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# Yeast in Sparkling Wines

Amateur Winemakers of Ontario

Sippers Symposium

June 24 2023

Hamilton, ON

# Escarpment Labs: Who Are We?

- Based in Guelph, ON
- We believe **fermentation should be easy**
- Focus on **knowledge, trust, and community**
- (mostly) beer yeast liquid cultures



1. What is different about traditional method sparkling wine making?
2. What traits does a sparkling wine yeast need to have to be successful?
3. Selecting yeast for sparkling wine
4. Engineering yeast for sparkling wine
5. Getting creative with sparkling wine yeast
6. Possible directions for the future

It looks like there was a prior presentation on general Sparkling Wine methods by Kevin Panagapka for Amateur Winemakers of Ontario

<http://www.makewine.com/winemaking/#winemaking-advanced>

## AWO Sparkling Wine Seminar

‘Keep it Clean and be Precise’

By Kevin Panagapka

2015-06-06

### Overview

- Equipment
- Base wine production
  - Pressing
  - Phenolic extraction
  - Juice Settling
  - Nutrient addition
  - Storage
  - Filtration
- Secondary Fermentation (Tirage)
  - Starter culture – Build up
  - Tirage Bottling
    - Nutrient, Aduvent, Additives
  - Aging on lees
- Disgorging
  - Dosage preparation

- BSc. Microbiology at University of Guelph
- MSc. Molecular Biology & Genetics at University of Guelph
- Thesis project - accelerated autolysis of sparkling wine
- Collaboration between UoG (George van der Merwe), Niagara College, and CCOVI/Brock U

Applied Microbiology and Biotechnology  
<https://doi.org/10.1007/s00253-018-9304-y>

APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

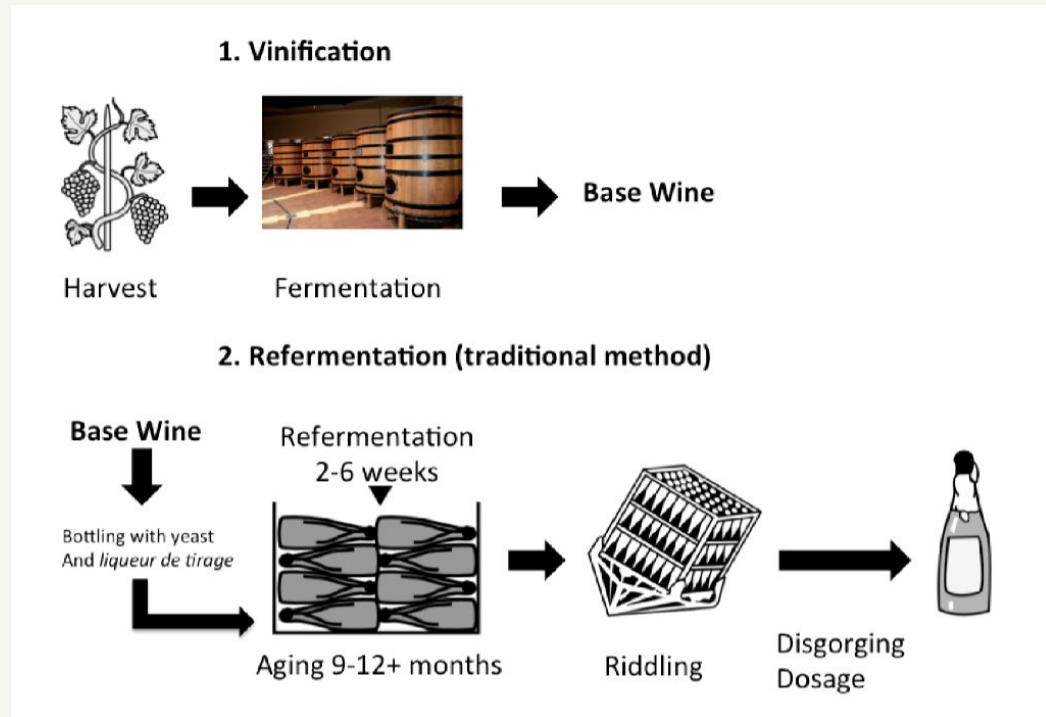


**Autophagy gene overexpression in *Saccharomyces cerevisiae* perturbs subcellular organellar function and accumulates ROS to accelerate cell death with relevance to sparkling wine production**

