Juice Microbiology and How it Impacts the Fermentation Process

Southern Oregon Wine Institute Harvest Seminar Series
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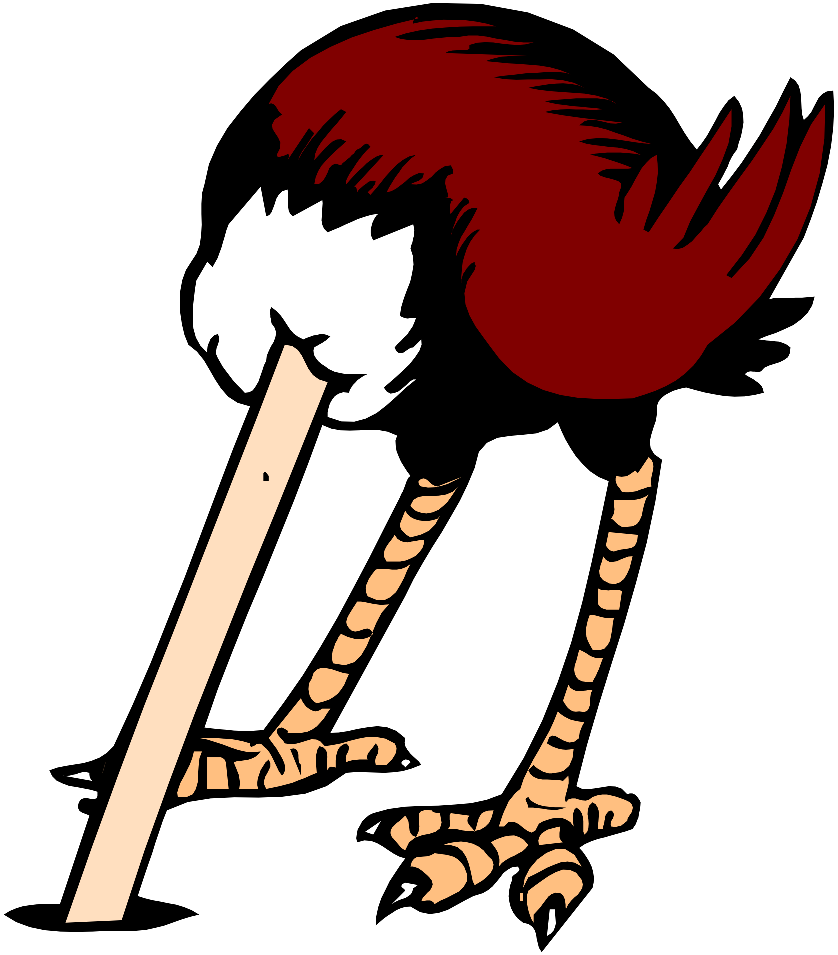
ETS Laboratories

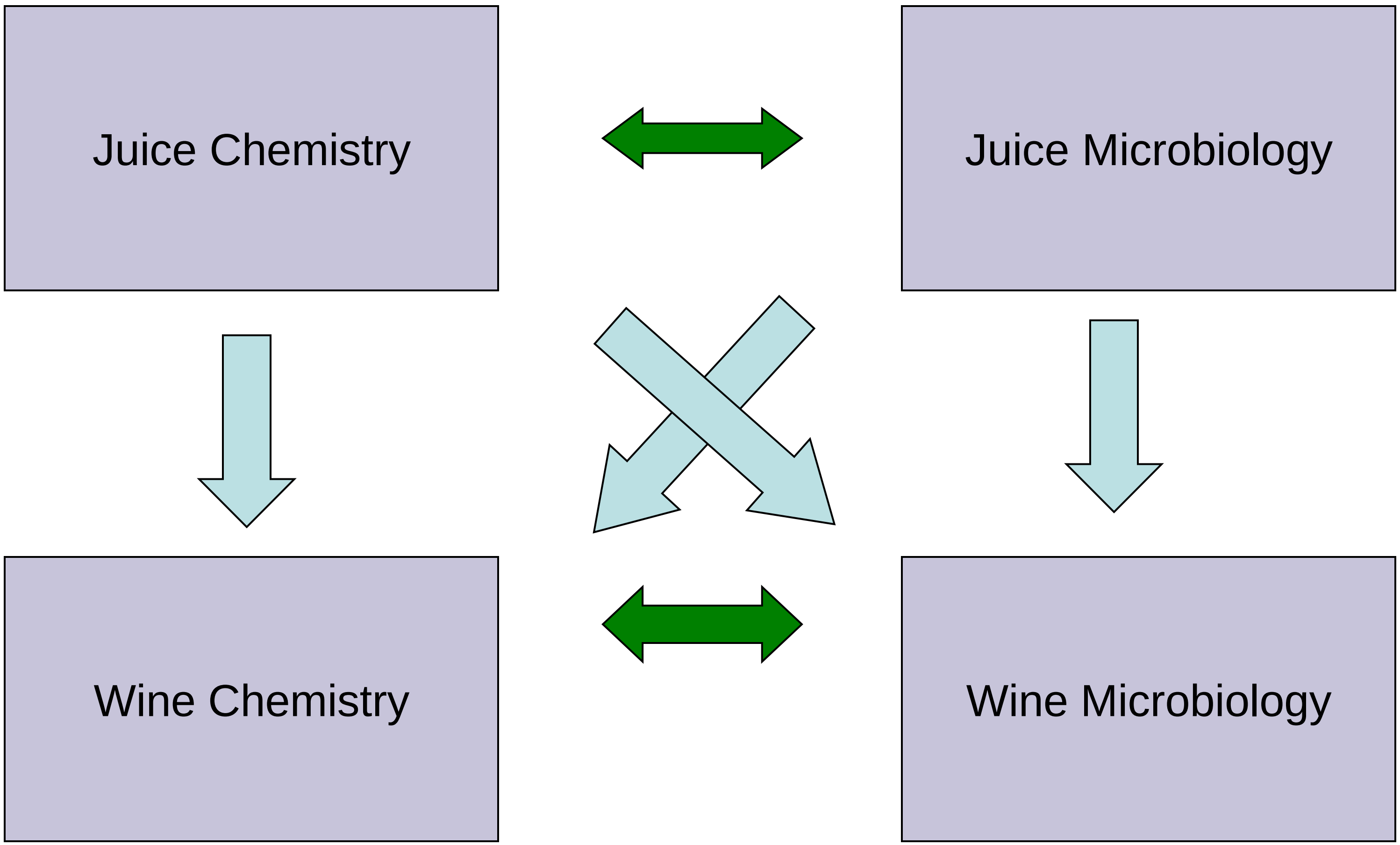
Monitoring Juice Microbiology:

Who Cares ?

Preemptive Screening
vs.
Forensic Analysis



What Happens in the Juice Does Not Stay in the Juice



Why should you care?

Risk Management

- Problem fermentations
- Negative impact on wine sensory due to production of metabolites
  - acetic acid
  - ethyl acetate
  - mousey
- Process problems
  - stuck/sluggish fermentations
  - filtration
- Loss of potential = Loss of $$$$

Juice Microbiology

- What is in the vineyard?
- What can they produce?
- What can they consume?
- How can they impact fermentation?
- What can you do to minimize this impact?
- New tools to monitor native fermentations

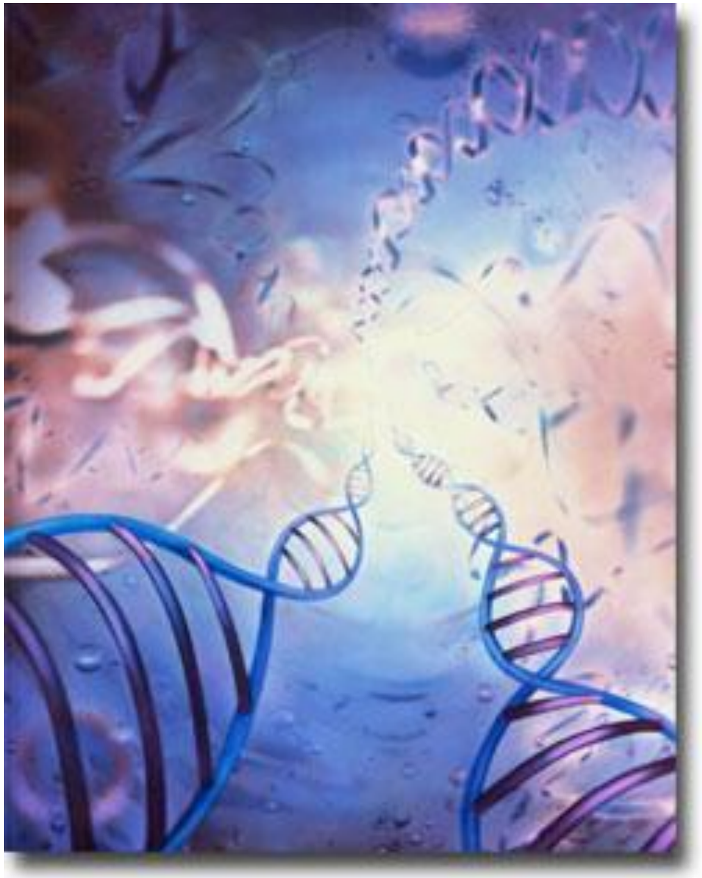
What Microbes are Found in the Vineyard

- Wild Yeasts
  - Hanseniaspora/Kloeckera
  - Pichia
  - Saccharomyces
  - Zygosaccharomyces
  - Brettanomyces
- Bacteria
  - Acetic acid bacteria
  - Lactic acid bacteria

What Methods for Detection?

