waiter.		X
B3	23 lovely grape, champion		210
B3	24 it's nice but not worth diluting the plan, next time?		→K
B3	25 Chozolately pith nice sour with tobacco.		210
B3	26 zingy tangy nice deep dark tart		210
W3	27 nice root beer, more over... sweet gummy		X →
U3	28 lovesies, grape apple nult.		210
W3	29 nice turn. going welcome back to the Oscar gange		X →
B3	30 herbal nice tart - dirt m'f'ing megirt		95
B3	31 dry beer pithy ok - waiter MAYBE YEP		→ 95
U3	32 ooofruity lovely gummy like Blackberry leopard		95
U3	21 same as short round HTTM!		65

1670

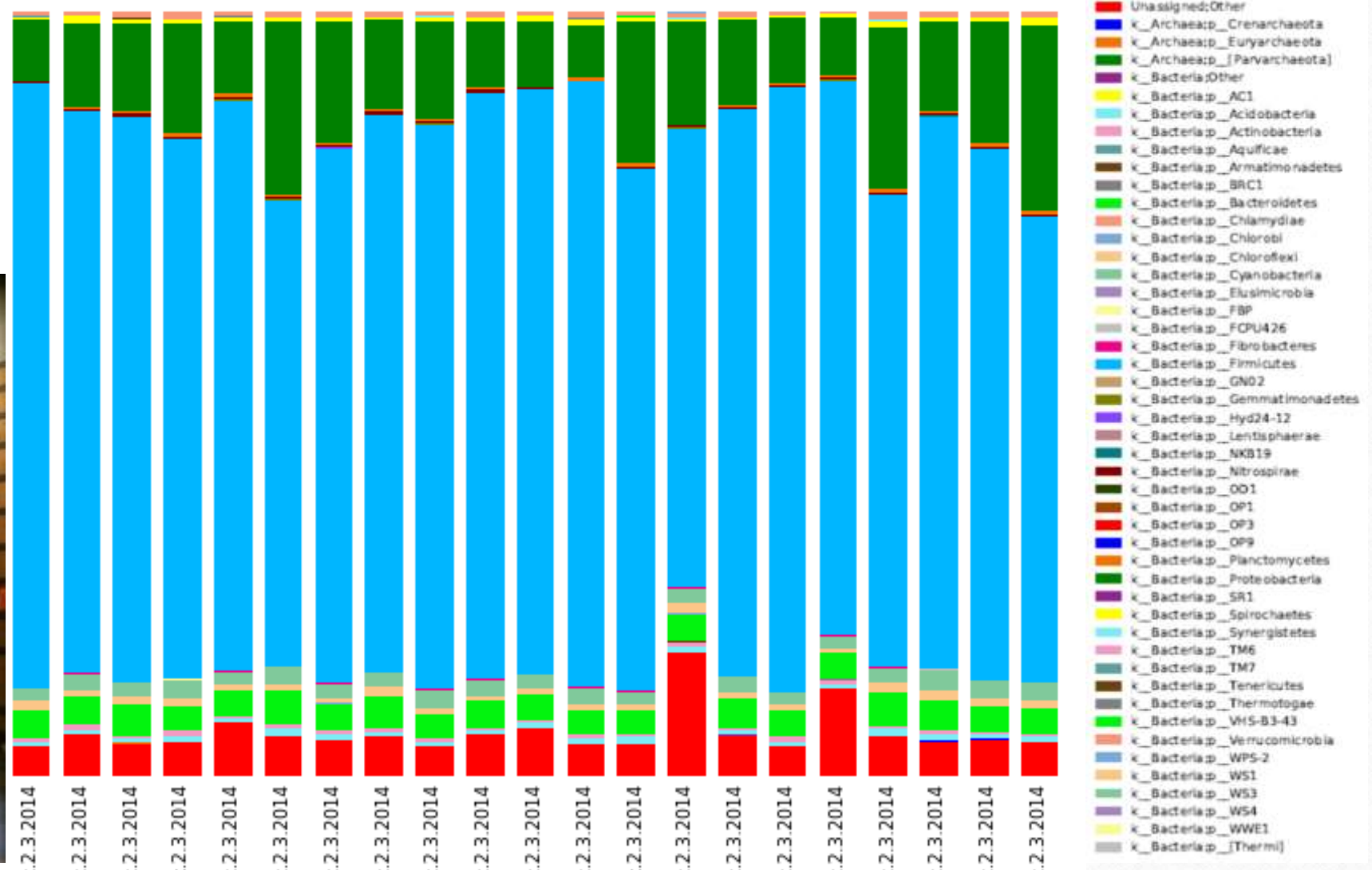
New Belgium's Sensory Evaluation Measurement

