ASBC Annual Meeting
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The Devil in the Details: *Saccharomyces cerevisiae* var. Diastaticus and Advanced Techniques for its Detection

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Saccharomyces cerevisiae var. Diastaticus

- First described by Andrews and Gilliland in 1952
- Originally named *Saccharomyces diastaticus*, later re-classified as a variant of *S. cerevisiae*
- Named for diastatic properties (ability to cleave dextrin)
- Also observed to produce phenolic aromas and flavors
- Similar cell morphology to Belgian strains

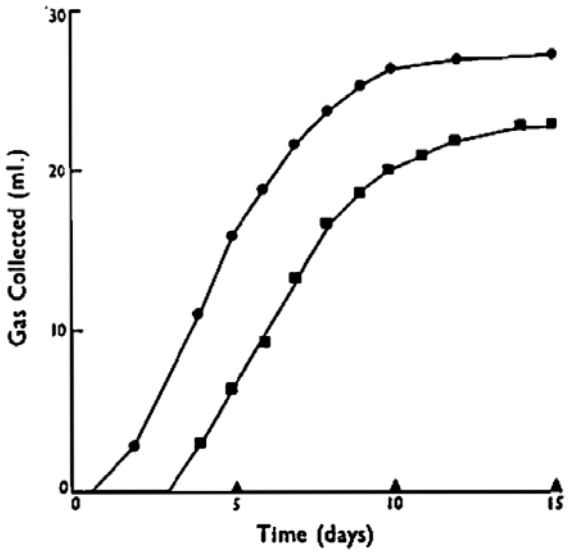


Fig. 1.—The production of gas from 15 ml. of 2% dextrin and starch by *S. diastaticus* and by *S. cerevisiae* in McCartney bottles. Each point is the average of five determinations.
S. diastaticus in dextrin —●—.
S. diastaticus in starch —■—.
S. cerevisiae in dextrin and starch —▲—.

Gilliland, RB. *Saccharomyces diastaticus* – a starch-fermenting yeast. J. Inst. Brew. Vol. 72. 1966.

A yeast by any other name…

- Not always a contaminant
- Can be used intentionally for a dry Belgian ale
- Use caution when using attenuative Belgian strains

Diastaticus contamination can wreak havoc

- Ability to ferment dextrins leads to superattenuation
- If attenuation does not finish in fermenter -> exploding packages
- Contaminated beer will **usually** be out of spec for ABV (high), AE (low) and have phenolic aromas and flavors
- Impossible to blend off out-of-spec beer unless pasteurizing

An ever more common issue

- Several product recalls and recoveries associated with this organism
  - Left Hand recall – nitro Milk Stout bottles
  - Bell's Winter White – discussed at CBC 2017
- Dogfish encountered in late 2016.

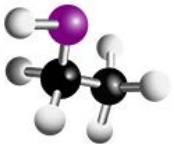
Suspicious colonies and puzzling sequencing data

- Namaste White, a Belgian witbier
- Observed growth on LCSM late in propagation
- Sent for sequencing and received:

28S DNA: 323 base pairs

FY28M2 DNA Match Report

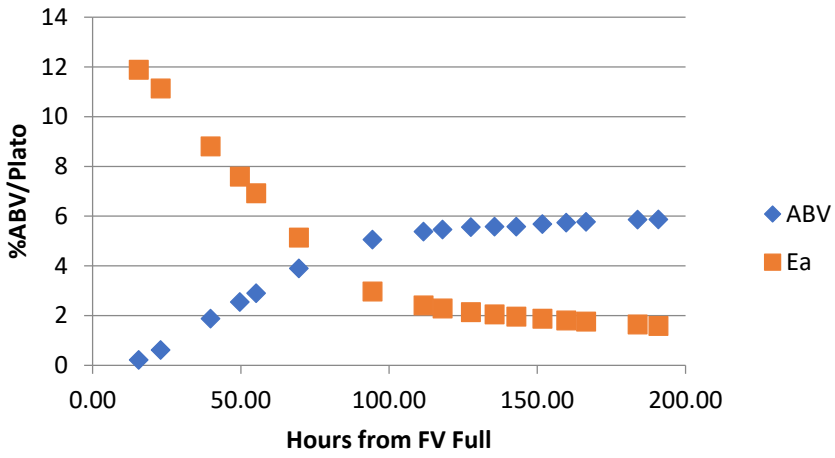
Match	%Diff	Length	Library Entry Name
1	0.00	323	Saccharomyces-boulardii
2	0.00	323	Saccharomyces-cerevisiae
3	2.79	323	Saccharomyces-bayanus/pastorianus
4	4.32	324	Zygosaccharomyces-microellipsoides
5	4.33	320	Kluyveromyces-africanus
6	4.63	324	Torulaspora-delbrueckii
7	4.94	324	Torulaspora-pretoriensis
8	4.95	323	Kluyveromyces-lodderii
9	5.26	323	Saccharomyces-unisporus
10	5.88	323	Saccharomyces-dairenensis