- Yeast and Bacteria
  - –Scorpions Juice Panel
    - –Yeast
    - –Bacteria
  - –Scorpions VA Producer Panel
  - –Chemical analysis (VA, ethyl acetate)

ETS Scorpions Molecular Diagnostics as a Detection Tool



- Identify microbes by detecting their DNA using the polymerase chain reaction (PCR)
- Adapted from food and medical fields, a mature technology
- Can detect VNC microbes
- Rapid, Sensitive, Accurate
- Enables early intervention

Scorpions Juice Panels

Juice Bacteria Panel

- 6 species of acetic acid bacteria
- 9 species of heterofermentive Lactobacillus
- 6 species of homofermentive Lactobacillus
- 5 species of Pediococcus

Juice VA Producer Panel

- 2 species of Hanseniaspora
- 6 species of acetic acid bacteria

Juice Yeast Panel

- 2 species of Hanseniaspora
- 2 species of Pichia
- 2 species of Zygosaccharomyces
- 2 species of Brettanomyces

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Survey of Juice Microbes

Organism	High	Low	Mean	Number	Number positive	Percent positive
Acetic acid bacteria	>1000000	10	9750	1451	1016	70
Lactobacillus	123600	10	6716	1451	102	17
Pediococcus	928860	10	197390	1451	87	6
Brettanomyces	8800	210	3012	1238	37	3
Zygosaccharomyces	733600	10	3965	1238	817	66
Pichia	>1000000	10	16708	1238	940	76
Hanseniaspora	>10,000,000	10	293960	1238	1002	81

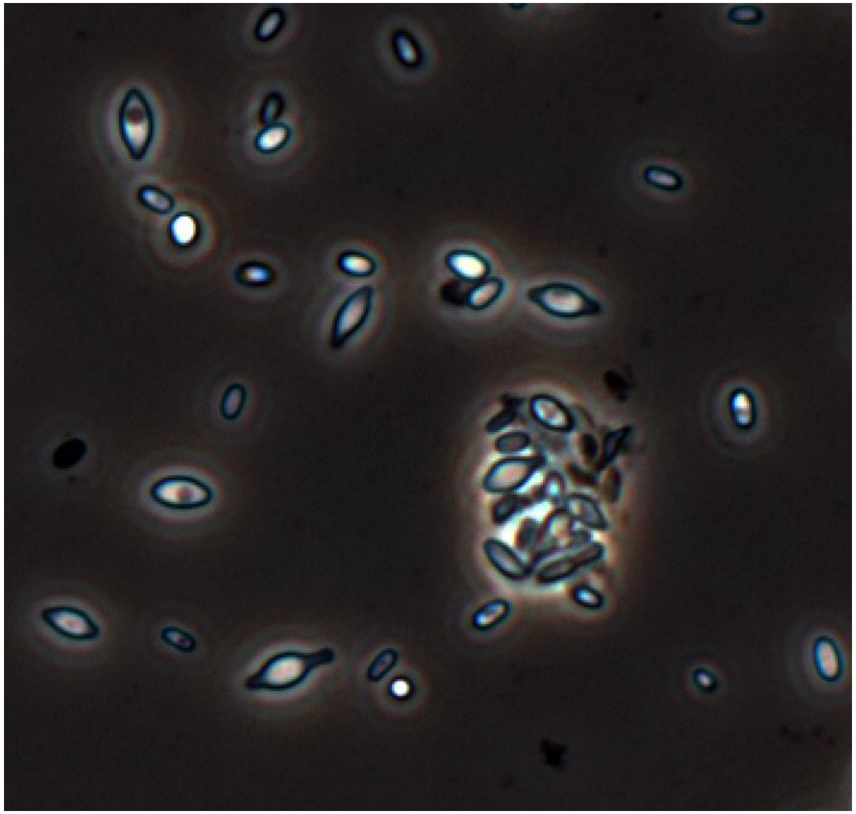
LABORATORIES

What Can Native Yeast Produce ?

- Ethyl Acetate
- Acetic Acid
- Enzymes
- Other compounds?
  - –Sulfides
  - –Inhibitory compounds (fatty acids, etc)

Native Yeast

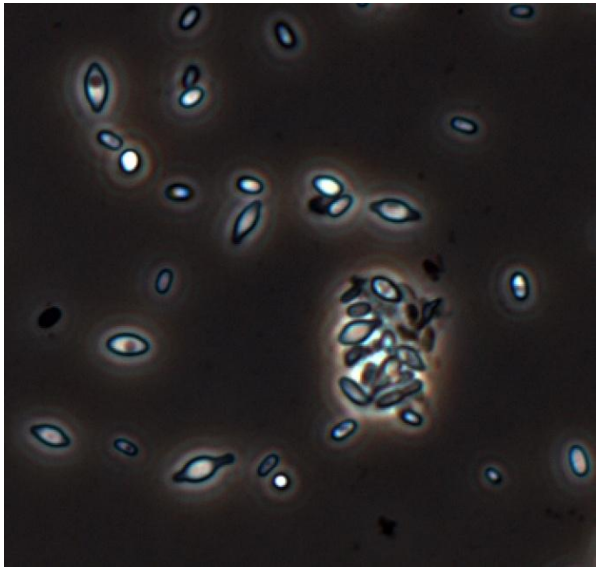
- *Hanseniaspora/Kloeckera*
  - Cold and SO_2 tolerant
  - Elevated ethyl acetate, amyl acetate, glycerol and acetic acid in juice or wine
  - Depletes YAN and thiamine
- Can inhibit growth of Saccharomyces
- High levels of protease activity
- Large differences in higher alcohols (Fusel oils) profile as compared to Saccharomyces

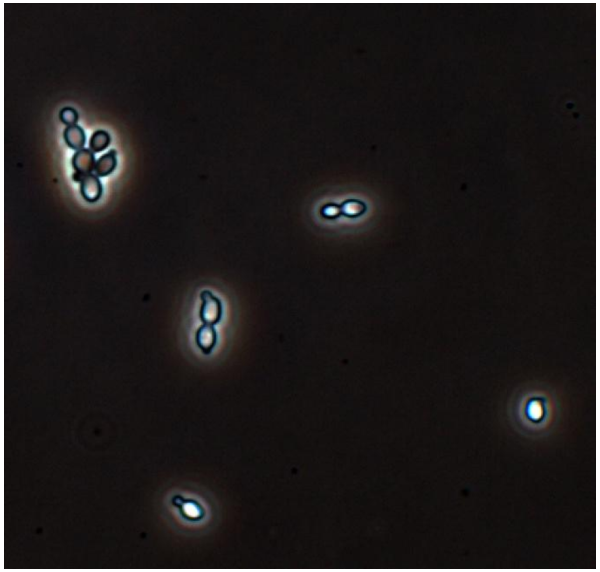


Native Yeast

Pichia species

- Most commonly associated with rotted or damaged fruit
- *P. guillermondii* shown to produce 4-EP/4-EG in must conditions
- Can tolerate ethanol levels up to 12.5% (v/v)
- Higher concentrations of propanol, phenylethyl acetate



