A locally isolated yeast that consumes acetic acid.

Is there application for this yeast in wine production for Icewine, appassimento wine, sour rot infected fruit, and sparkling wine?

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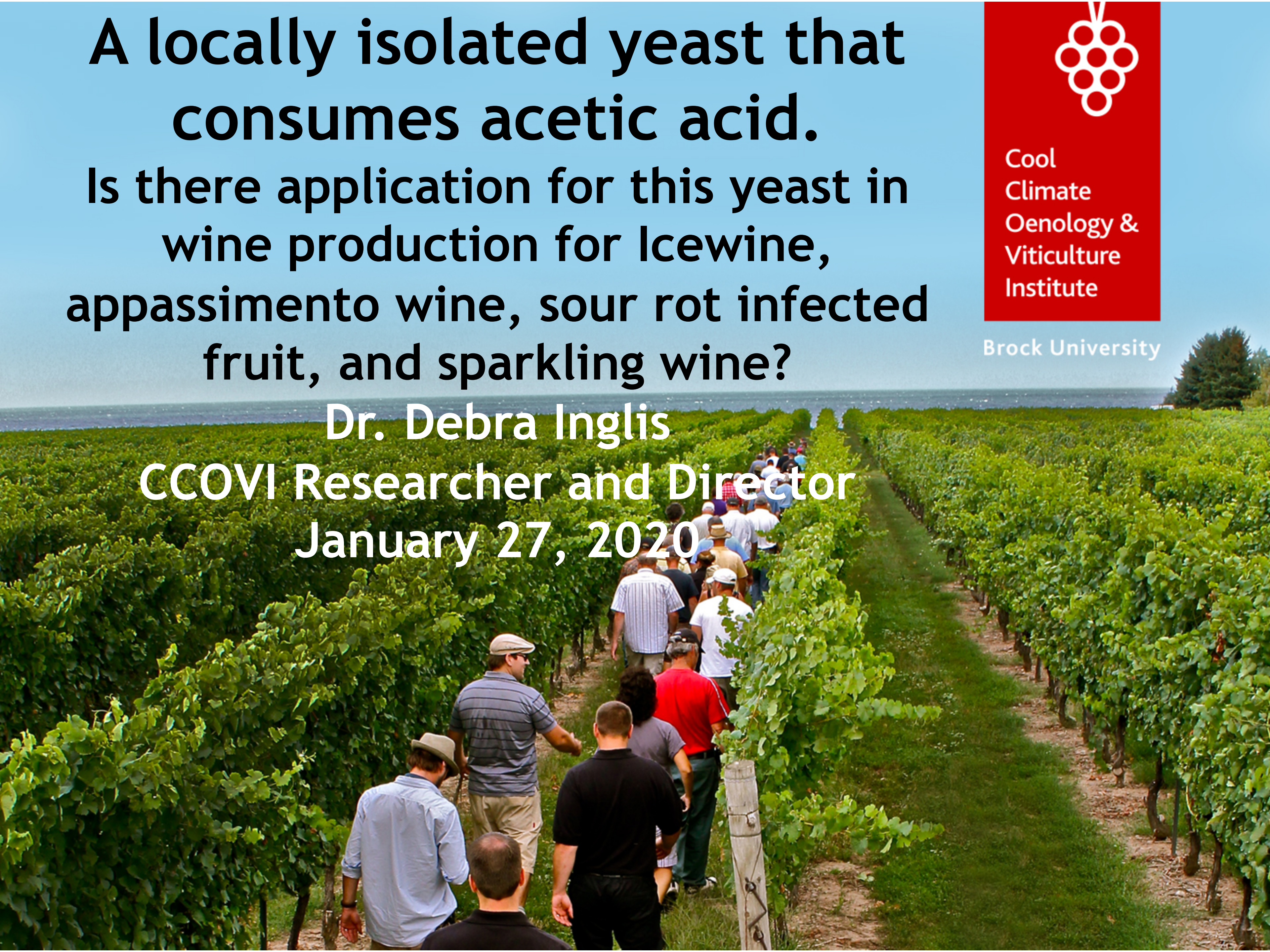
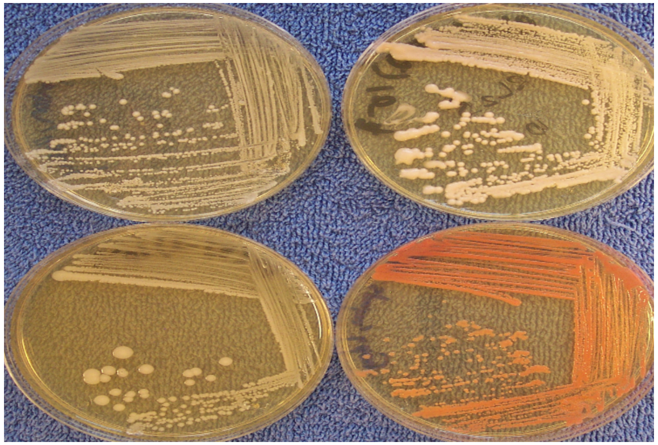


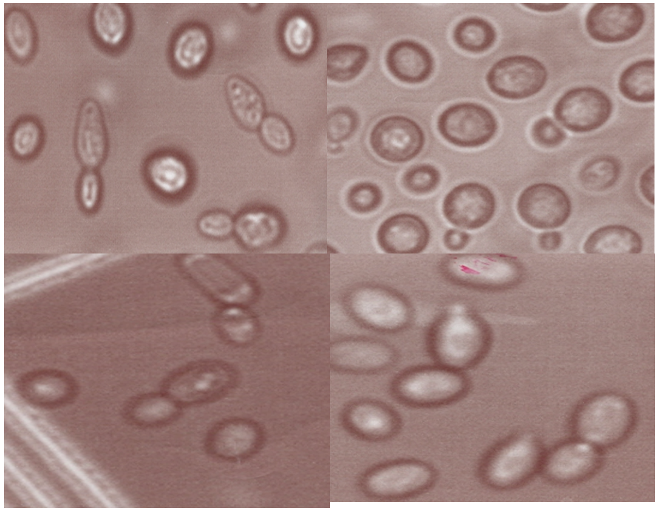
Characterization of a locally isolated yeast

- Started this project 15 years ago
- There was little information about the diversity of yeasts and their impact on flavour and aroma compounds associated with Icewine produced in the Niagara Region, ON, Canada.
- We embarked on a project to isolate yeast from Riesling and Vidal and characterize their fermentation performance
- Post-doctoral Fellow Dr. Canan Nurgel, Dr. Gary Pickering and Dr. Debra Inglis