- **'User'**- Sour Barrel. Most if not entire flavor profile is dominated by acid.
- **'Blender'**- Sour perception with other interesting flavors (esters, brett, malt) present.
- **'Waiter'**- Minimal to no sour flavor perception. young, 'beery'.
- 😊- great; happy with the flavor profile, fun, delicious, best case scenario.
- 😐- ok to good; if others are better, I'll choose it, not 'bad' – usually used to prioritize.
- ☹- huh, um; used usually when unexpected flavors are found. Investigation.
- Other denotations- BB, SPD, ♥, ➡

How we measure- Microbiology & Analytical Chemistry

Diversity of Life in the Spontaneous Ferments of One Brewery

In the ~50 barrels of one brewery, ~200 organisms from ~50 different groups of Bacteria & Archaea were found.



DNA Sequencing

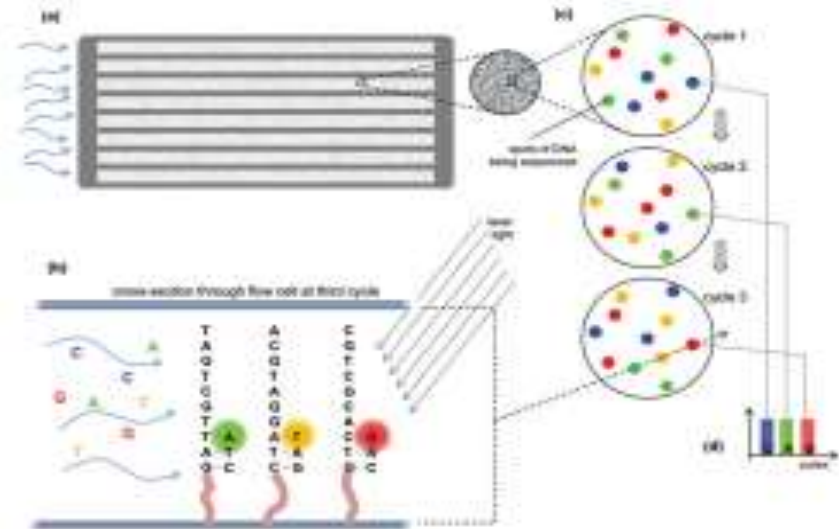
Individual strands of DNA obtained from a mixed culture are attached to a glass slide.