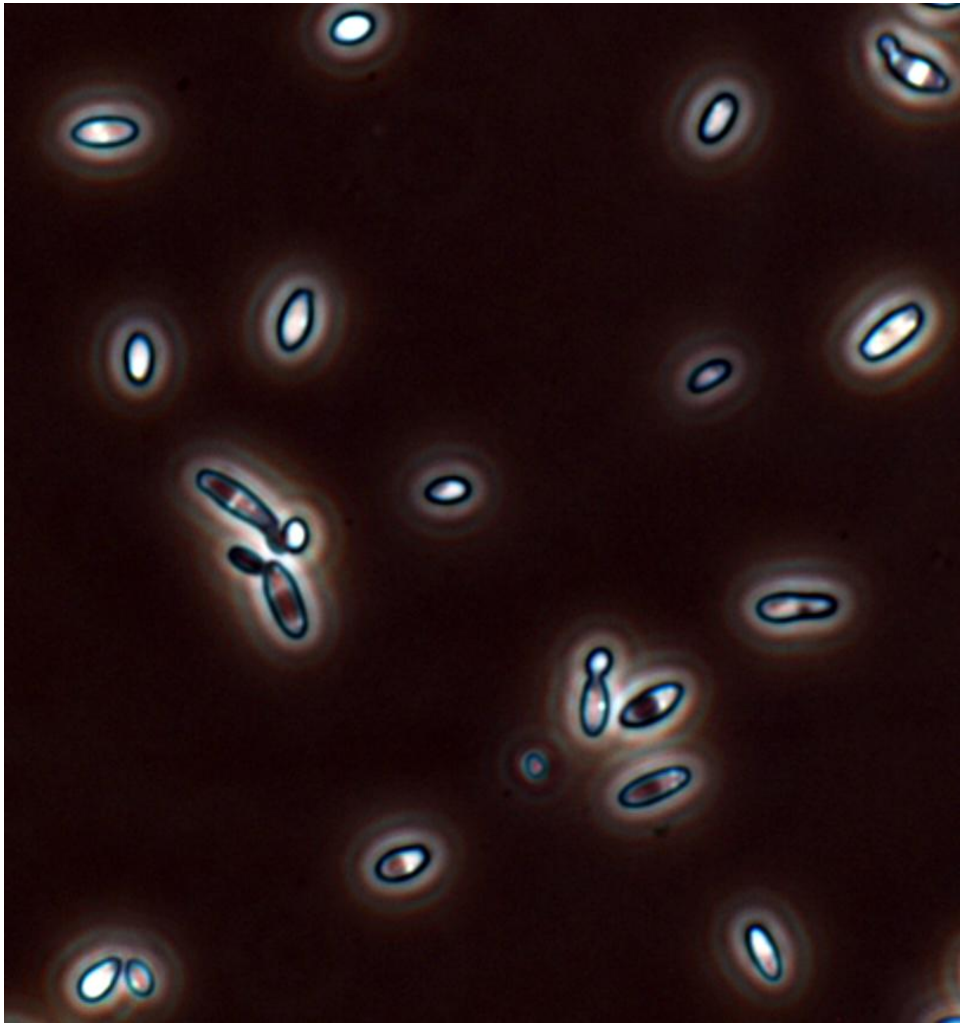


Native Yeast

Brettanomyces bruxellensis

- Aroma defects in wine:
  - horse sweat
  - band-aid
  - medicinal

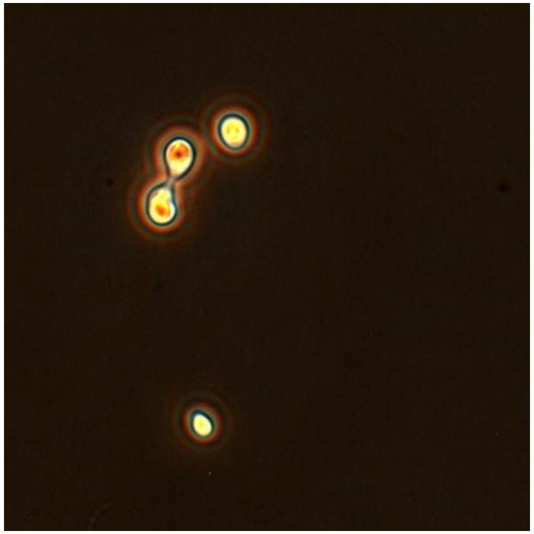
- Grapes, equipment, primarily cooperage

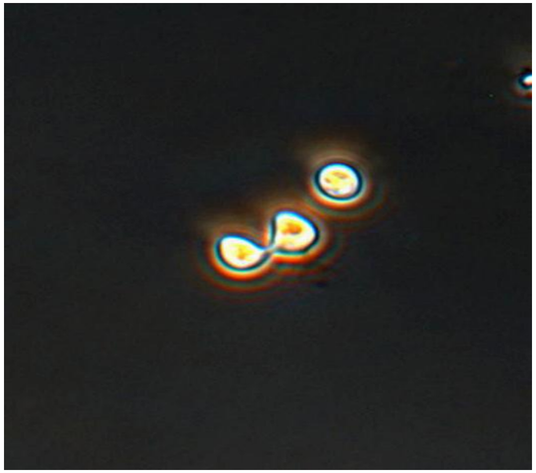


Native Yeast

Zygosaccharomyces bailii

- Turbidity, CO_2 in bottled sweet wines
- Found in cellars, bottling lines, & concentrates
- Grapes, equipment
- Resistant to elevated levels of alcohol, SO_2 and sorbate





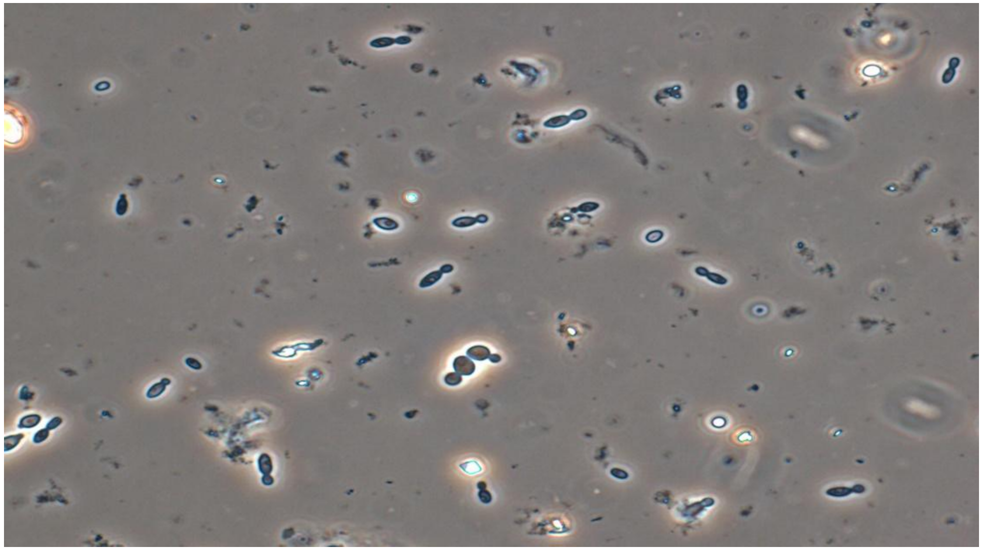
Elevated Ethyl Acetate in Must

910030211

Brix	20.1 g/100mL	10/3/09
volatile acidity (acetic)	0.63 g/L	10/3/09
ethyl acetate	194 mg/L	10/3/09
yeast assimilable nitrogen	89 mg/L (as N)	10/3/09

909290453

Brix	18.4 g/100mL	9/29/09
volatile acidity (acetic)	0.81 g/L	9/29/09
ethyl acetate	296 mg/L	9/29/09
yeast assimilable nitrogen	59 mg/L (as N)	9/29/09

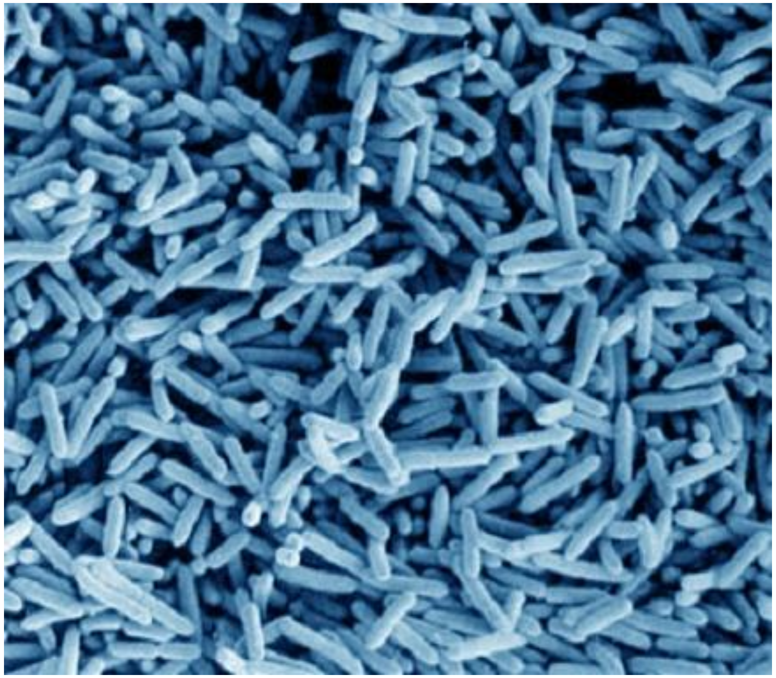


What Can Bacteria Produce ?