Isolated yeast from Riesling and Vidal Icewine grapes

- Yeast were isolated from the juice of Riesling and Vidal Icewine grapes and also throughout spontaneous fermentations by spreading samples onto agar plates
- The various colony shapes were separated, colony colours were noted, colonies were coded and representative colonies were isolated
- Morphology of cells were determined under microscope
- Initial identification was carried out using API identification kits (Biomerieux, France) and biochemical tests (Barnett *et al*., 2000)
- Later, identification was verified using molecular methods



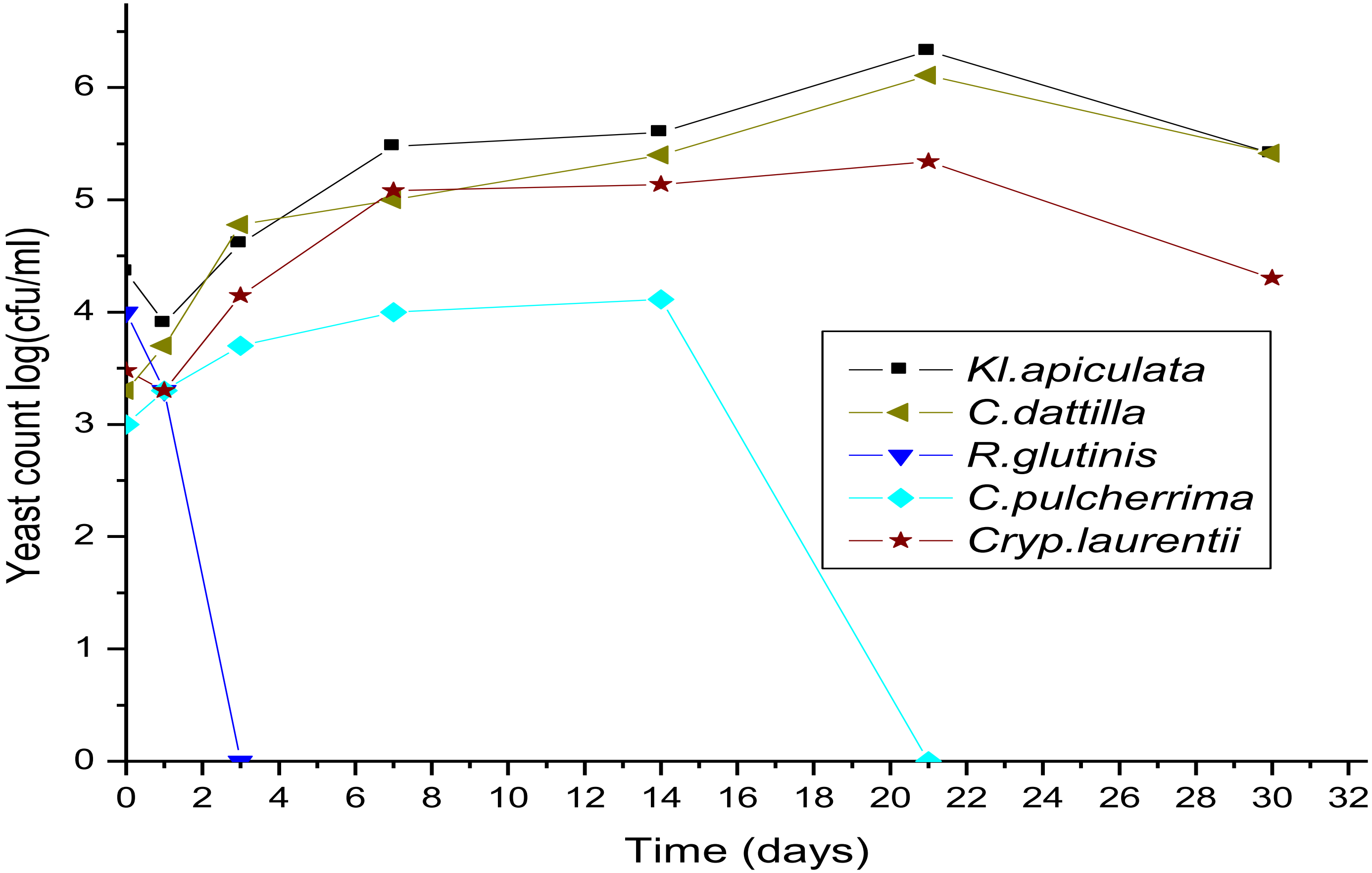


Yeast isolated from Riesling Icewine grapes

Isolate # from Riesling Icewine grapes	**Yeast Identification via API kit (BioMerieux, France)**
1	*Candida dattila*
2	*Candida pulcherrima*
3	*Kloekera apiculata*
4	*Cryptococcus laurentii*
5	*Rhodotorula glutinis*

Nurgel, C., Inglis, D.L., Pickering, G.J., Reynolds, A., and Brindle, I. (2004) Dynamics of Indigenous and Inoculated Yeast Populations in Vidal and Riesling Icewine Fermentations.   Am. J. Enol. Vitic. 55: 435A.

Riesling Icewine yeast flora during spontaneous fermentation
(identification based on biochemical tests)



Nurgel, C., Inglis, D.L., Pickering, G.J., Reynolds, A., and Brindle, I. Dynamics of Indigenous and Inoculated Yeast Populations in Vidal and Riesling Icewine Fermentations. Presented at the American Society of Enology and Viticulture Eastern Section 29th Annual Meeting, Roanoke, Virginia, July 2004.