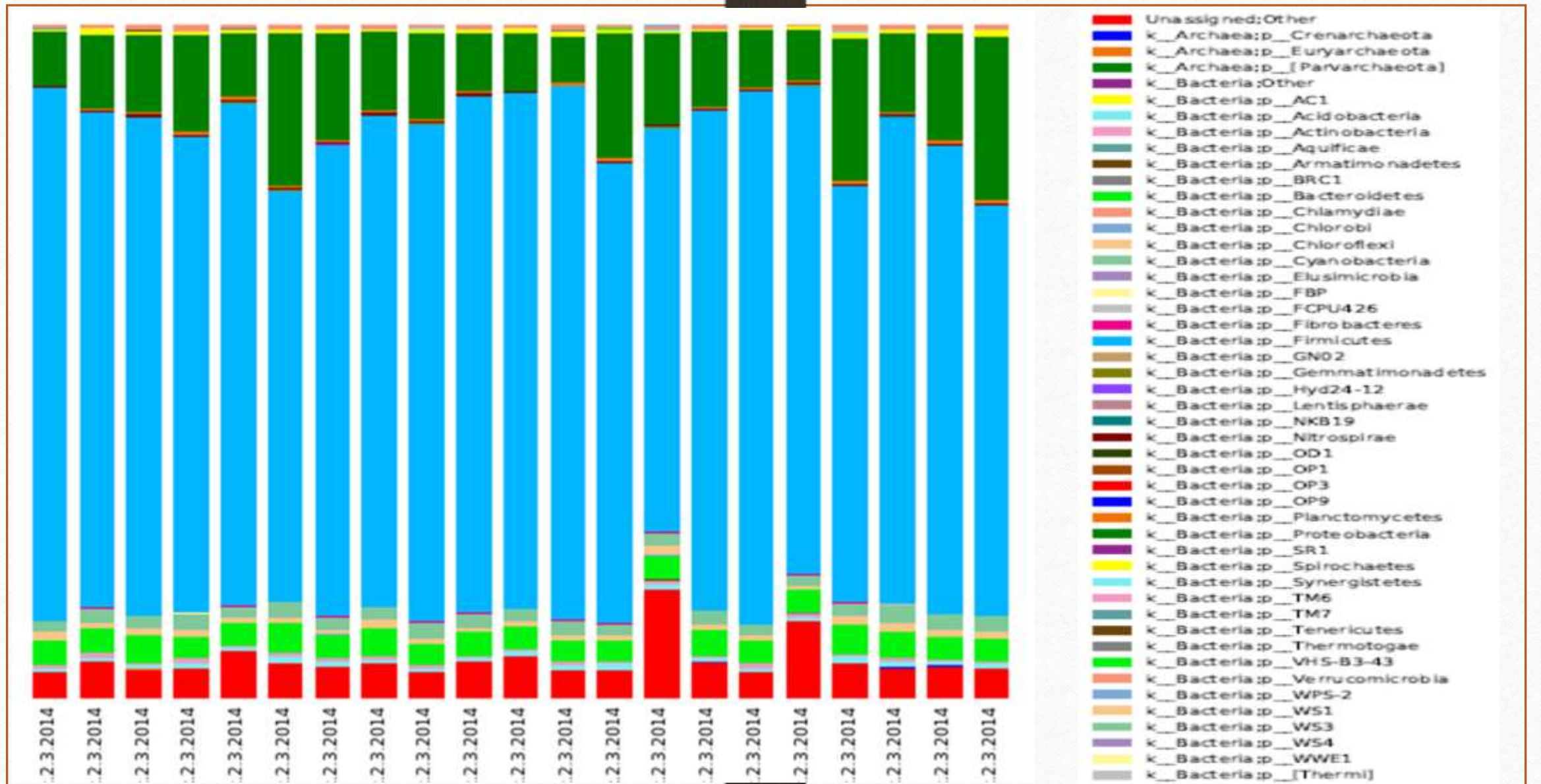
A A D A B
E B D A D A
A D A D A A
E D A E C

A computer identifies the location of each DAN strand using a laser and fluorescent light detector.

The DNA strands are replicated on the glass plate in a way that reveals the sequence of each strand.

The number of times each given DNA sequence is found in the set of sequences is a close approximation of the relative number of each species in the population.