- Acetic acid
- Biogenic amines
- Tetrahydropyridines (mousy)
- Other compounds?
  - lactic acid
  - yeast inhibitory compounds
  - sulfides

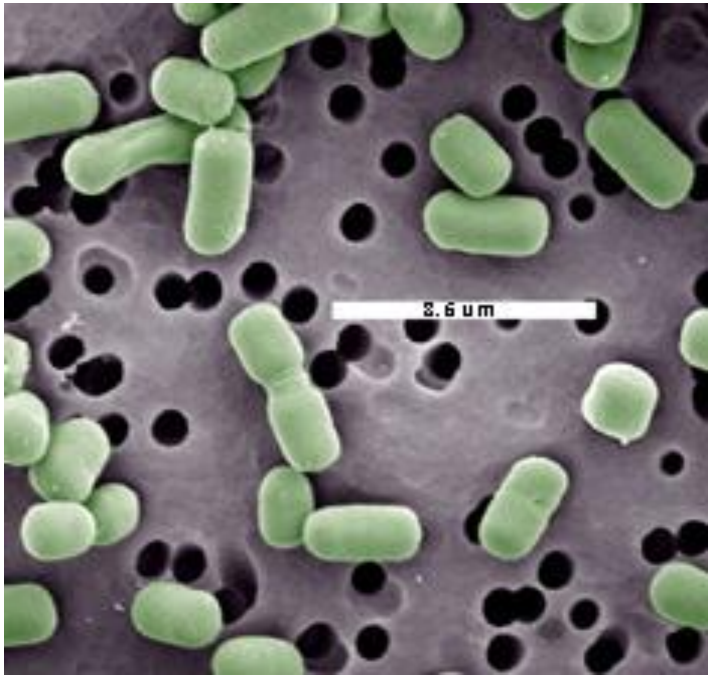
Acetic Acid Bacteria

- *Gluconobacter* species
- *Acetobacter* species
- *Gluconacetobacter* species
- Produce VA in juice or wine
- Elevated numbers associated with damaged/moldy grapes



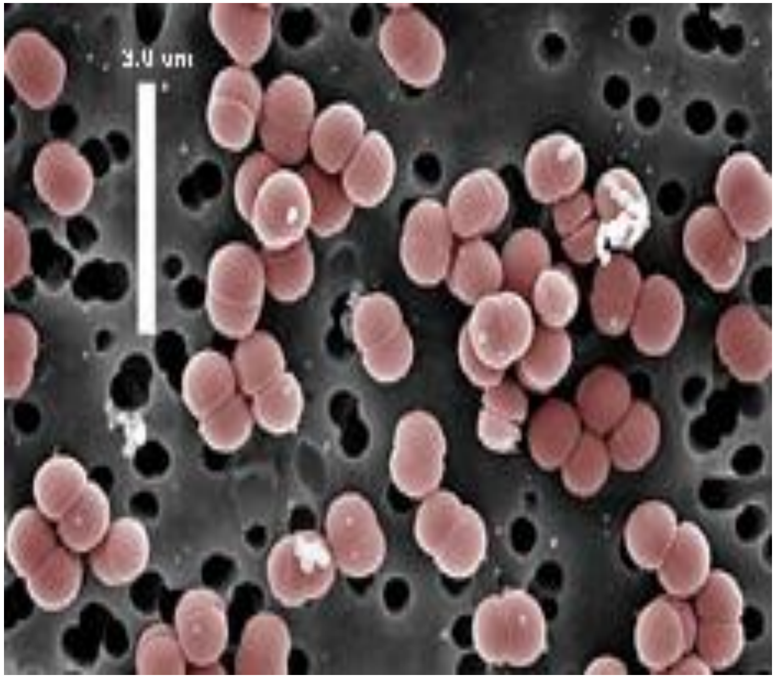
Lactobacillus species

- Biogenic amines
  - histamine
  - cadaverine
  - putrescine
  - tyramine
- Problem during sluggish/stuck ferments
  - heterofermentive *Lactobacillus spp*.
  - homofermentive *Lactobacillus spp.*
- Elevated VA in wine can contribute to stuck ferments



Pediococcus species

- *Pediococcus damnosus*
  - can cause ‘ropiness’
- *Pediococcus parvulus*
  - warm climate species
- Both produce biogenic amines which can cause covert and overt spoilage
  - histamine
  - cadaverine
  - putrescine
  - tyramine



High VA Must

Analyte	Result	Analysis Date
810030211 PN-1 08 (Fermenting)		
volatile acidity (acetic)	0.81 g/L	10/3/09
glucose + fructose	12.4 g/100mL	10/3/09
ethanol at 20C (FTIR)	4.98 % vol	10/3/09
810030212 PN-2 08 (Fermenting)		
volatile acidity (acetic)	0.96 g/L	10/3/09
glucose + fructose	10.1 g/100mL	10/3/09
ethanol at 20C (FTIR)	5.39 % vol	10/3/09
810030213 PN-3 08 (Fermenting)		
volatile acidity (acetic)	0.97 g/L	10/3/09
glucose + fructose	15.9 g/100mL	10/3/09
ethanol at 20C (FTIR)	1.62 % vol	10/3/09

What Do Microbes Consume ?

- Nitrogen
- Essential micronutrients
- Sugars and organic acids

Depletion of Yeast Assimable Nitrogen

- YAN represents total N available in the must
- Sufficient YAN levels are necessary for Saccharomyces to complete fermentation
- Vineyard microbes can utilize 80-90% of the YAN available in the grape
- YAN deficiencies can result in stressed yeast and stuck fermentations

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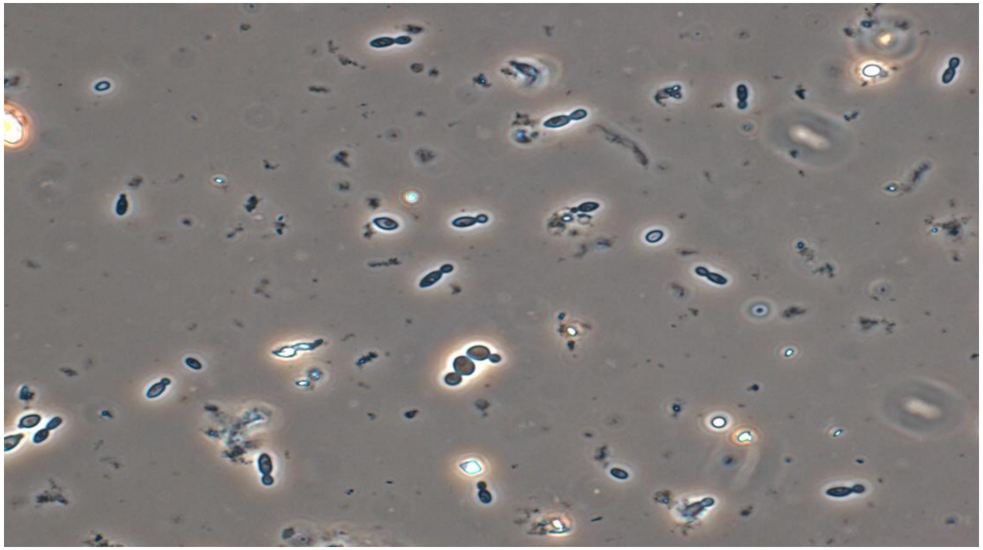
Variation in Yeast Assimable Nitrogen

Analyte	Result	Analysis Date
810030211		
Brix	24.1 g/100mL	10/3/09
alpha-amino compounds	85 mg/L	10/3/09
ammonia	54 mg/L	10/3/09
yeast assimilable nitrogen	129 mg/L (as N)	10/3/09
809290453		
Brix	23.4 g/100mL	9/29/09
alpha-amino compounds	140 mg/L	9/29/09
ammonia	210 mg/L	9/29/09
yeast assimilable nitrogen	309 mg/L (as N)	9/29/09
810270342		
brix	23.2 degrees	10/27/09
alpha-amino compounds	183 mg/L	10/27/09
ammonia	64 mg/L	10/27/09
yeast assimilable nitrogen	236 mg/L (as N)	10/27/09

Loss of YAN to Native Yeast in Must

910030211		
Brix	20.1 g/100mL	10/3/09
volatile acidity (acetic)	0.63 g/L	10/3/09
ethyl acetate	194 mg/L	10/3/09
yeast assimilable nitrogen	89 mg/L (as N)	10/3/09

909290453		
Brix	18.4 g/100mL	9/29/09
volatile acidity (acetic)	0.81 g/L	9/29/09
ethyl acetate	296 mg/L	9/29/09
yeast assimilable nitrogen	59 mg/L (as N)	9/29/09



Essential Micronutrients

- Vitamins and Minerals
  - –Thiamine
  - –Niacin
  - –Biotin
  - –Folic acid
  - –Pantothenic acid
  - –Magnesium
  - –Pyridoxine
  - –Myo-Inositol
- These compounds are difficult to measure and there are no rapid test methods to determine if levels are deficient

How Can They Affect Fermentation ?