Further Yeast identification from Riesling Icewine grapes

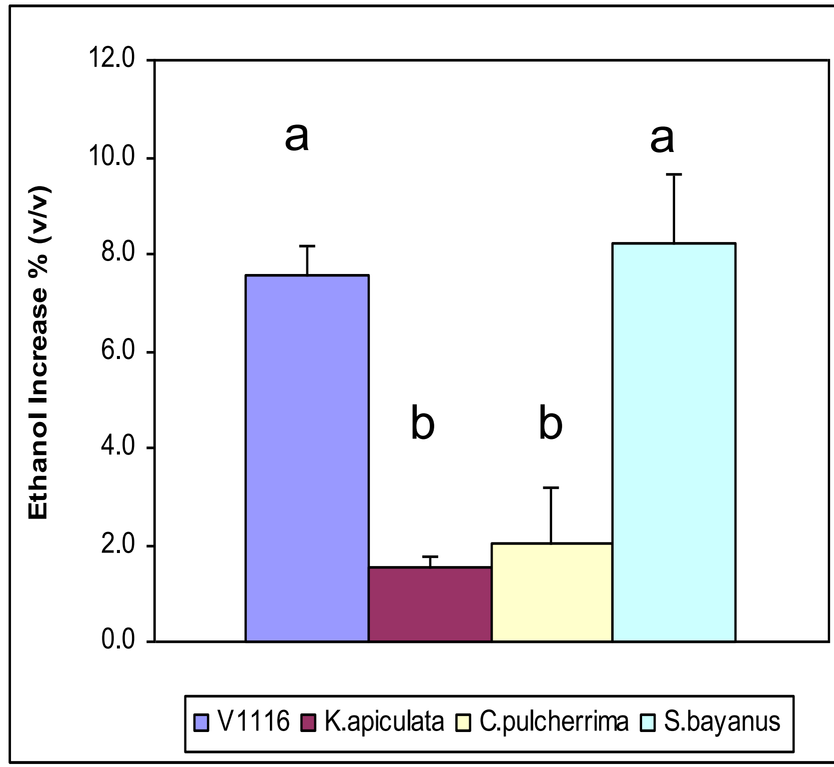
(Canan Nurgel, Jamie Quai, Shiri Sauday, Ai-Lin Beh, Jen Kelly)

Isolate # from Riesling Icewine grapes	Classical methods	Analysis of the 5.8S rRNA-ITS region		DNA Sequencing of *B-tubulin* and *COXII* genes
	API (BioMerieux, France)	PCR-RFLP	DNA sequencing	
1	***Candida dattila***	***Saccharomyces bayanus, Saccharomyces pastorianus***	***S. pastorianus S. bayanus***	***S. bayanus****
2	*Candida pulcherrima*	*Metschnikowia pulcherrima*	*M. pulcherrima*	
3	*Kloekera apiculata*	*K. apiculata/ Hanseniaspora uvarum*	*H. uvarum*	
4	*Cryptococcus laurentii*	*Cr. magnus Cr. ater*	*Cr. magnus Cr. ater*	
5	*Rhodotorula glutinis*	*R. glutinis*	*R. glutinis*	

*Kelly J, Yang F, Dowling L, Nurgel C, Beh A, Di Profio F, Pickering G and Inglis D. (2018). Characterization of *Saccharomyces bayanus* CN1 for Fermenting Partially Dehydrated Grapes Grown in Cool Climate Winemaking Regions. Fermentation. 4(77): 1-13.

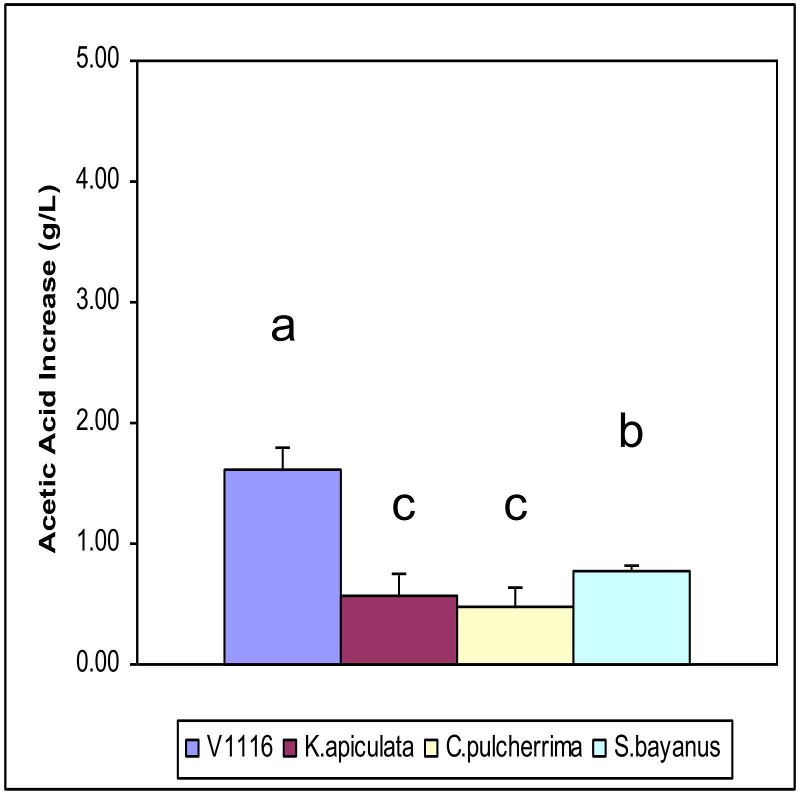
Initial Icewine Fermentations 40˚Brix, sterile-filtered Riesling Icewine juice-27 days (J. Quai, 2006)

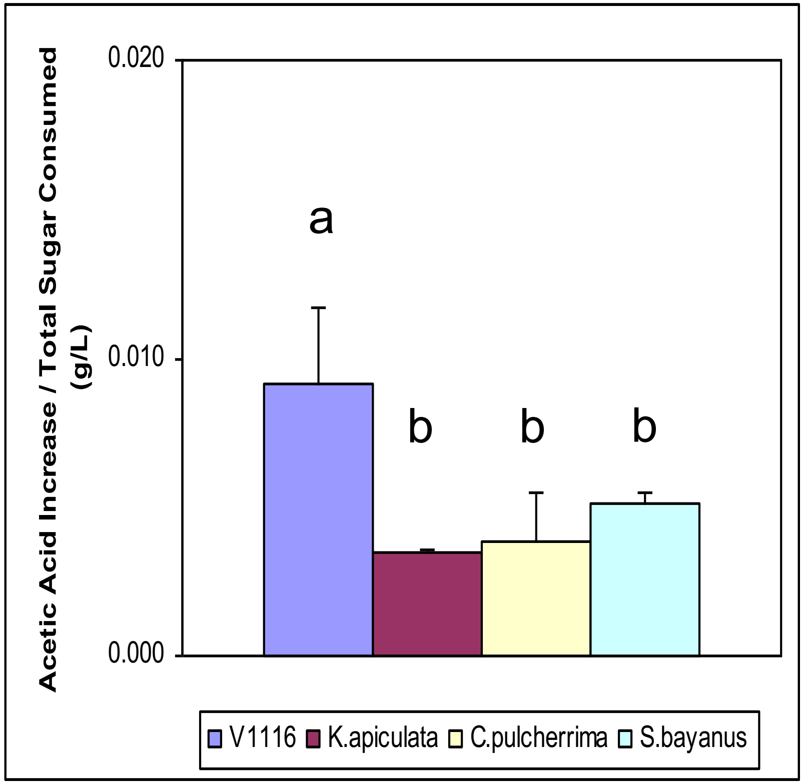
Parameter	Riesling Icewine Juice Value
Soluble Solids (ºBrix)	40.2±0.2
Titratable Acidity (g/L)[1]	9.1±0.1
pH	3.27±0.00
Amino Acids (mg N/L)	507±12
Ammonia (mg N/L)	124±10
Total YANC (mg N/L)	632±22
Acetic Acid (g/L)	0.12±0.00
L-Malic Acid (g/L)	4.7±0.1
Glycerol (g/L)	5.7±0.2
Acetaldehyde (g/L)[2]	ND
Succinic Acid (g/L)	0.28±0.00
Ethanol (%v/v)[2]	ND