Richard Preiss<sup>1</sup> · Caroline Tyrawa<sup>1</sup> · George van der Merwe<sup>1</sup> 



- “Traditional method” means that re-fermentation happens in the bottle
- This is the method made famous by Champagne, but also used in Cava and other regions
- Extended aging of yeast lees in contact with wine creates beneficial flavour - **autolysis**











- High acidity (low pH)
- Alcohol ~9-11%
- Low residual YAN
- This is a *stressful* environment for yeast to thrive in!

**Table 1** Compositional analysis of base wine.

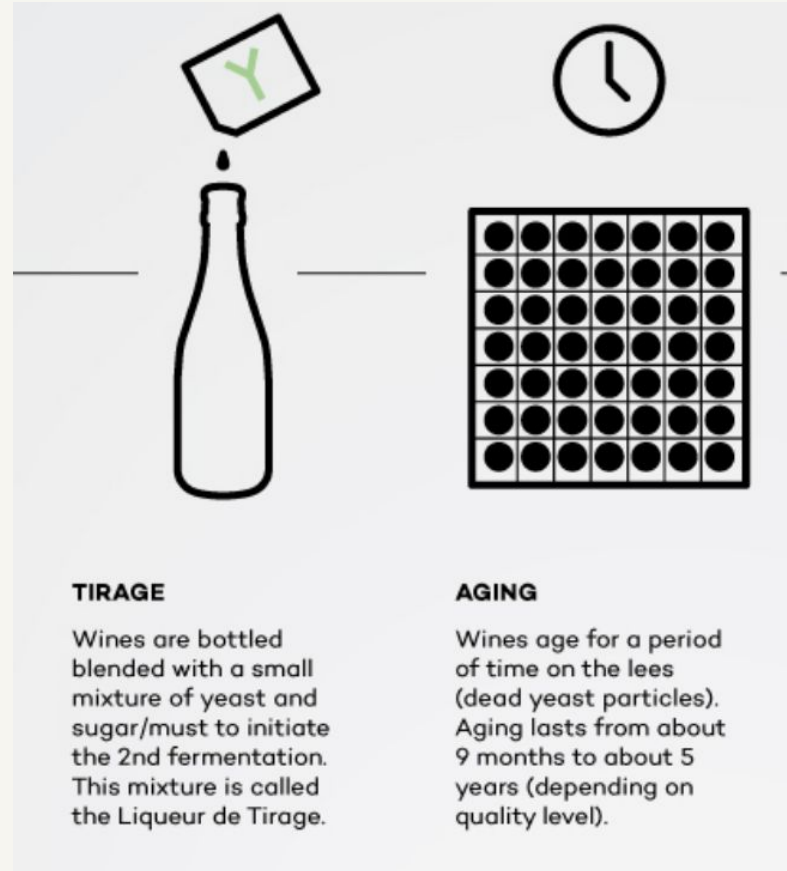
Variable	Mean measured values ± standard deviation
pH	3.11
Titrateable acidity (g/L Tartaric)	8.8±0.2
Ethanol %	10.7±0.1
Amino Acids (mg/L)	54.3±1.6
Ammonia (mg/L)	11.5±0.7
Protein (mg/L)	241±26
Free SO <sub>2</sub> (mg/L)	10.2±0.3
Total SO <sub>2</sub> (mg/L)	62.8±0.1
Total Residual Sugar (g/L)	2.8±0.1
Total Residual Sugar (g/L)*	24.0±0.1

\* Indicates Total Residual Sugar after chaptalization in preparation for secondary fermentation

1. Experimenting with terroir - Ontario cold climate is suited to production of high quality sparkling wines
  - a. Especially Chardonnay
2. Gluttons for punishment
3. Seriously, if you like to do a lot of extra work when you make your wine - this is the wine for you.