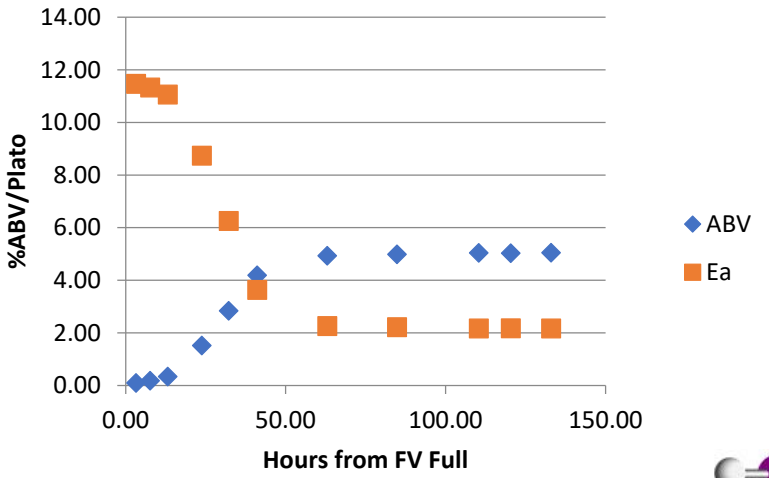
Let's keep an eye on it...

- Canceled harvest of affected tank and started new propagation from slurry
- Next propagation also showed colonies late in growth
- First fermenter finished 0.9% ABV above target

Affected tank

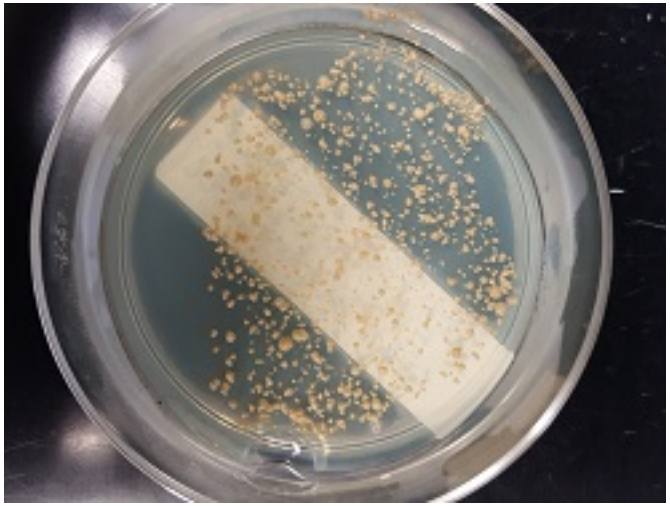


Clean tank



The trouble continued

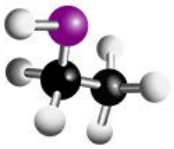
- Saw wild yeast growth in more fermenters
- No smoking gun common link between incidences
- Colonies appearing late in propagation
- Suspected Diastaticus based on attenuation



Diastaticus is genetically different from typical *S. cerevisiae* at one locus

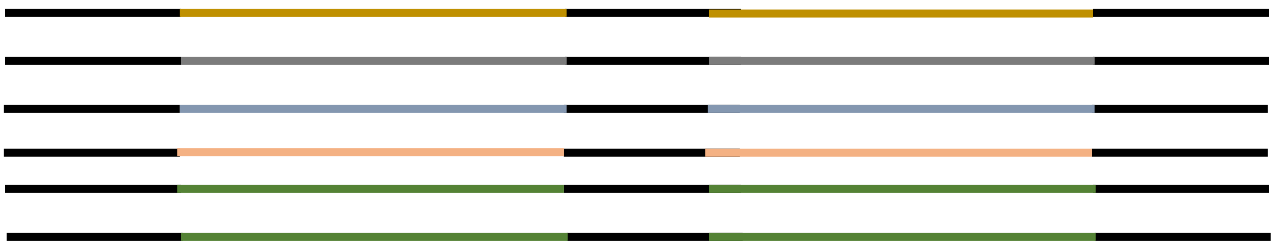
- Expression and secretion of glucoamylase differentiate Diastaticus from typical *S. cerevisiae*
  - Unbranched a-(1->4) glucosidic chains