S. bayanus produced comparable ethanol to commercial yeast K1-V1116

Acetic Acid Production during Riesling Icewine Fermentation (J. Quai, 2006)





S. bayanus isolate produced significantly less acetic acid during the fermentation, even after normalizing for the amount of sugar consumed

Focus on *S. bayanus* isolate

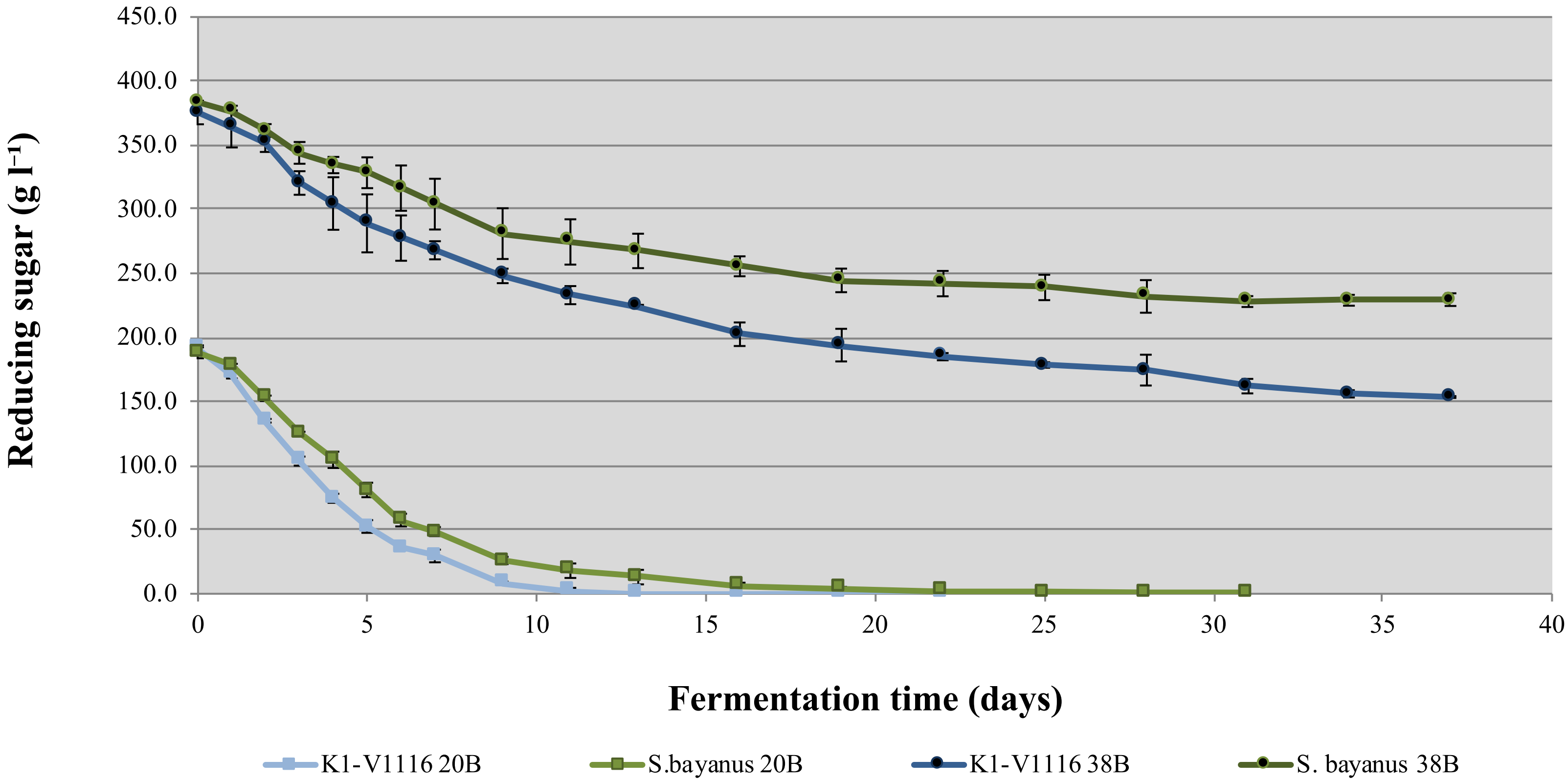
- Based on fermentation performance in Icewine juice, future experiments focused on the *S. bayanus* isolate
  - Comparable fermenter to commercial yeast strain K1-V1116
  - Low producer of acetic acid

- Next step: Compare performance of *S. bayanus* isolate to commercial yeast under Icewine and table wine conditions
  - Vidal juice
  - C. Heit, 2011

Vidal Icewine juice (38°Brix) and Diluted Vidal juice (20°Brix) (Heit, 2011)