Analytical Chemistry

Densitometer/ Ancolyzer	Auto- Titrator	VDKs by GC	Volatiles by GCMS- same principles as GC with different detector.	Acids by UPLC
pH- inline probe measures diff in voltages produced varying conc H ions	Titratable Acidity	Quantification of 2,3 butanedione (diacetyl) and 2,3 pentanedione	Gas Chromatograph Mass Spectrometer- determine the mass-to-charge ratio and analyzing resultant motion.	Reverse Phase Ultra Performance Liquid Chromatography- same principles- measure liquid phase using extreme pressure- the acids will elute a specific retention time (spectra)
Color	Uses a pH probe/stir plate to determine an intial pH and delivers sodium hydroxide at steady rate to titratable endpoint (pH=8.2)	Gas Chromatograph with an electron capture detector (ECD)- gaseous headspace is analyzed and extrapolated via a known calibration curve to identify and quantify each compound	Acetaldehyde	Lactic acid
ABW%			isoamyl aceate, isoamyl alcohol	
EA			ethyl acetate, phenethyl acetate, ethyl hex, ethyl butyrate	
BCOG			4-vinyl guaiacol	
Specific Gravity				
ER/RDF				
calories				Succinic acid

TA and pH

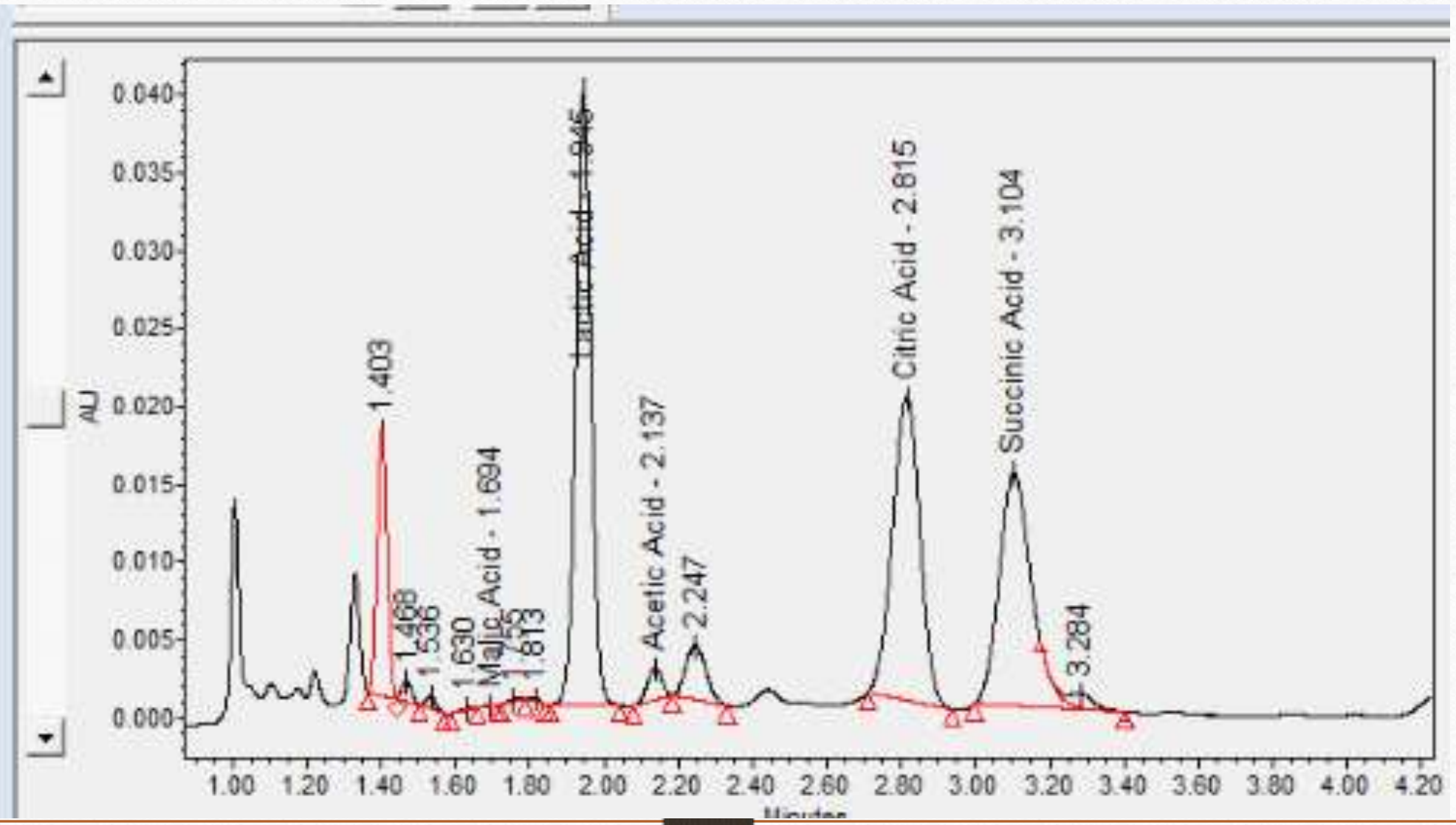
- Titratable Acid – any acid that can lose a proton in an acid-base reaction.
- Titratable Acidity – is the measure of the amount of these acids in a solution.
- pH – is the measure of the strength of the acid in a solution.
- These are two **VERY DIFFERENT** measurements in that two beers can have the same TA and very different pHs.
- In brewing we use the ASBC method BEER 8