- Yeast must overcome:
  - high acidity (low pH)
  - Alcohol ~9-11%
  - Low residual YAN
- How do we do it?
  - We “train” the yeast
  - This is known as “tirage”



1. Take a "pinch" of rehydrated dried yeast, or 5 mL of liquid yeast and pitch into 100 mL of **YPD + Go-Ferm Media**. Grow for ~24 hours on a stir plate.
2. Transfer the starter into 100 mL of **Adaption Media 1**. Grow for ~24 hours on a stir plate.
3. Transfer the starter into **Adaption Media 2** (whatever volume is needed for batch size). Grow for ~24 hours without stirring.
4. Inoculate ~1 million cells per mL into wine that is being carbonated, along with priming sugar.

Each adaptation step is “stronger” in terms of acid and alcohol. This helps adapt the yeast to the harsh environment.

## **YPD + Go-Ferm Media (boiled or filter sterilized)**

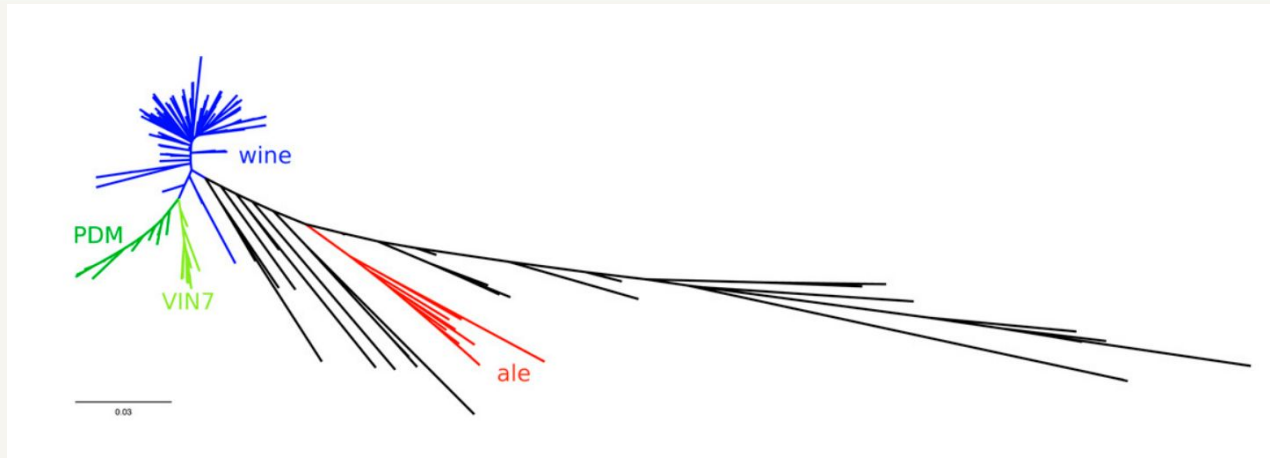
- 0.02375 g/L Go-Ferm
- 10 g/L yeast extract
- 20 g/L peptone
- 20 g/L dextrose

## **Adaption Media 1 (boiled or filter sterilized)**

- 10% base wine
- 20 g/L sucrose
- 0.02375 g/L Go-Ferm

## **Adaption Media 2 (boiled or filter sterilized)**

- 70% base wine
- 20 g/L sucrose
- 0.2 g/L Fermaid



- Most yeasts aren't up to the job
- Enter the “Prise de Mousse” (PDM) genetic family
- Interesting genetic features including genes from other species

Example strains:

LVCB, DV10, Elegance, 4F9, Rhone4600, EC1118, QA23, N96, IOC18-2007, PDM, and PC

PDM clade has 34 non-standard genes obtained from other yeast species. Help confer stress resistance, sulfite tolerance, and more!

