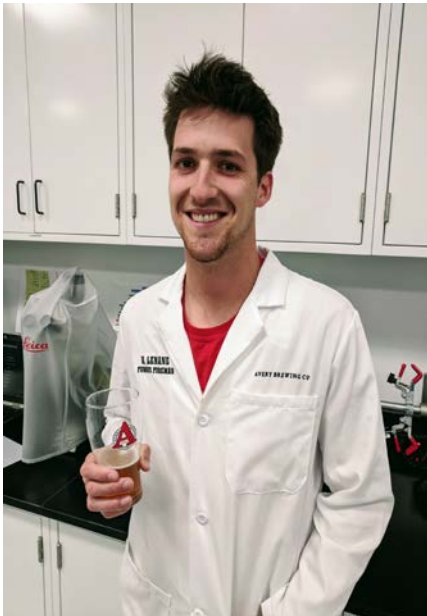




Diastaticus 101: Background and Detection Methods

Intro

- Kyle Lenane



- B.S. Fermentation Science and Technology, CSU, 2015
- Avery Brewing Company: Fungus Foreman (Laboratory Technician)

- Matthew Peetz, MS



- Masters in Cell Biology, UCCS, 2014
- Inland Island: Yeast Whisperer
- Regis University: Director of Applied Craft Brewing Certificate



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Hot Tonic or Old Hat?

Table 1. Number of negative and positive findings of *S. cerevisiae* var. *diastaticus* per year from January 2008 to June 2017

Year	Negative finding	Positive finding	Total per year
2008	0	1	1
2009	0	1	1
2010	4	4	8
2011	11	4	15
2012	4	5	9
2013	7	4	11
2014	3	3	6
2015	10	17	27
2016	18	19	37
01-06/2017	7	4	11
Total 01/2008 to 06/2017	64	62	126
Mean/month for 114 months	0.56	0.54	1.11



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- *Saccharomyces cerevisiae*
 - Sucrose 100%
 - Glucose 100%
 - Fructose 100%
 - Maltose 100%
 - Maltotriose *Variable*
 - Dextrins 0%
- *Saccharomyces cerevisiae* var. *diastaticus*
 - Sucrose 100%
 - Glucose 100%
 - Fructose 100%
 - Maltose 100%
 - Maltotriose 100%
 - Dextrins ???%

Percent composition

Glucose	10 - 15
Fructose	1 - 2
Sucrose	1 - 2
Maltose	50 - 60
Maltotriose	15 - 20
Dextrins	20 - 30



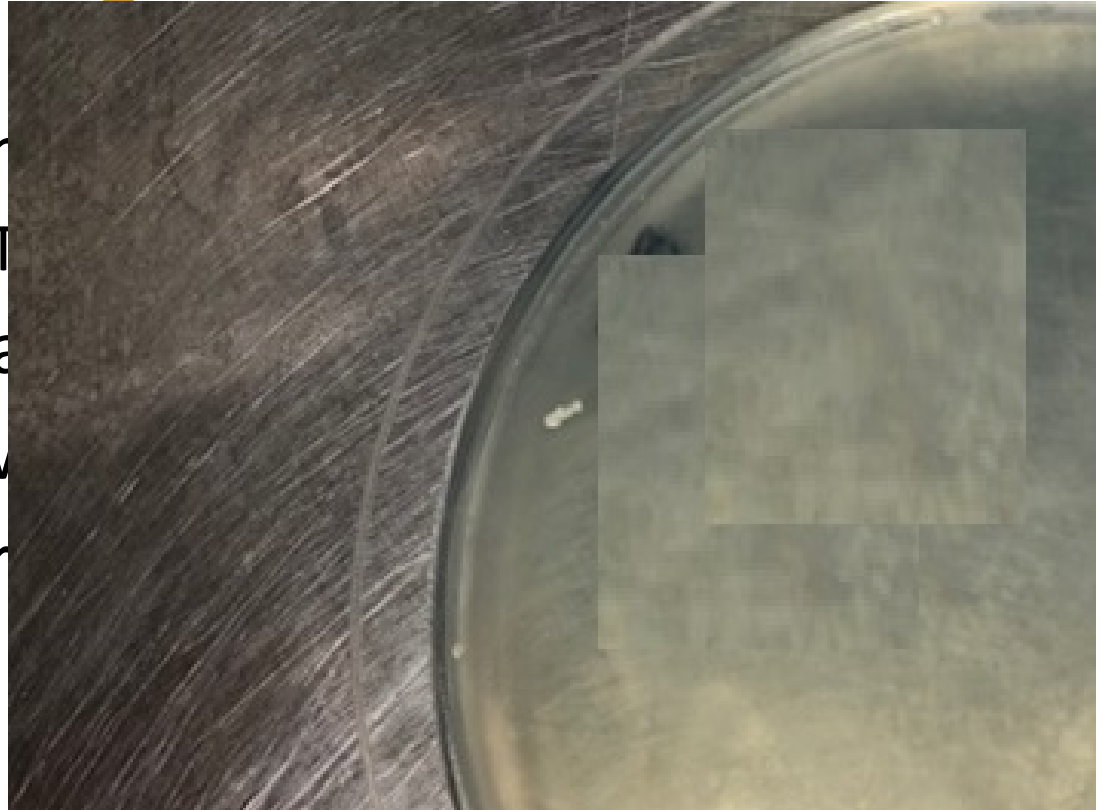
So What Is It?