- Microbes from the vineyard can have a negative impact on primary fermentation
  - scavenge available nutrients resulting in lack of sufficient nutrients for Saccharomyces/Oenococcus to complete fermentation
  - production of inhibitory compounds
- Can cause problems during stuck or sluggish fermentations
  - production of acetic acid

Stuck/Sluggish Fermentation Case Study

Client had a fermentation that stopped and then started to move slowly……

Chemical analysis

volatile acidity(acetic)	0.091 g/100mL	10/23/09
glucose + fructose	3.8 g/100mL	10/23/09
ethanol at 20C (FTIR)	11.98 % vol	10/23/09

'Scorpion' Stuck and Sluggish Spoilage Panel

Lactobacillus brevis/hilgardii/plantarum	800 cells/mL	10/25/09
Pediococcus damnosus/parvulus	<10 cells/mL	10/25/09
Acetobacter/Gluconobacter	3000 cells/mL	10/25/09
Oenococcus oeni	1,680,000 cells/mL	10/25/09

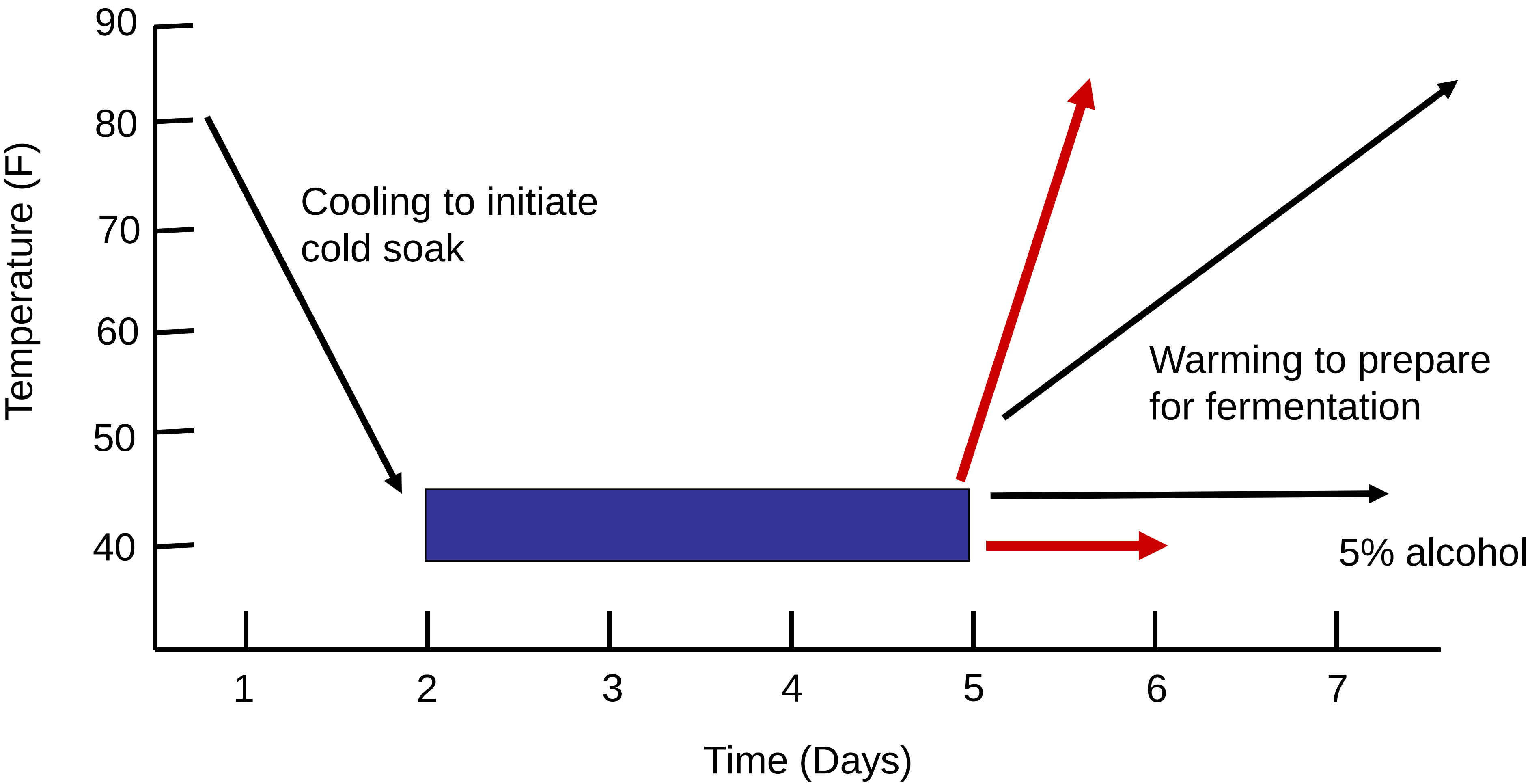
What Can you do about Vineyard Microbes ?

- Minimize use of rotten fruit
- Identify the presence of potentially problematic microbes
- Intervene when and where possible

Scorpions Juice Panel Results

Analyte	Result	Analysis Date
810090254 PN-2 08		
'Scorpion' Juice Spoilage Panel		
Lactobacillus brevis/hilgardii/plantarum	17800 cells/mL	10/10/09
Pediococcus damnosus/parvulus	<10 cells/mL	10/10/09
Acetobacter/Gluconobacter	962000 cells/mL	10/10/09
Brettanomyces bruxellensis	<10 cells/mL	10/10/09
Pichia membranifaciens	70 cells/mL	10/10/09
Hanseniaspora uvarum	193700 cells/mL	10/10/09
Zygosaccharomyces bailii	1600 cells/mL	10/10/09
810090255 PN-3 08		
'Scorpion' Juice Spoilage Panel		
Lactobacillus brevis/hilgardii/plantarum	6400 cells/mL	10/10/09
Pediococcus damnosus/parvulus	<10 cells/mL	10/10/09
Acetobacter/Gluconobacter	2300000 cells/mL	10/10/09
Brettanomyces bruxellensis	<10 cells/mL	10/10/09
Pichia membranifaciens	1200 cells/mL	10/10/09
Hanseniaspora uvarum	171400 cells/mL	10/10/09
Zygosaccharomyces bailii	6200 cells/mL	10/10/09

Intervention at the cold soak stage



Intervention Using Additions

- Appropriate use of SO_2
- Use of commercial strains of yeast and bacteria
- Appropriate nutrient additions
- Acid adjustments

Juice Microbiology
and
Native Fermentations

LABORATORIES

DNA Fingerprinting of *Saccharomyces cerevisiae*

AKA - Multi-locus, Variable Number, Tandem Repeat, DNA Analysis

Why did we develop the assay?

- Clients want to isolate “the yeast” from a great wine resulting from a native fermentation
- Clients want to know if their commercial yeast are working and or why fermentation results are inconsistent
- Until now, the tool to provide this information was not available commercially in the US

LABORATORIES

What Drives the Fermentation ?

- Initial fermentation primarily non-Saccharomyces yeast
- The yeast population is dynamic, Saccharomyces strains represent a small percentage of the total yeast population
- Once ethanol reaches 5-6%, Saccharomyces has a competitive advantage over the majority of non-Saccharomyces yeast
- Individual strain tolerance can result in a dynamic Saccharomyces population

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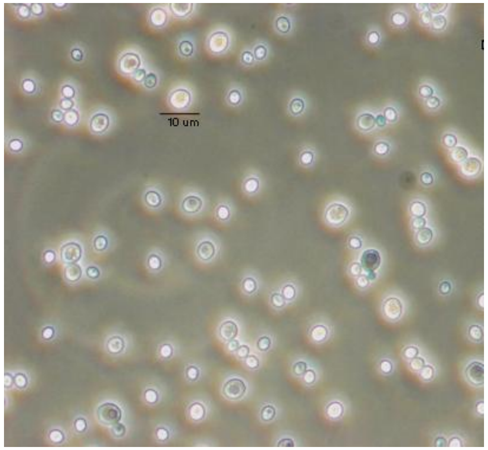
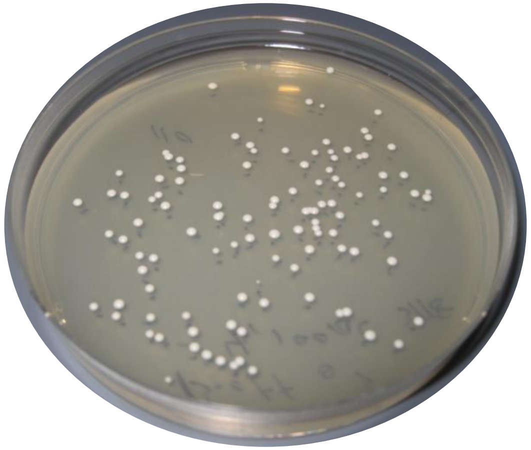
The Saccharomyces VNTR Process