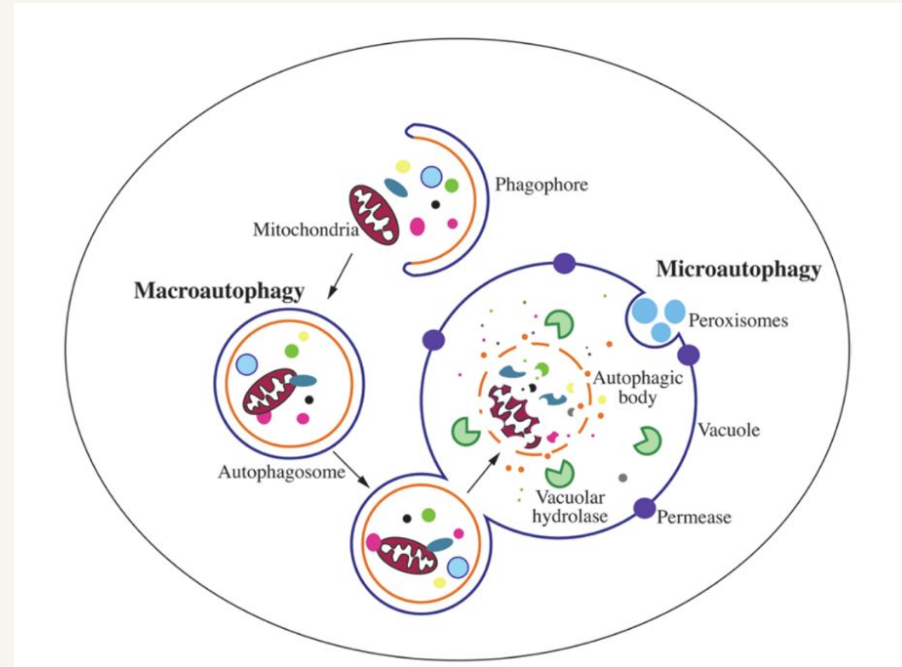


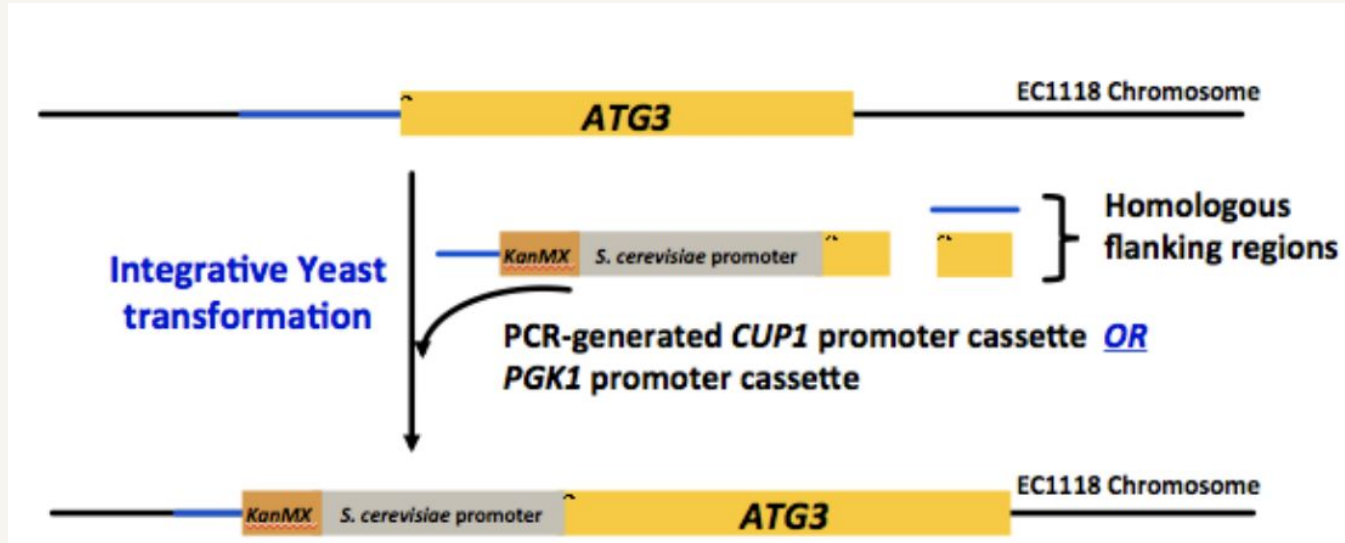
- The challenge:
  - Industry would benefit from accelerated aging characteristics
  - We need yeast to complete re-fermentation, then die fast
  - Faster death = faster autolysis = tastier wine faster on a commercial scale
- The strategy:
  - Disrupt gene(s) that isn't needed during fermentation but is needed for post-ferment survival