- *Saccharomyces cerevisiae* var. *diastaticus*
- A yeast containing 1 of several additional genes coding for Glucoamylase
 - STA1 (DEX2), STA2 (DEX1), STA3
- Releases glucose from the non-reducing ends of starch and dextrin
- Hydrolyzes only 1-4 bonds
- Almost always contains POF gene
 - Ferulic Acid to 4-VG (clove)



Handling in a Yeast Lab

- Separate Equipment
 - Piping, Tubes, Taps
- Autoclave / Steam
- Rinse, Caustic wash
- Plating (Time Critical)
 - Starch Plates
 - Dextrin Plates
 - Lin's Cupric Sulphate Media (LCSM)
- PCR (Only Test for STA1)



The Usual Suspects

- Saisons
 - Blaugies, French Saison, Dupont, etc.
- Other Belgians?
 - Belgian Golden Ale
- Other POF Strains?
- Highly Attenuating English Strains?
 - Thomas Hardy “Super High Gravity” (WLP 099)



Avery's Experience with Diastaticus



- First discovered in June of 2017
- Over attenuated beer
 - 1 degree plato below our typical AE
 - Increase in ABV
- Sensory
 - Did not stand out with the phenolic character of White Rascal



Avery's Experience Continued

- Plated on all media types
- PCR
 - Two distinct colony morphologies, both PCR positive.
- Screening identified diastaticus in subsequent fermentations
 - Pasteurization utilized to prevent diastaticus getting into package.



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Avery's Experience Continued

- Environmental micro testing
 - Included all areas of production
 - Yeast plant aeration line as source
- Corrective actions taken
 - Aeration line CIP protocol was introduced
 - Sterile filter was added inline from O2 source



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Experiments with Diastaticus

Media Study

5 different strains of *S. diastaticus* were plated on 5 different media types.

- The types of media used were :
 - WLN
 - LCSM
 - LWYM
 - Lysine (@pH 3.1, @pH 5)

Strain	WLN		LCSM		LWYM		Lysine @ pH 3.1		Lysine @ pH 5	
	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours	48 hours	96 hours
007	X	X	X	X	X	X	No growth	No Growth	No growth	X
008	X	X	X	X	X	X	No growth	No Growth	No growth	X
009	X	X	X	X	No growth	X	No growth	No Growth	No growth	X
010	X	X	X	X	No growth	X	No growth	No Growth	No growth	X
011	X	X	X	X	X	X	No growth	No Growth	No growth	X