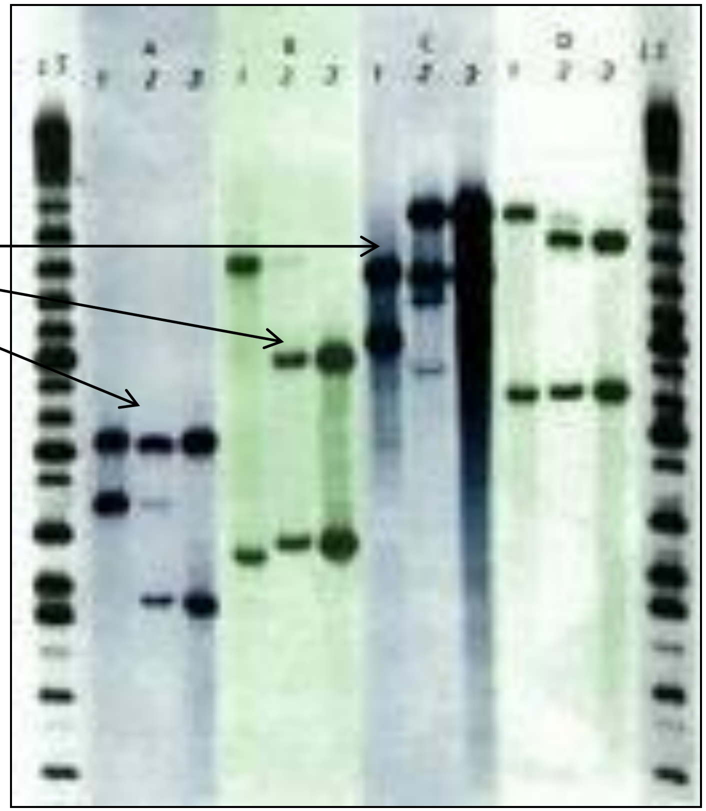




“Old School” DNA Fingerprinting

4 different fingerprint loci

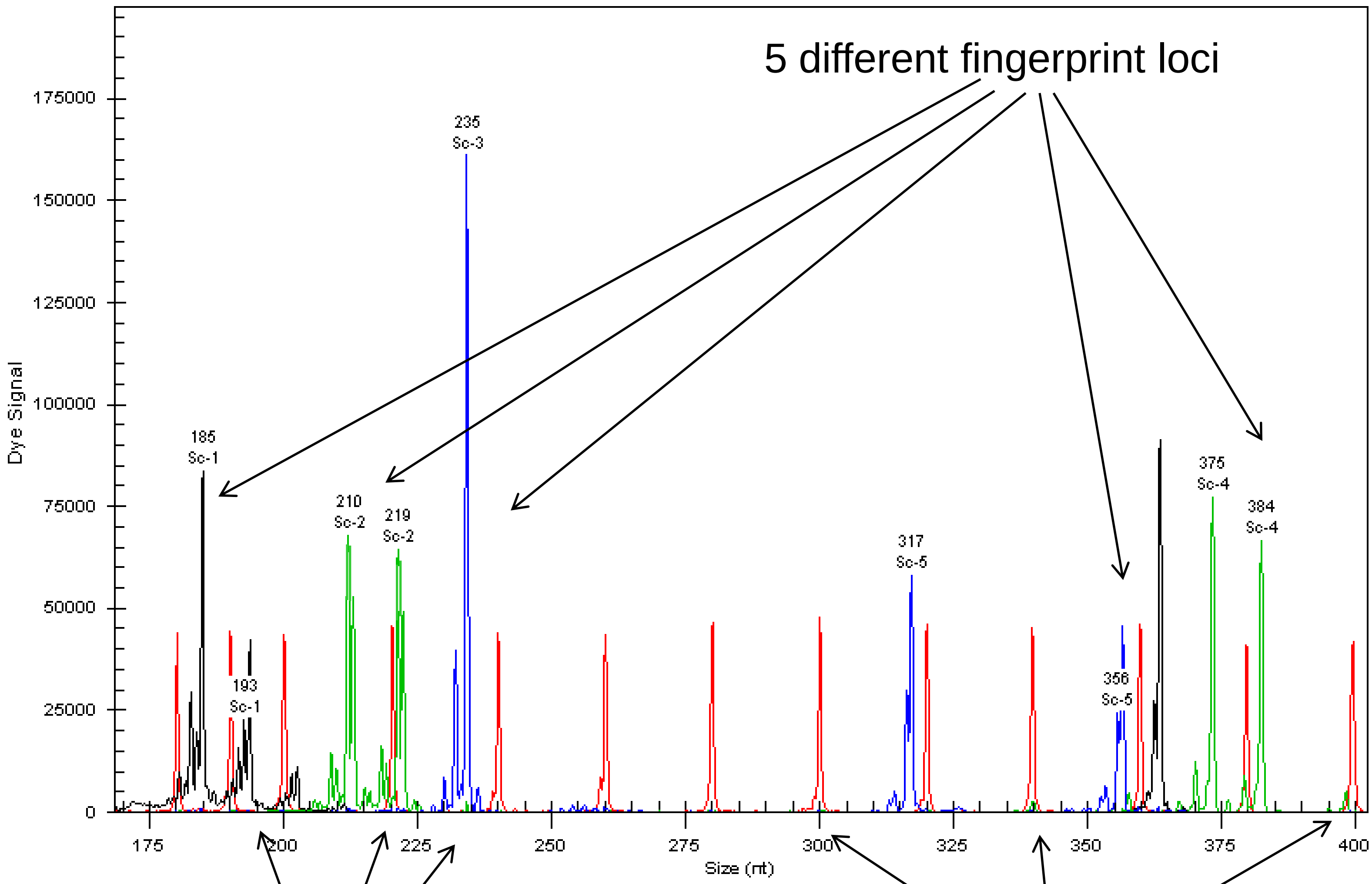
DNA fragments of different sizes



Molecular weight markers

Saccharomyces cerevisiae VNTR Profile

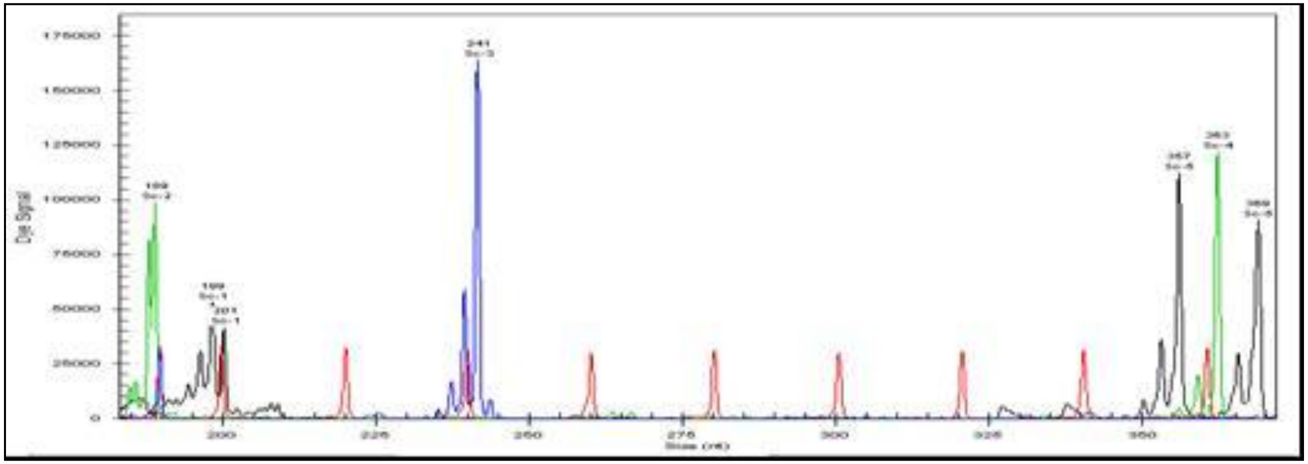
5 different fingerprint loci



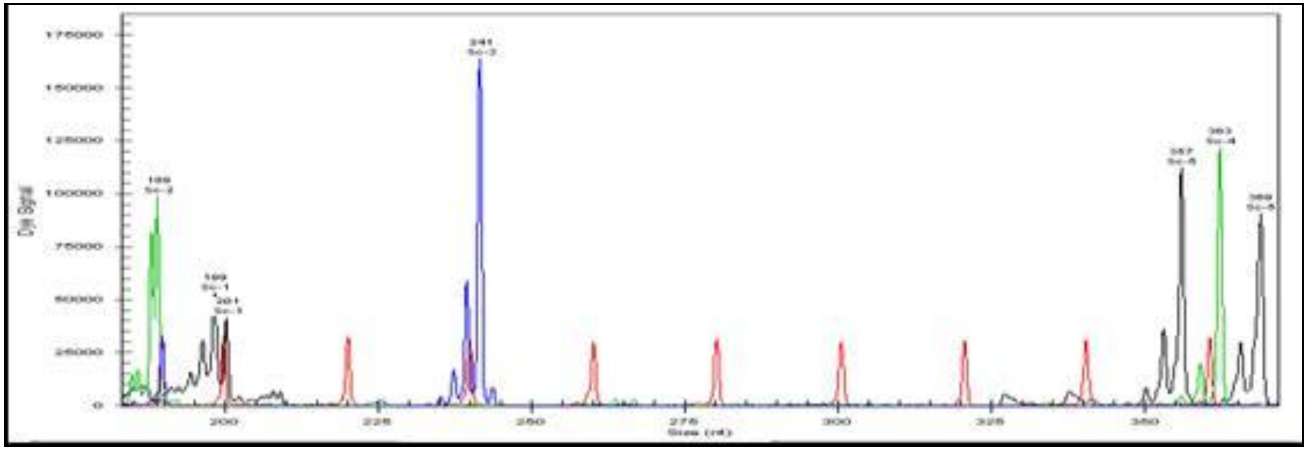