Souring Acids

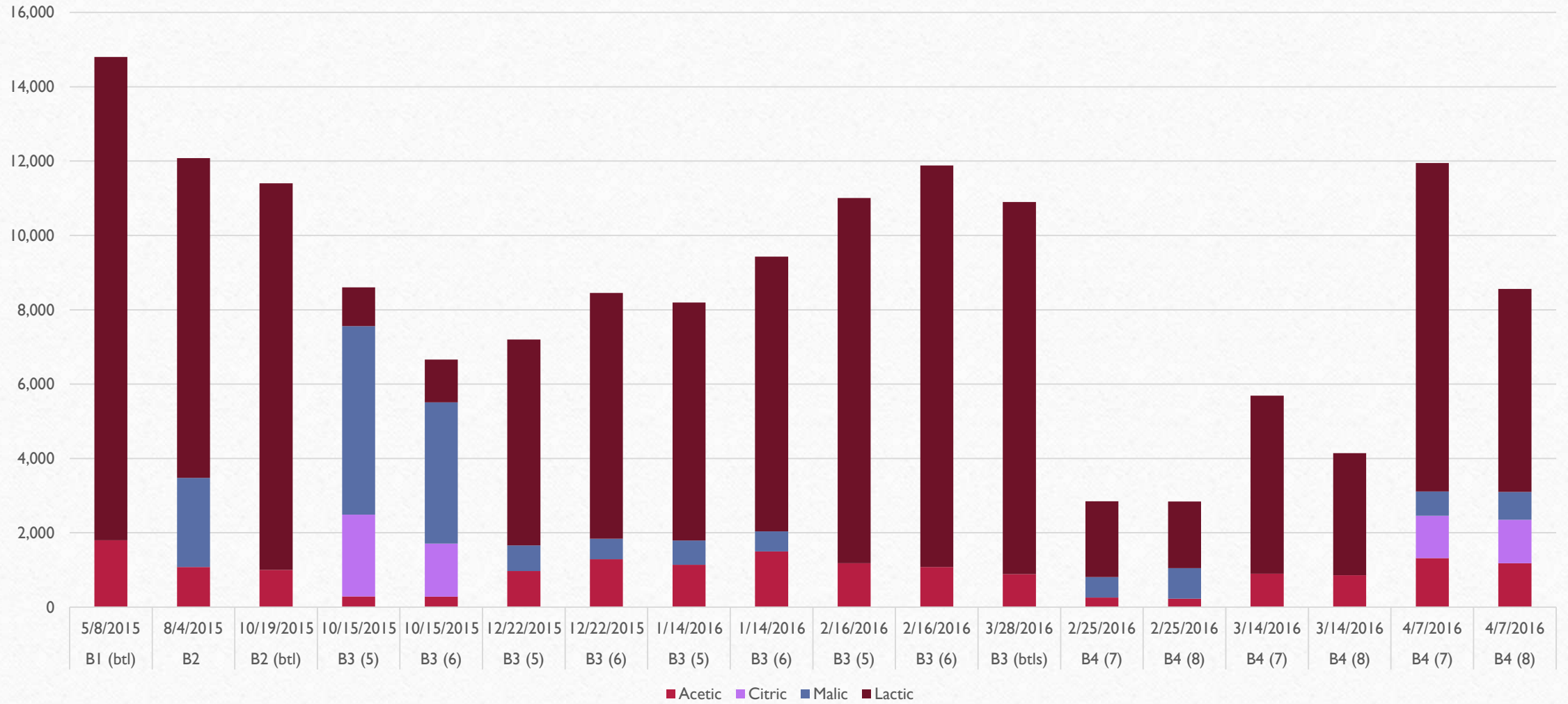
- Lactic Acid- ($C_3H_6O_3$), lactic acid bacteria (LAB), in our case lactobacillus and pediococcus convert sugar and malic acid into lactic acid, the latter through MLF.
 - Flavor threshold & profile: ~400ppm. Aroma evaluated in it's raw form, lactic acid almost has a cooked, brothy or brown sugar aroma. It's said to have a yogurt or soured milk flavor with a more 'pleasing' sour taste.
- Acetic Acid- ($C_2H_4O_2$), acetic acid is produced by acetobacter during or after the fermentation period if exposed to steady supply oxygen. It is the most volatile of the primary acids associated in our process.
 - Flavor threshold & profile: 70-200ppm. Tasted at 600ppm, intense vinegar aroma, round harsh sour.