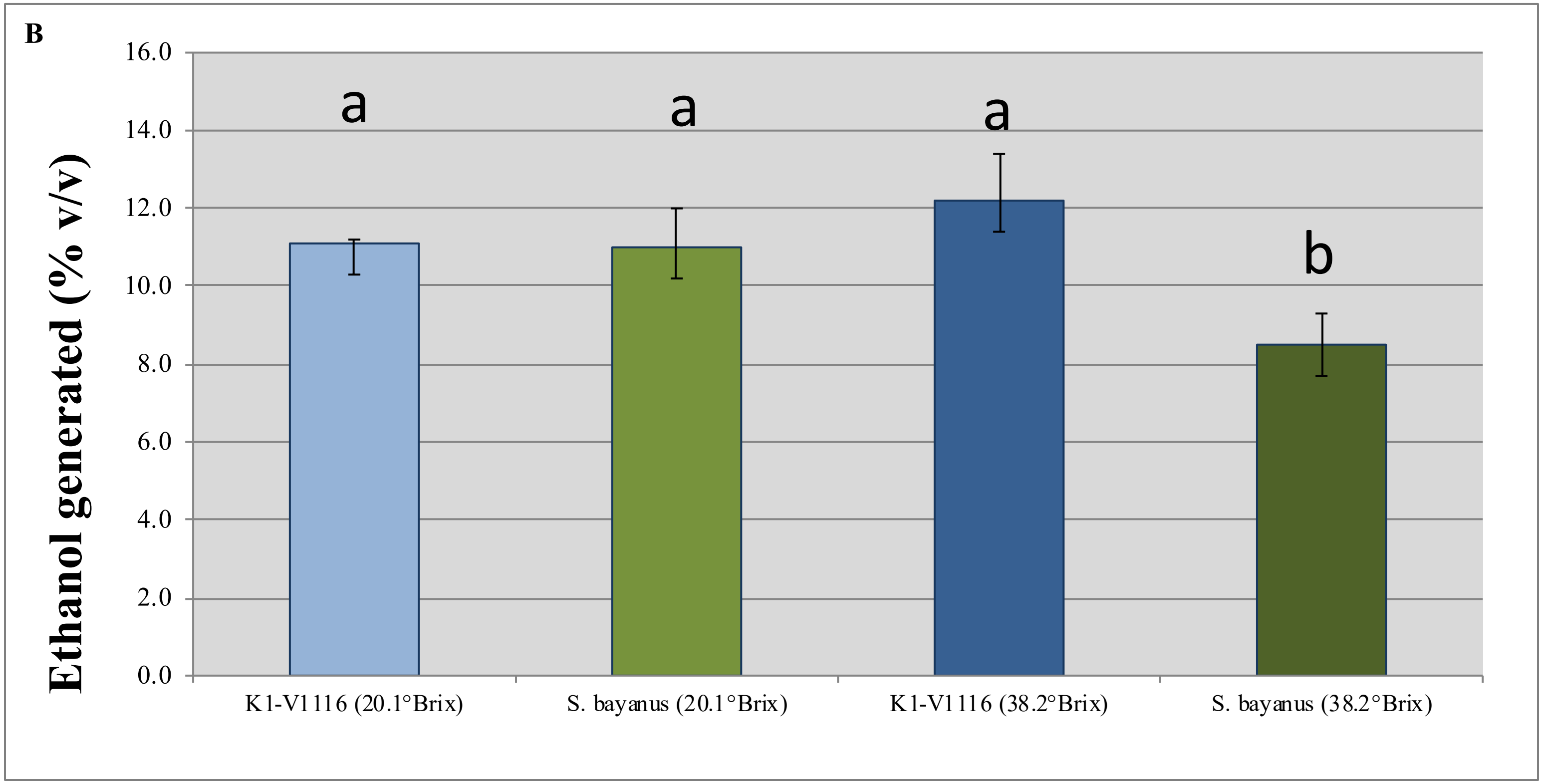
Juice Measurement	38.2°Brix	20.1°Brix
Reducing sugar (g l^{-1})	403 ± 2	201 ± 1
Titratable Acidity (g l^{-1})	11.2 ± 0.0	5.30 ± 0.02
pH	3.33 ± 0.01	3.43 ± 0.01
Ammonia Nitrogen (mg N l^{-1})	87 ± 3	60 ± 1
Amino Acid Nitrogen (mg N l^{-1})	583 ± 0	275 ± 6
YANC (mg N^{-1})	670 ± 3	335 ± 6
Glycerol(g l^{-1})	1.40 ± 0.02	0.7 ± 0.03
Acetaldehyde (mg l^{-1})	6.7 ± 0.0	5.6 ± 0.0
Ethanol (% v/v)	0.2 ± 0.0	0.2 ± 0.0
Acetic acid (g l^{-1})	<0.02 ± 0.00	<0.02 ± 0.00
Ethyl acetate (mg l^{-1})	0 ± 0	0 ± 0

Sugar consumption during Icewine and table wine fermentation K1-V1116 vs *S. bayanus* isolate (C. Heit)



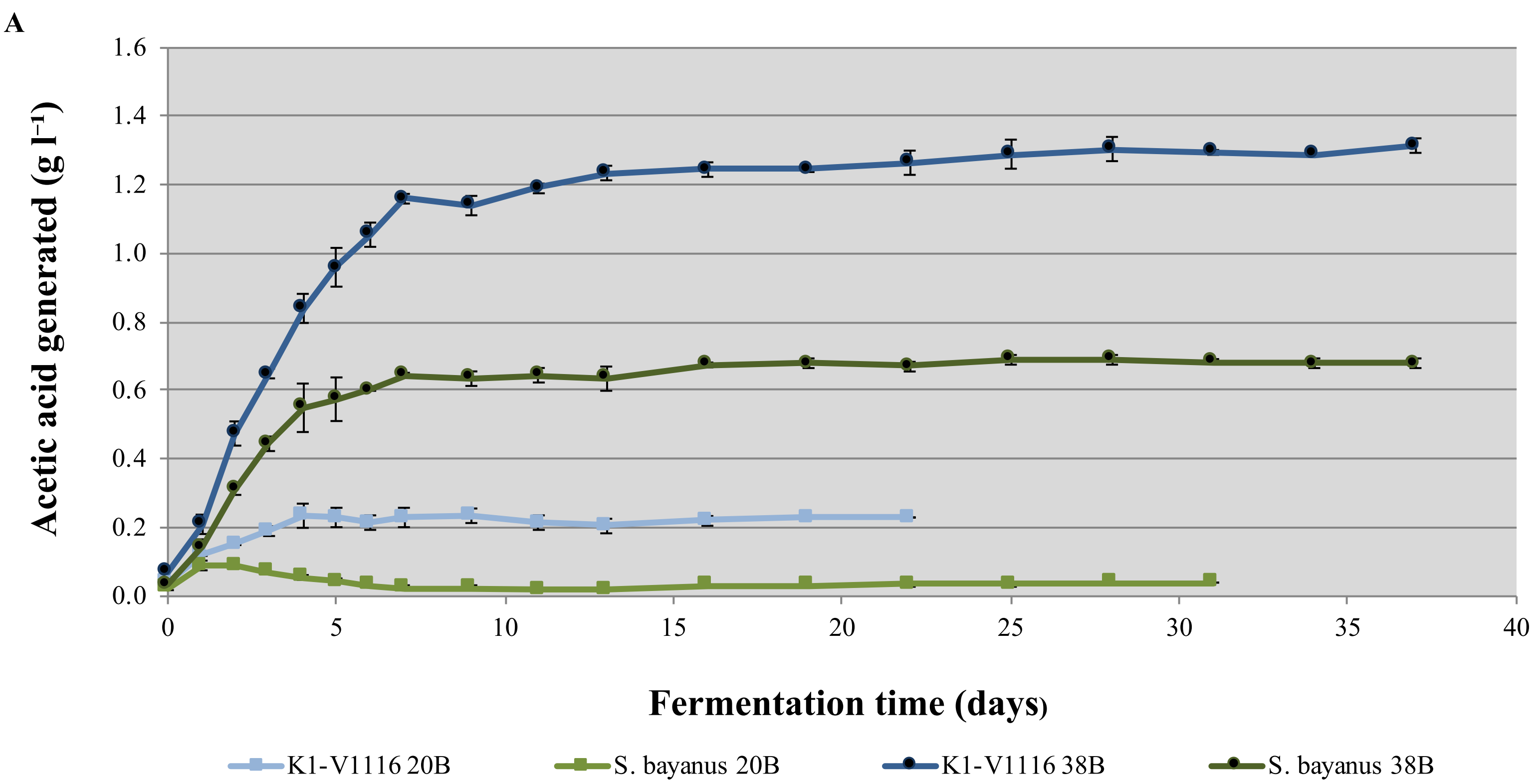
S. bayanus isolate fermented 20ºBrix juice to dryness but lagged behind K1-V1116 in 38ºBrix juice