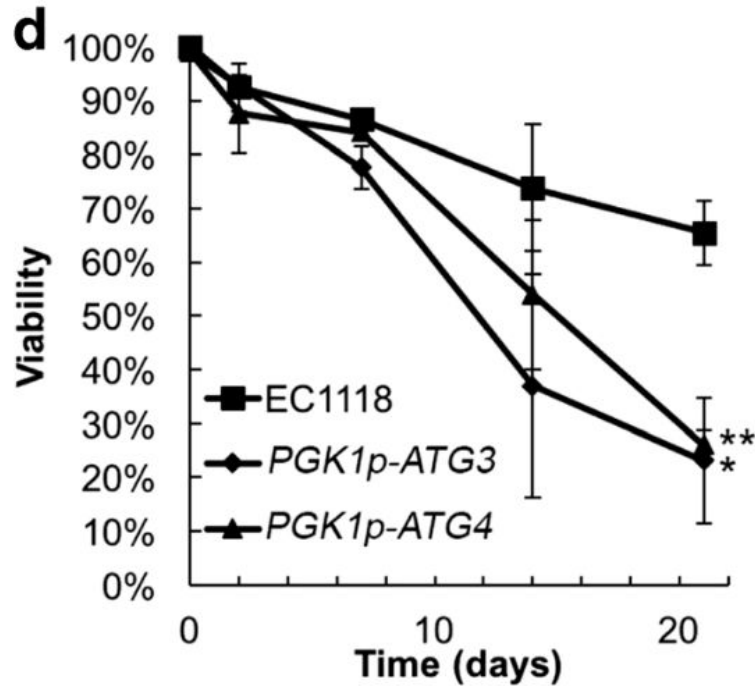
- Autophagy (“self-eating”) is the yeast cell’s recycling system
- Needed to survive starvation (e.g. low YAN)
- Deleting these genes makes yeast ferment poorly ❌
- Over-expressing autophagy genes disrupts this system and makes yeast die faster ✅





*Expression of genes was changed  
by replacing the gene promoter*

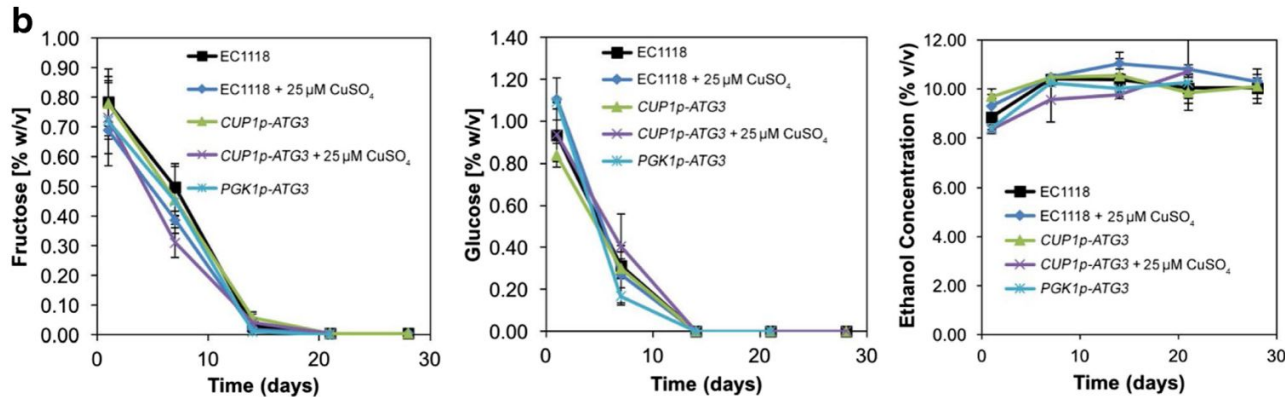
*Promoters are the “advertising  
system” of gene expression*



*EC1118 with overexpression of autophagy genes dies faster in nitrogen starvation*