The rest of the (relevant) acids

- **Malic Acid-** ($C_4H_6O_5$), this acid is most talked about in fruit fermentation (MLF). Levels of malic acid in grape berries are at their peak in concentrations as high as 20 g/L. As the vine progresses through the ripening stage, malic acid is metabolized in the process of respiration, and by harvest, its concentration falls to around 1-4 g/l.
 - Flavor threshold & profile: ~350ppm. Tasted at 350ppm has a somewhat citric aroma with harsh puckering mouthwatering sour.
- **Citric Acid-** ($C_6H_8O_7$), highest natural levels in citrus fruits- lemon= Note that citric acid may be converted by LAB to acetic acid and diacetyl.
 - Flavor threshold & profile: ~300ppm. Tasted at 300ppm, citrus fruit aroma, pleasant citrus-like biting sour.
- **Succinic acid-** ($C_4H_6O_4$), is an intermediate of the TCA cycle and one of the fermentation end-products of anaerobic metabolism. *Saccharomyces cerevisiae* can produce succinic acid under aerobic and/or anaerobic conditions and is a major influencing factor of the pH drop in fermentation.
 - Flavor threshold & profile: ~125ppm. Tasted at 250ppm no discernable aroma, candy-like initial sour to intense bitter/astringent mouthfeel.



Avery/Raspberry Sour Organic Acid Analysis (ppm)



Typical Organic Acids on Raspberry Sour

	Lactic (ppm)	Malic (ppm)	Citric (ppm)	Acetic (ppm)
Fermenter (after fruit)	1100	4100	1700	300
Month 2	7100	700	1100	800
Month 3	13700	400	900	1000
Month 4/package	13300	400	800	1100

Some testing variation is inevitable, but nearly all acid production/reduction is done around month three

Where Science, Art and Sensory Collide

Each brewery's research is underway. How are we using the results to make better sour in the future?