Ethanol production in Icewine and table wine fermentation K1-V1116 vs *S. bayanus* isolate (C. Heit)



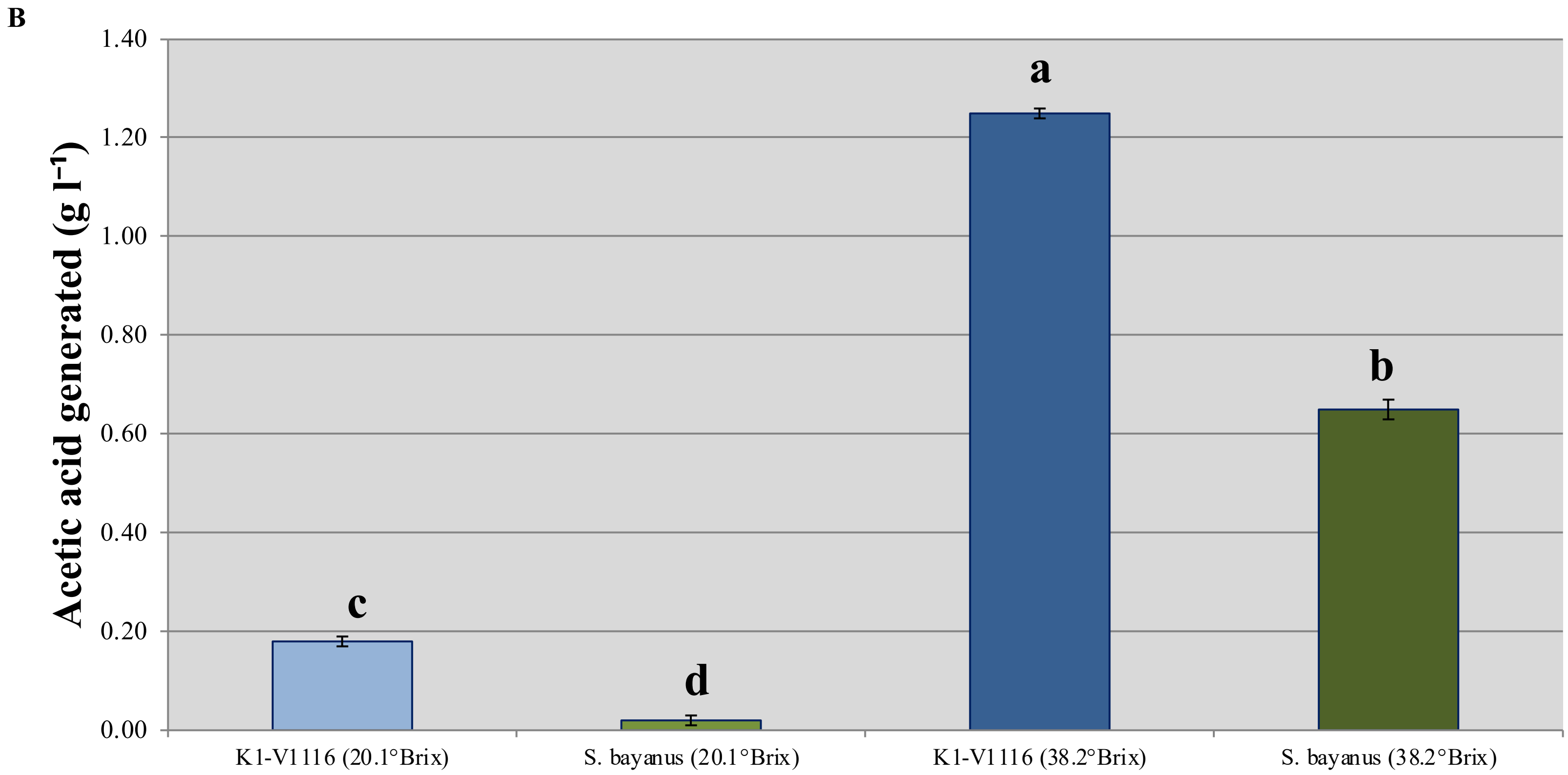
S.bayanus isolate made comparable ethanol from 20ºBrix juice but lower ethanol in Icewine juice (38ºBrix) in comparison to K1-V1116

Acetic acid production during Icewine and tablewine fermentation K1-V1116 vs *S. bayanus* isolate (C. Heit)