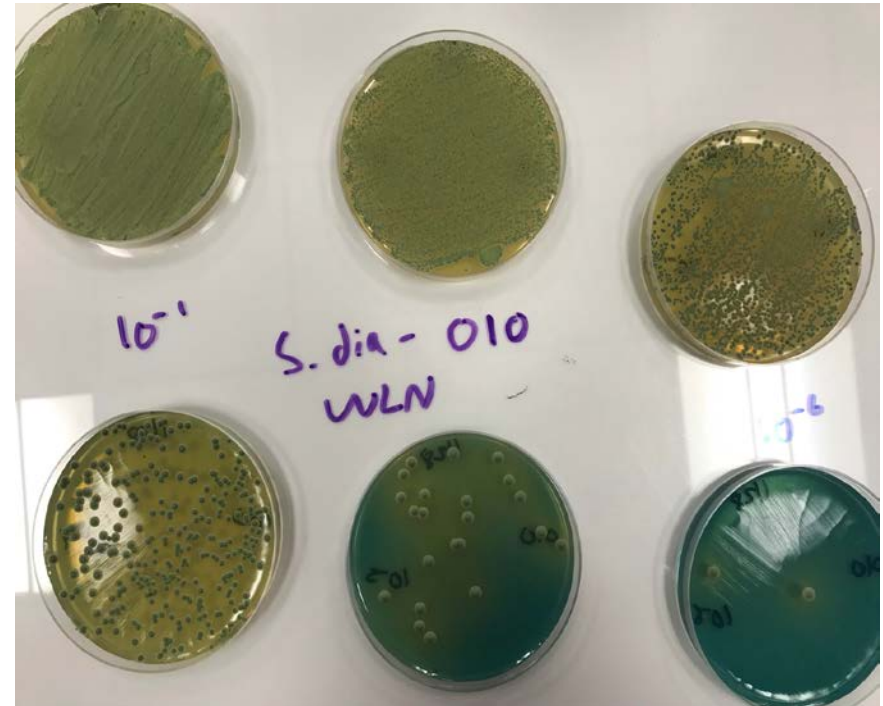


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Experiments with Diastaticus

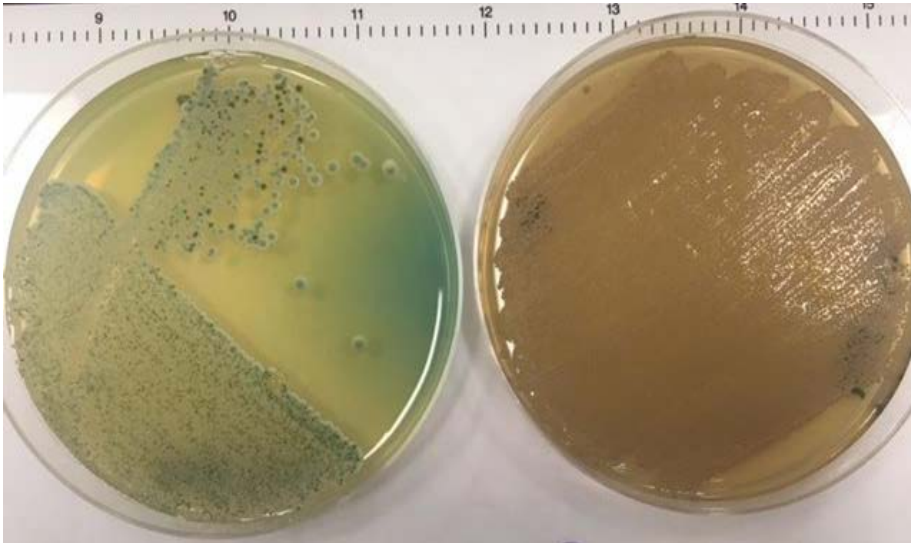
Detection Limit Study

- Each of the 5 diastaticus strains were inoculated into sterile DI water.
 - Avg. starting cell count: 71.5 million cells/mL
- Each sample was diluted by a factor of 10 until a dilution factor of 10^{-9} was achieved
- Each dilution for each strain was plated on LCSM and WLN media.
- Detection limit: 570cells/mL



Experiments with Diastaticus

Differentiation Study



- All 5 strains of diastaticus were mixed with all of our house yeast strains and plated on LCSM and WLN media.
- Every combination grew on both agar types with some interesting results:
 - WLN plate PCR results were negative for diastaticus.
 - On LCSM the diastaticus outcompeted our house yeast strains with full size colonies @48hrs



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Avery Protocol

Micro Testing:

- Plating on LCSM agar
 - Propagation
 - Fermentation
 - Package
 - Incidence of *Saccharomyces cerevisiae* var. *diastaticus* in the Beverage Industry Cases of Contamination, 2008–2017
- QPCR for confirmation
 - Applied Biosystems Real-Time PCR
 - Pika Weihenstephan detection kit.
 - DNA preparation includes:
 - A step to control the amount of DNA in sample
 - All samples contain an internal positive control
 - Prep DNA with both positive and negative controls.



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Prevention

- Regular CIP protocol should be enough... Just yeast folks!
 - Monitor chemical concentrations
 - Time
 - Temperature
- Preventative maintenance
 - Gaskets
 - Hoses
 - Pump (pump head)
- Quantitative data to confirm effective CIP protocol
 - RLU CIP sani
- Visual inspection
 - No biofilms



Summary

- It Is Just Yeast
- Questions?



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