Examples From Avery

- Oxygen ingress and off-flavors – just like an all-steel beer, you need to know how off-flavors are generated. Since excessive oxygen and time are the most common factors, we are working to shorten the time in the actively drying barrel without compromising quality
- Using lab results to train our palates – Immitis
- Using pH and gravity results to do detective work – Insula
- Raspberry Sour, organic acid testing. We planned on a six month minimum stay in the barrels, but the malolactic fermentation seems to be done by month three and sensory tests say it's done by month four. With more experimentation and testing we might be able to shorten the time further. Too much barrel time can mean more acetic acid production and the potential for serious off-flavors

Examples From Avery

- The final test is simply sampling each barrel with multiple tasters, but lab and sensory testing help improve our predictability and forecasting
- How do you know when a beer is ready? When it tastes great. If your fermentation and acid production are done, it's probably not going to get much better and the risks become bigger with every month.

NBB chemical analysis report

Le Terroir blend info

The World Brewing Congress Blend

The goal: either a light colored quaffable sour with mild acidity or a dark, acidic palate wrecker, depending on subjective choices by an extensive two-person panel.

Avery

-**Bolder Weisse** - - 5% blonde sour, neutral barrels. Primary fermentation of 3 Brett strains, 1 Saison strain. Funkiest of the group. Cheese, lemon, leather, Pear, lime, cellar/wet concrete, minerality.

-**Mojito** - 8% orange sour, fresh rum barrels, Sacch primary, Brett secondary. Orange, big lactic, caramel, slight acetic burn, hint of oak tannins, coconut, lemon/lime curd, caramel and oak- zing, sour.

-**Raspberry Sour** - 6.5% red sour, neutral barrels, raspberry and Brett in secondary fermentation. Clean, lactic, vanilla, cocoa dust, underripe raspberries (because of super sour taste), underlying/lingering bretta- or maybe simply styrene from fruit. Tangy.

New Belgium: Oscar- dark sour, Felix- light sour

-**Oscar 16** - astringent, black cherry, young

-**Oscar 22** - toasted crackers, lactic, clean

-**Oscar 24** - huge 14+ month Flanders Red-esque monster

-**Felix 44** - xxxtra big lactic bomb, slight acetic

-**Felix 48** - Milder, honey, peach, slight Brett aroma

-**Felix 52** - gueuze-esque, Lemon, complex Brett aroma

The Blend

With all samples on the table, we tasted each, evaluated separately, compared notes, and started blending. After a few dozen shared blends that didn't inspire, we ended up with two winners:

- Light sour, with 45% Felix 52, 45% Bolder Weisse, 10% Mojito
- Orange sour, with 50% Felix 52, 25% Mojito, 25% Raspberry Sour

WBC BLEND and its COMPONENTS

sample	malic acid	lactic acid	acetic acid	citric acid	succinic acid	Lactic/Acetic	TA
WBC Blend	112.13	15442.56	674.48	4519.99	6446.51	22	1.36%

sample	malic acid	lactic acid	acetic acid	citric acid	succinic acid	Lactic/Acetic	TA
Foeder 52	9	16105	531	4493	8310	30	1.35%

sample	malic acid	lactic acid	acetic acid	citric acid	succinic acid	Lactic/Acetic	TA
Raz - 234	218.12	15323.75	1492.42	4682.92	5025.96	10	1.44%
Raz - 235	221.98	15648.25	1158.92	4627.81	4997.03	13	1.41%

sample	malic acid	lactic acid	acetic acid	citric acid	succinic acid	Lactic/Acetic
moj 123	61.13	14022.59	366.11	4875.59	9179.9	38
moj124	66.24	13793.18	565.56	4774.37	8841.12	24

Take Aways

- Predictions, Correlations, understandings, do stuff better.
- Do specific base sour at the 'same' time? Is this beer or location oriented?
- Do 'same' size of 'same' age sour sour at the same time
- Correlate waiter, blender, user with TA.
- With our current methods, oxygen and time are the biggest enemies. Sensory and science are helping us minimize them
- More detailed testing and tasting of organic acids will improve predictability

Q&A/ Acknowledgements



Jeff Irby-Analytical Chemist
Eric Salazar- Wood Cellar Manager
Wood Cellar Crew
Dr. Paul Ogg



The Avery crew, lab, and
barrel folk (Thomas Ledder,
Leigh Audin, Travis Rupp)