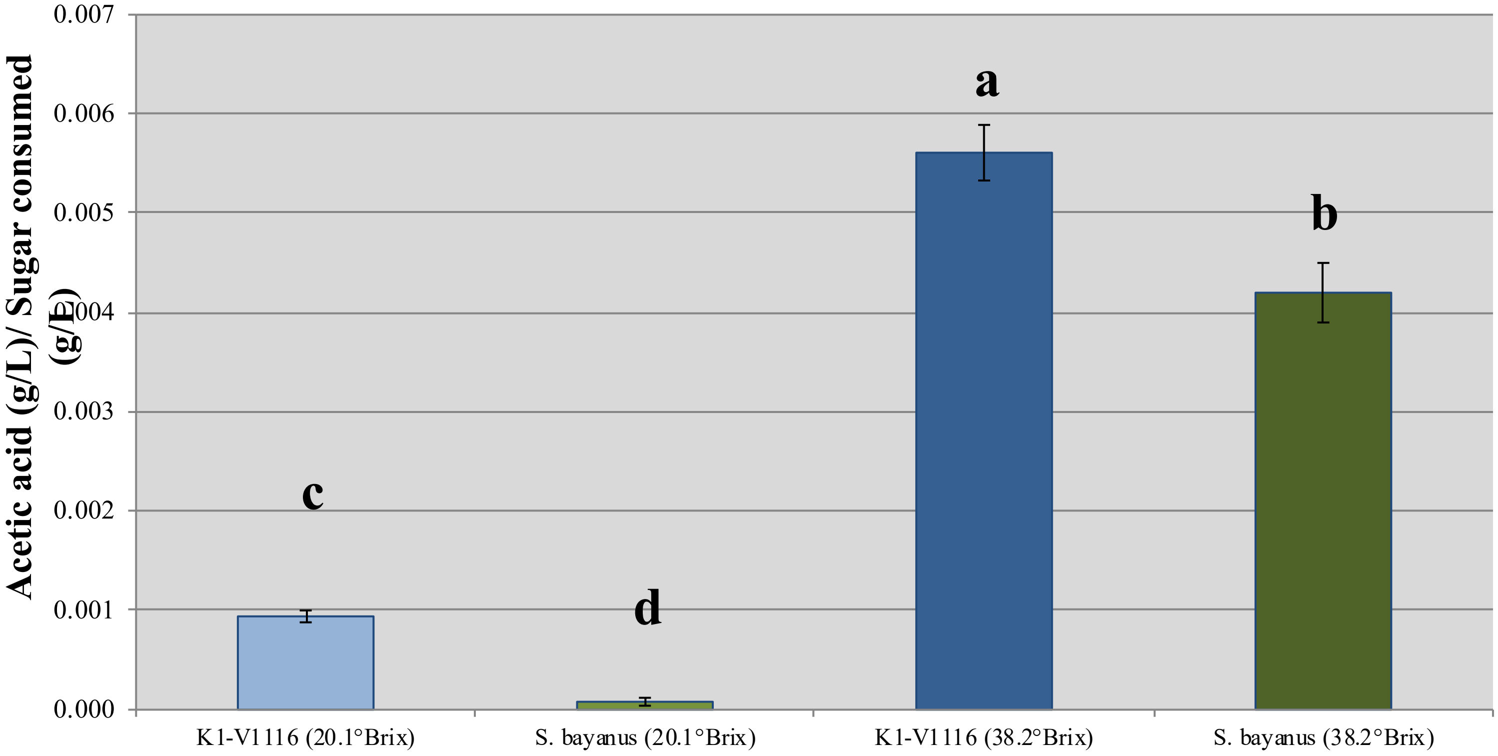
S. bayanus isolate produces less acetic acid and consumes acetic acid in lower Brix fermentations

Acetic Acid in table wine and Icewine K1-V1116 vs *S. bayanus* isolate (Heit, 2011)



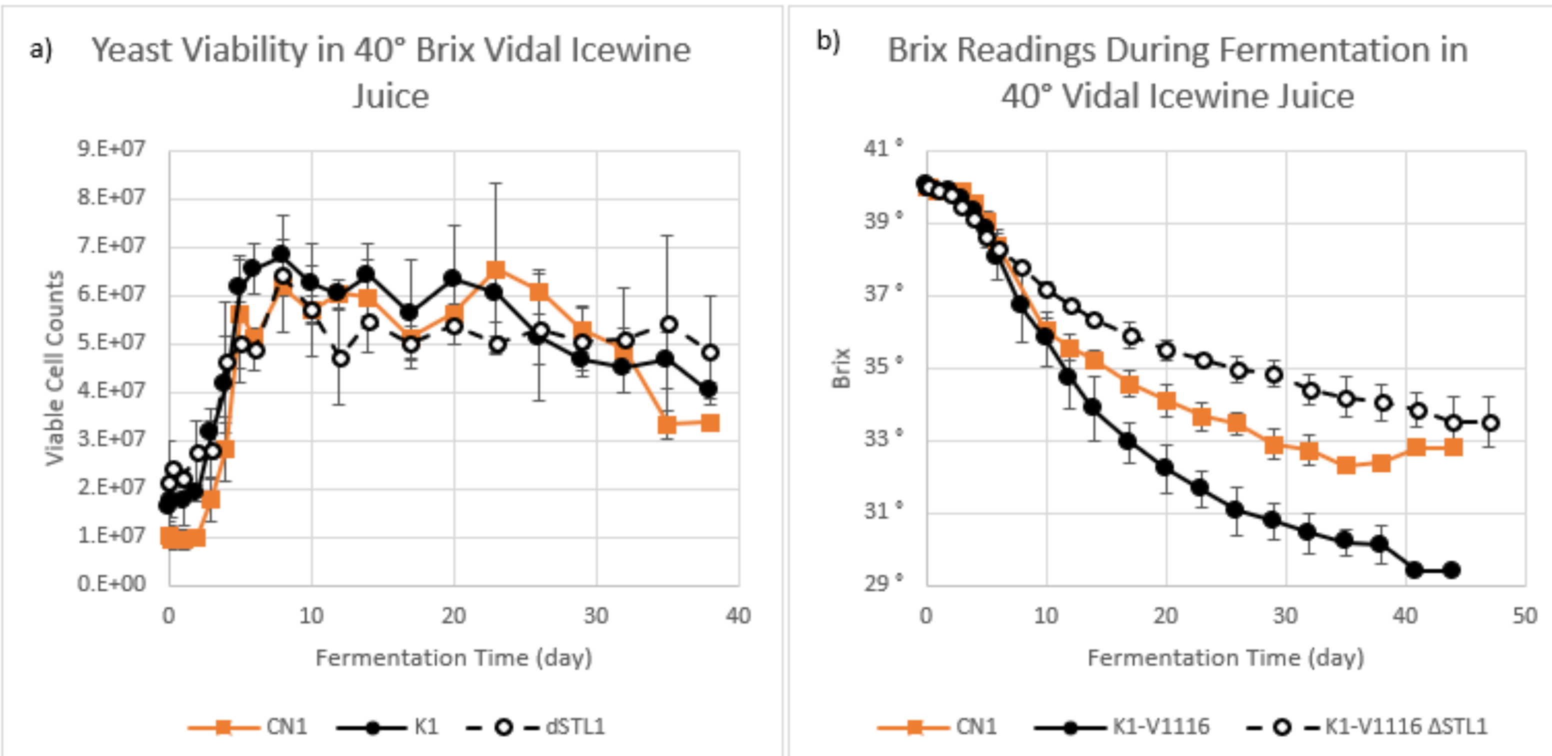
S. bayanus isolate produces less acetic acid from 20ºBrix or 38ºBrix juice compared to K1-V1116