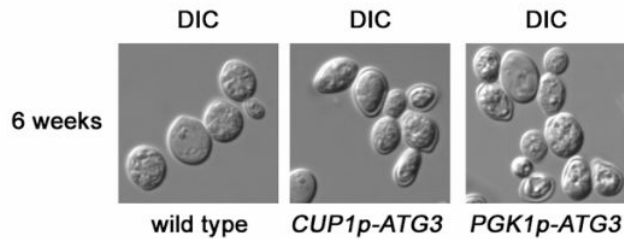
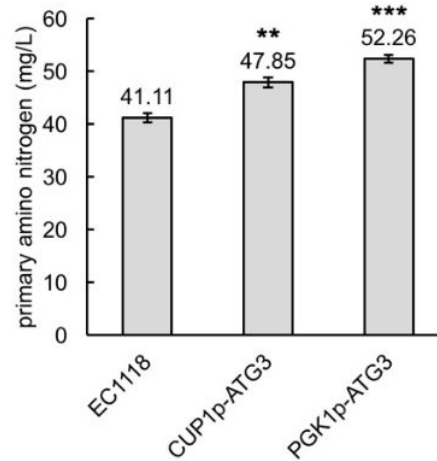
**a**

Genotype	YPD	AM1	AM2
EC1118	100%	93.9% +/- 1.32%	90.4% +/- 3.43%
EC1118 <i>CUP1p-ATG3</i>	100%	93.8% +/- 1.23%	93.1% +/- 0.63%
EC1118 <i>PGK1p-ATG3</i>	100%	89.6% +/- 2.45%	85.9% +/- 4.48%