DNA fragments of different sizes

Molecular weight markers

VNTR Profile of Yeast Strain from Native Fermentation

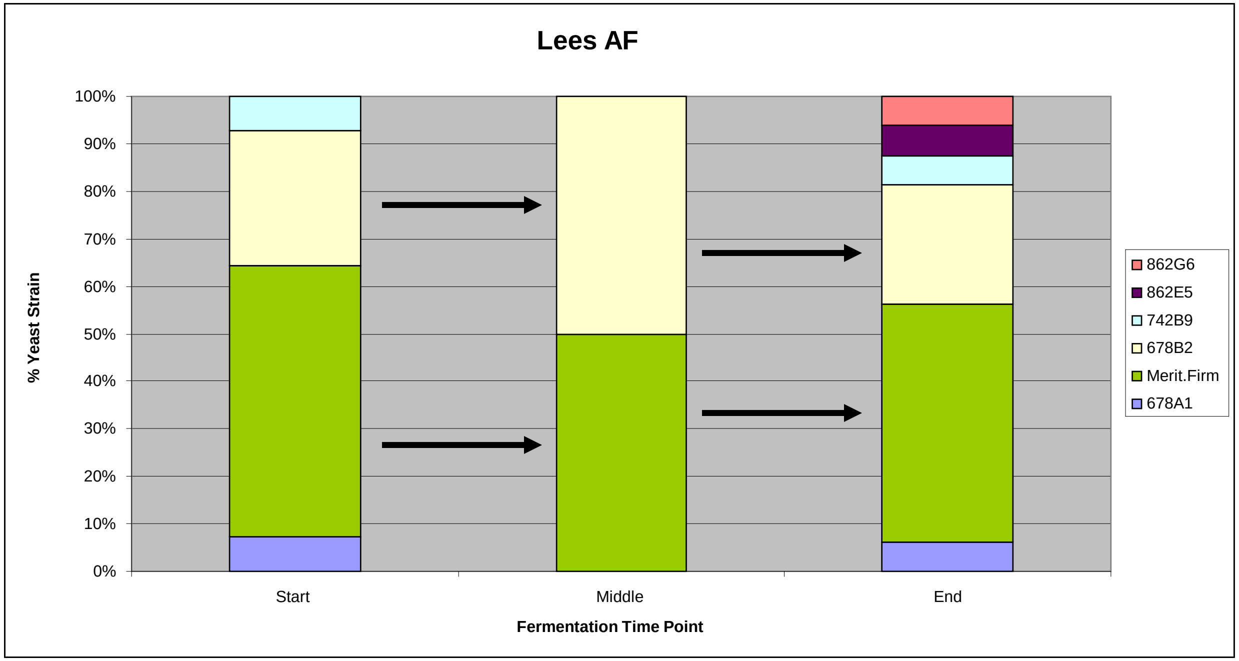


Dominant yeast strain from "Native" fermentation



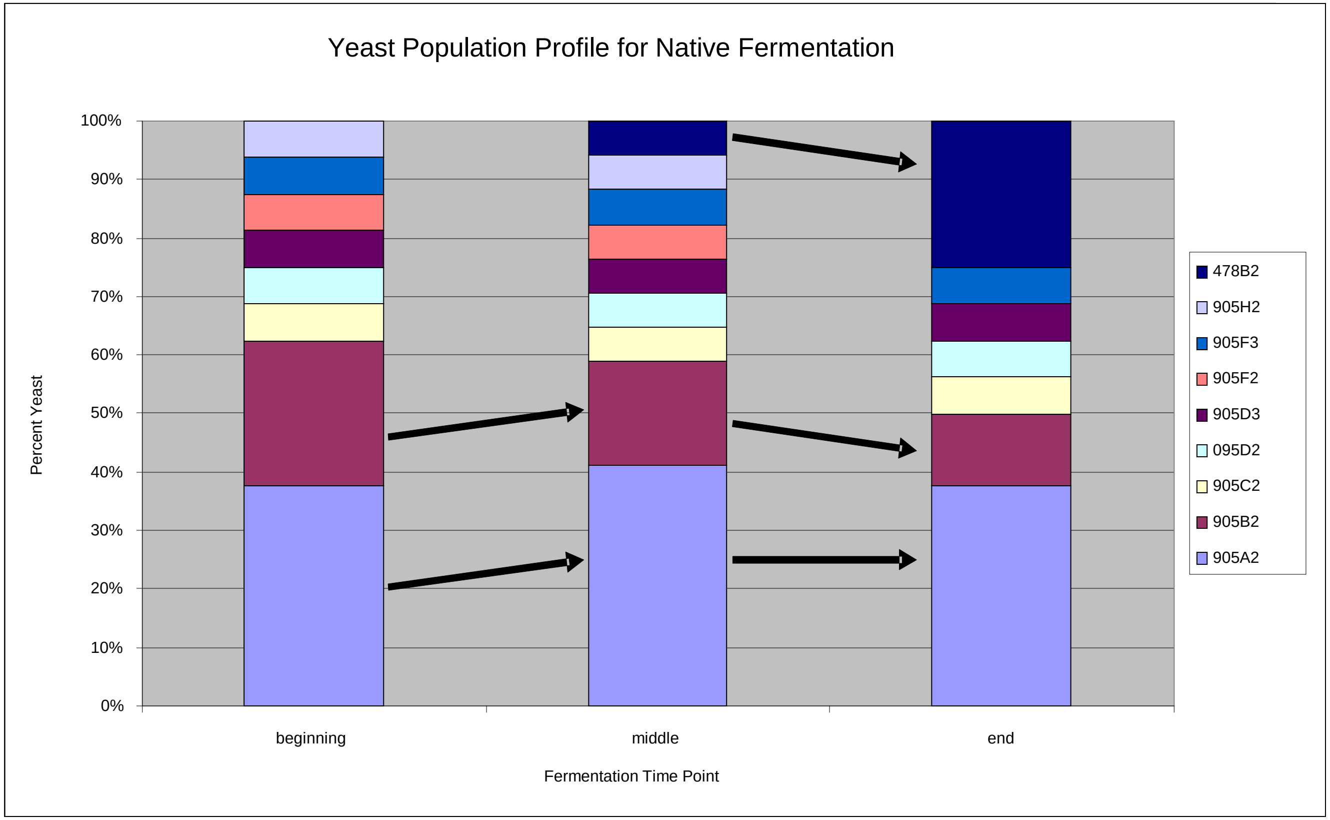
Commercial yeast strain CH Merit.firm used at the winey the previous year