- Phenolic off-flavor (POF1) gene encoded next to glucoamylase genes
  - Decarboxylation of phenolic acids in wort -> production of 4-vinyl phenol and 4-vinyl guiacol



Subtle genetic differences complicate Diastaticus detection

- Typical sequencing based ID methods use 16S ribosomal DNA
- 16S region used is 100% identical between typical *S. cerevisiae* and Diastaticus variant
- Must use PCR with primers designed for the specific locus that is different
  - PIKA Weihenstephan and Biotecon offer detection kits to be used with an array of thermocycler platforms
  - Can send in samples for analysis if only a few



More sensitive detection needed!

- Diastaticus can grow on Lin's Cupric Sulfate Medium

- Testing slurry for presence of Diastaticus is difficult if it is present at very low levels
  - Typical practice – dilute slurry 1:40 before plating
  - Plating 100 μl – limit of detection is 400 cfu/ml

- Needed a way to see beyond culture yeast

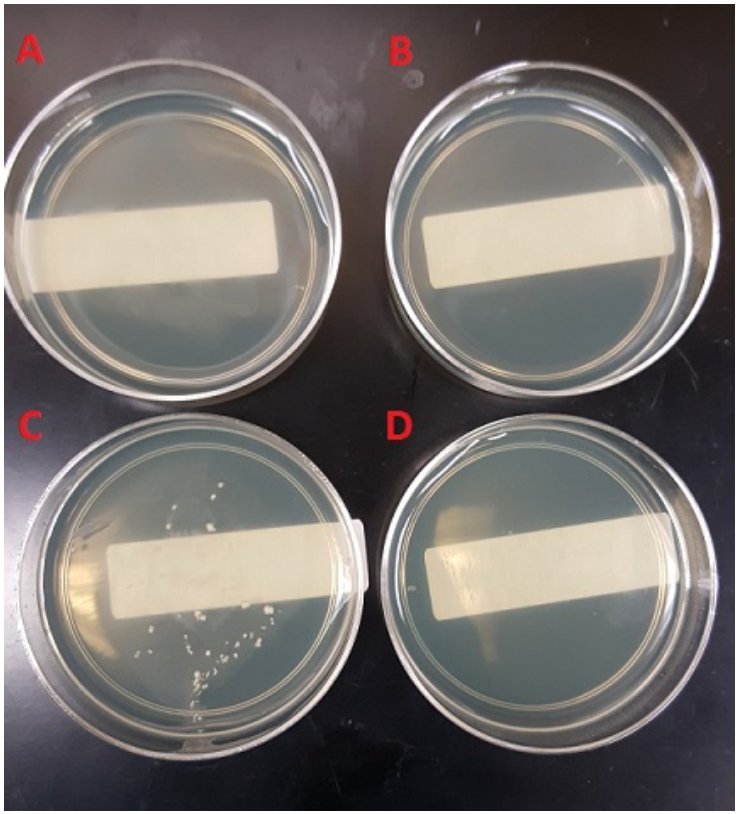
Enrichment screening method

- Assuming desired yeast is not capable of growth on LCSM (copper sensitive):
  - Inoculate slurry sample into an autoclaved malt starter supplemented with cupric sulfate
    - CuSO4 concentration in ASBC methods (Microbiological Control 5).
  - Grow aerobically with sampling and plating on LCSM every 24 hours.

The presence of cupric sulfate is required for enrichment of Diastaticus in low concentrations

- Colonies appeared after 48 hours of enrichment culture
- Matches observations during propagation
- Without cupric sulfate, unable to outcompete culture yeast

DME/water + slurry DME/water



DME/water/CuSO4 + slurry DME/water/CuSO4

Moving forward: lessons learned

- Dumped affected fermenters
- Added PCR to our testing repertoire
- In-house yeast propagation with Diastaticus detection testing

Takeaways

- Diastaticus is a real issue that is becoming more prevalent
- Safety issues a major concern
  - Increased ABV – TTB compliance
- Difficult, but possible to detect
- Importance of knowing growth phenotypes, morphology, and typical performance of house strains

Thanks!!

Questions??