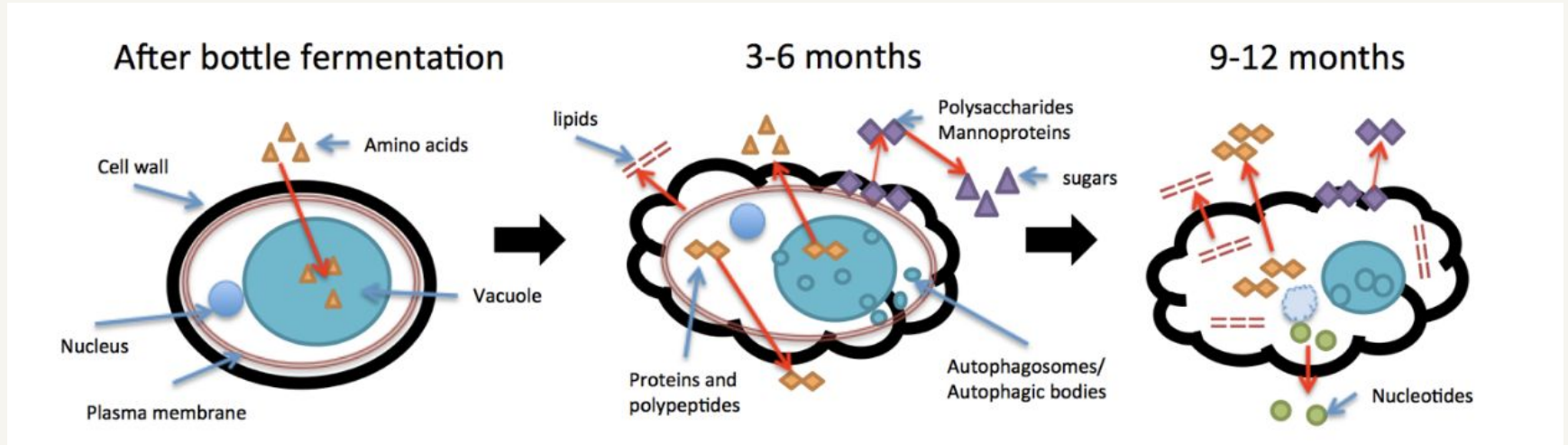


***Modified EC1118 with autophagy gene overexpression completes sparkling wine refermentation without problems***



**A**

**B**
**9 months sur lie aging**


***Modified EC1118 with autophagy gene overexpression degrades faster and releases primary amino nitrogen faster***



**Proteins/polypeptides** contribute to foam and body

**Polysaccharides** contribute to texture and body

**Mannoproteins** contribute to foam and body

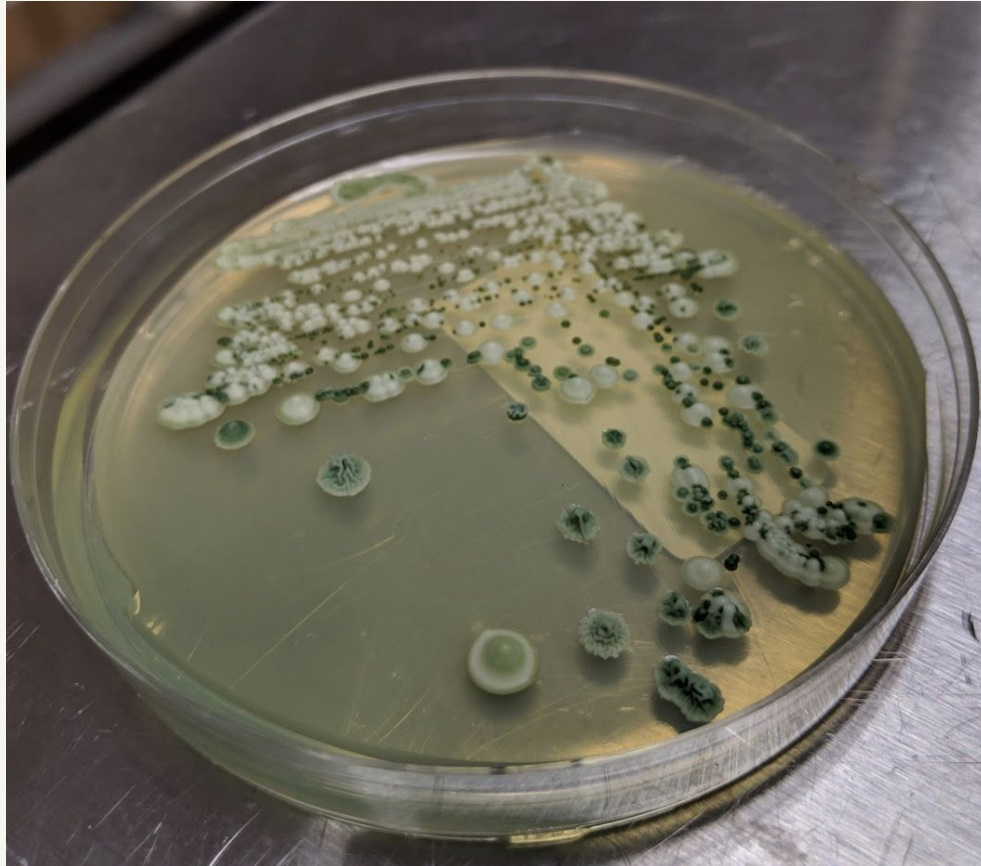
**Amino acids and polysaccharides** contribute to flavour (brioche/toast - Maillard reactions)

**Nucleotides** contribute to flavour - umami

- Grant funding ran out
- Never commercialized
- Wine industry less receptive to genetic engineering/GMO than beer industry
- Even though PDM yeasts are already naturally transgenic (has genes from other species)
- So I mostly focus on beer...



**Interesting yeasts**



**Are everywhere!**



- Small project with commercial partner in Niagara region
- Isolated from “spontaneous” wine/no added yeast
- Selected yeast from wine fermentations and tested in Chardonnay base wine refermentation
  - Slower but with interesting taste notes

**20 g/L sugar dosed into  
bottle fermentations**

<b>Strain</b>	<b>R.S. 4 weeks</b>	<b>R.S. 8 weeks</b>	<b>Tasting notes</b>
EC1118 (control)	5.0 g/L	0.1 g/L	neutral
#1545	15 g/L	4.8 g/L	Light brioche
#1546	13 g/L	4.3 g/L	neutral
#1547	10 g/L	2.6 g/L	Ginger, apple, pear



## Philosophic moment: do we have to make wine with wine yeast?

- Some commercial wine yeasts are not genetically wine yeasts
  - E.g. 71B closer to bread yeast (1)
- Beer strains can be used in wine making if they have enough alcohol/pH tolerance
- We have used beer yeasts in a variety of wines with success!



Saison yeast +  
Riesling or  
Chardonnay



Thiol Libre +  
Sauvignon Blanc

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Also check out our Knowledge Base  
on our website!