Fermentation Profile for a Lees Initiated Fermentation

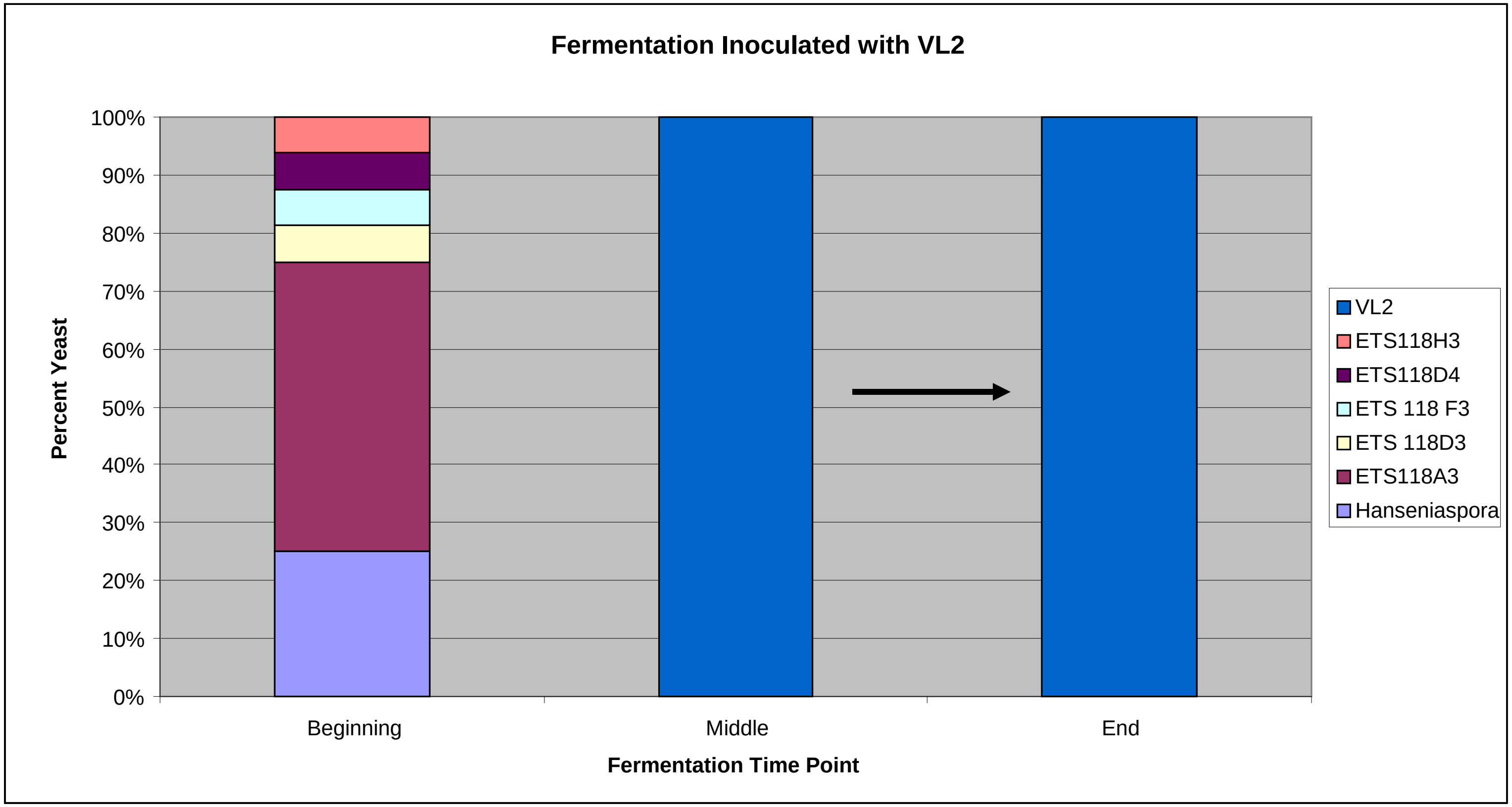


Yeast Strain	Fermentation Sample Point		
	Start	Middle	End
678A1	6%	0%	6%
Merit.Firm	50%	50%	50%
678B2	25%	50%	25%
742B9	6%	0%	6%
862E5	0%	0%	6%
862G6	0%	0%	6%

Fermentation Profile for a Native Fermentation

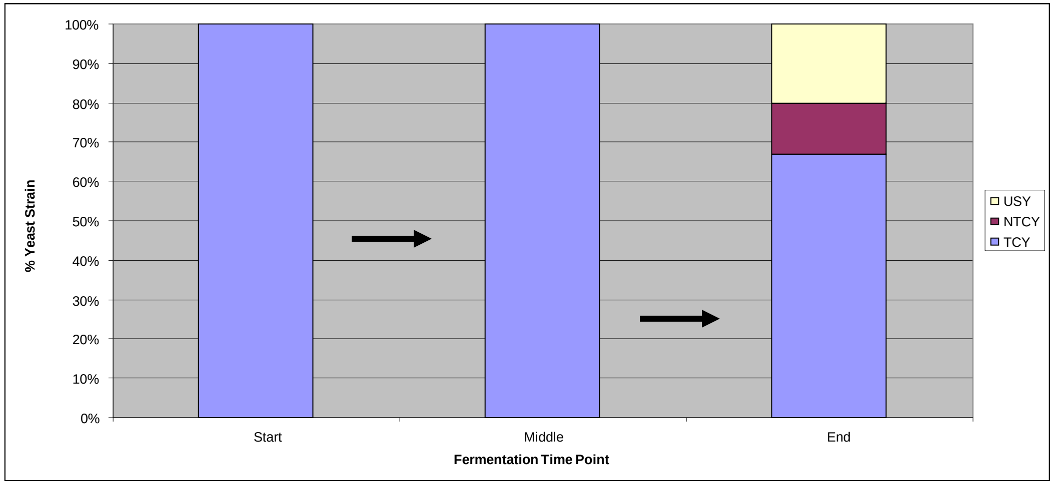


Fermentation Profile for VL2 Inoculated Fermentation

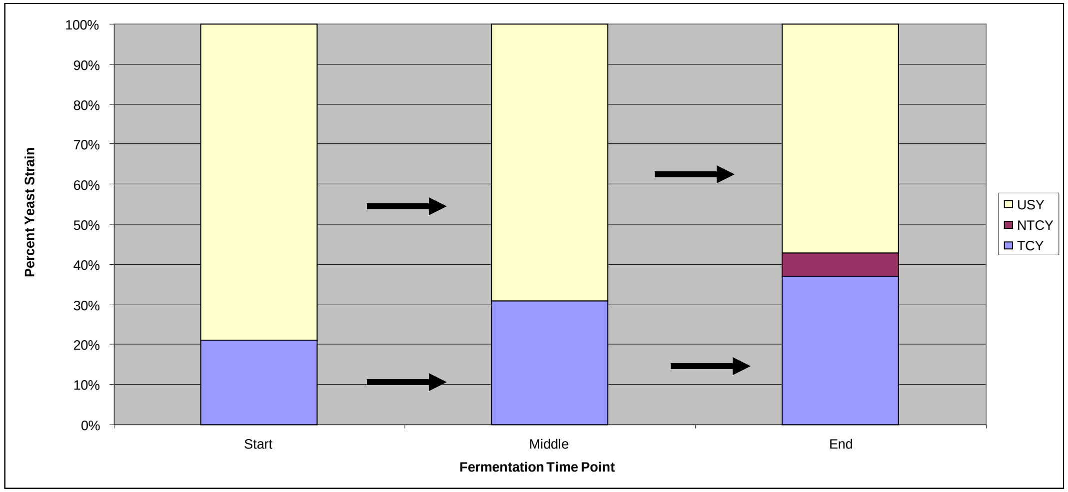


Fermentation Profiles for Two CY3079 Inoculated Fermentation

Tank A



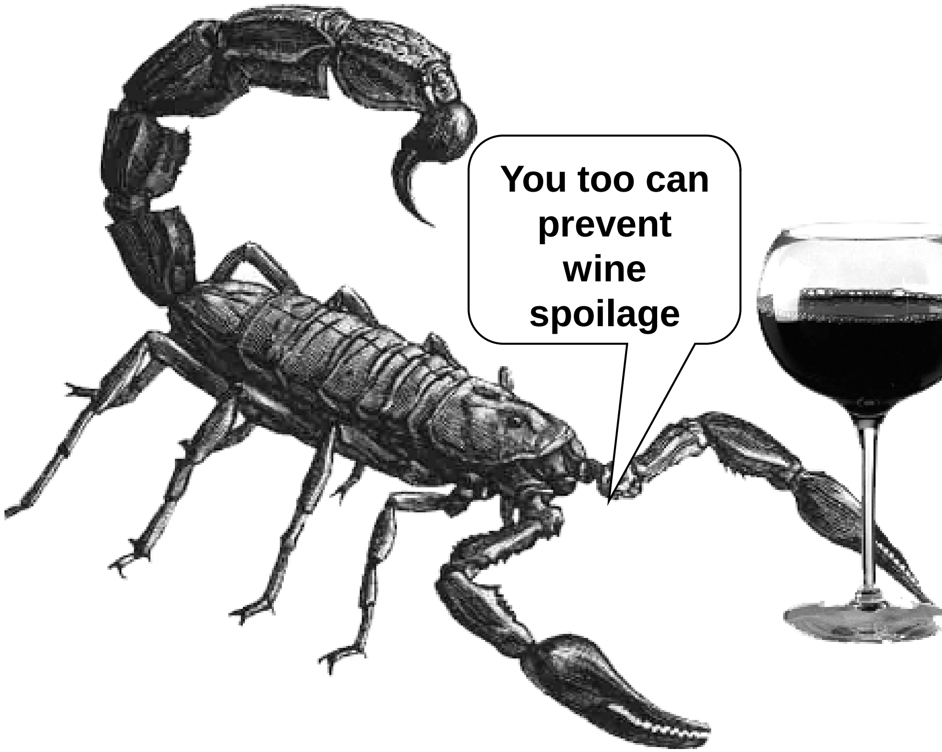
Tank B

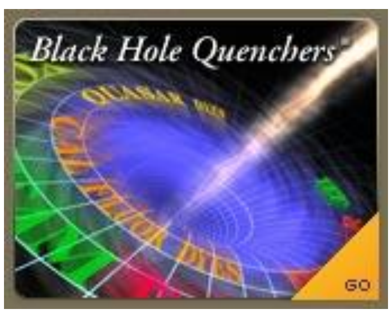


Why Use VNTR Analysis?

- Monitor the populations of beneficial microbial strains in fermentations
  - monitor yeast populations in native fermentations
  - determine the efficacy of inoculations with commercial strains
- Strain selection can be based upon their ability to provide a specific wine style
- Can also be used as a quality control tool to verify that yeast strains are dominating the fermentation, resulting in more consistent fermentations

Thank You





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