Acetic Acid in table wine and Icewine Normalized to Sugar consumed K1-V1116 vs *S. bayanus* isolate (Heit, 2011)



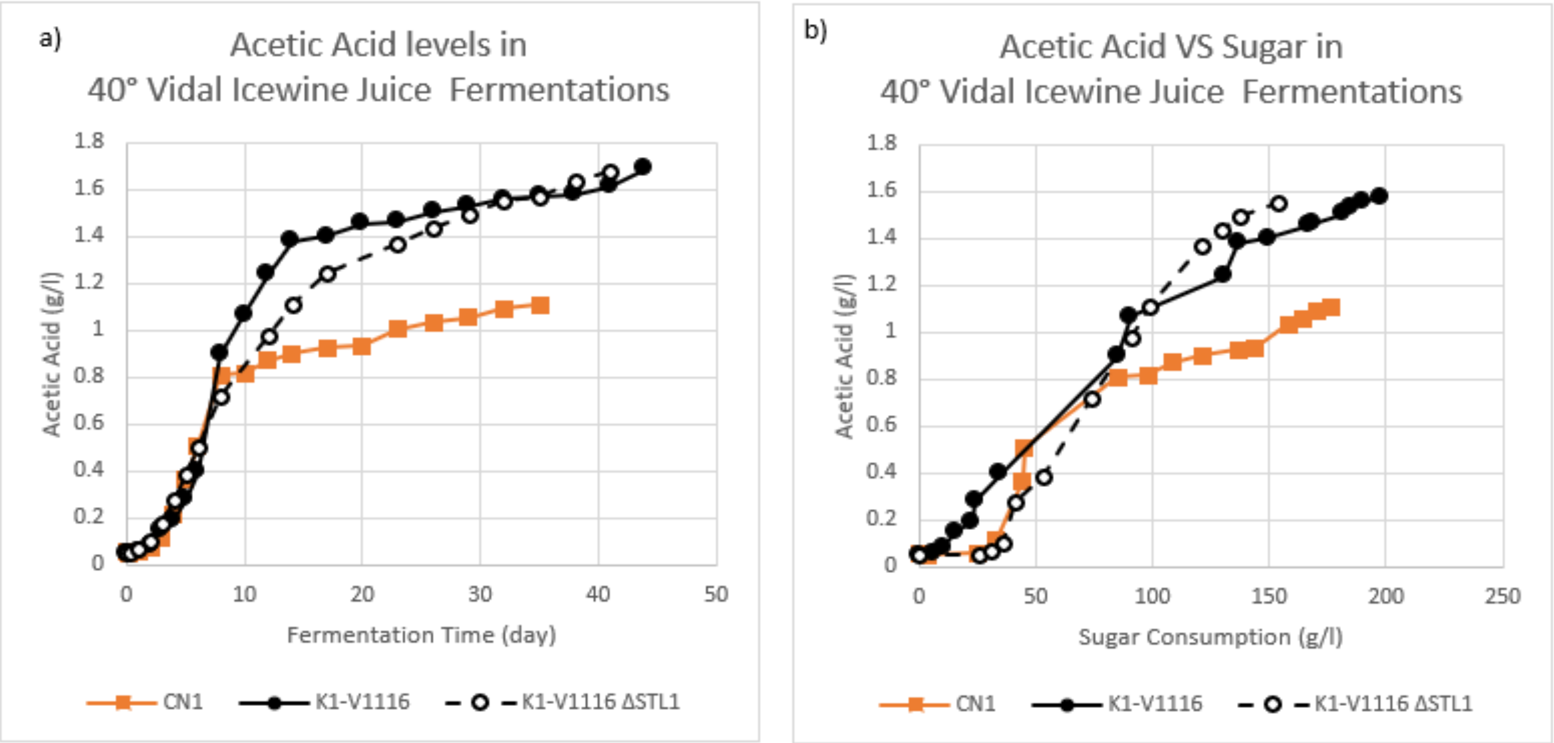
When normalized to sugar consumed, *S. bayanus* isolate still produces less acetic acid from 20ºBrix or 38ºBrix juice compared to K1-V1116 (90% less in low brix fermentation to 25% less in Icewine)

More recent Icewine Fermentations
(R. Allie, 2019)



Yeast Cell Viability and Brix Readings During Fermentation in 40° Brix Vidal Icewine Juice

Low acetic acid production by *S. bayanus* isolate as a function of time and as a function of sugar consumed



Same results as previously reported for Icewine, *S. bayanus* isolate consumes less sugar during Icewine fermentation but produces ~30% less acetic acid when normalized to sugar consumed

Limitations of using *S. bayanus* in Icewine

- **Not as strong a fermenter as commercial *S. cerevisiae* yeast in osmotically stressful conditions of Icewine, typically producing Icewine with ~8-9% v/v alcohol**
- **But the yeast is a significantly lower producer of acetic acid, after normalizing for sugar consumed**
- **Would the *S. bayanus* isolate have an application in other wine styles?**
  - Appassimento wines where sugar is concentrated in the grape up to 28°Brix and starting juice can be high in VA
  - Sour rot infected grapes with high starting acetic acid concentration

Application of *S. bayanus* isolate for other wine styles

-Appassimento wines
-Sour rot infected grapes: red wine
-Sour rot infected grapes: sparkling wine

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Application of *S. bayanus* isolate to Appassimento wines

- **Started working with the industry in 2011 on appassimento wines**
- **Grapes dried post harvest to further ripen fruit off-vine**
  - full bodied, red wines, higher in alcohol (>14% v/v alcohol)

Challenges to overcome

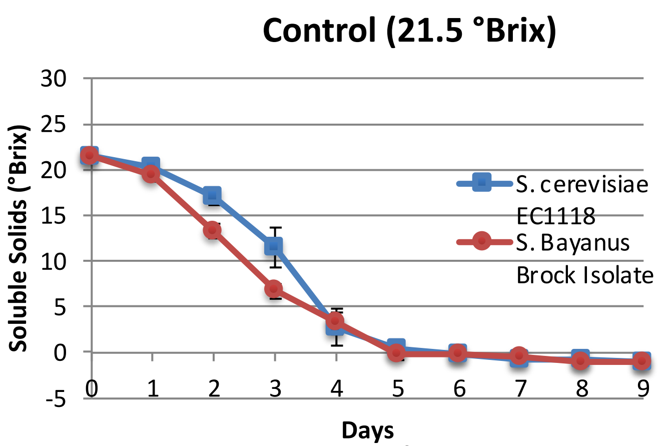
- **Target of 28°Brix for upper sugar level**
  - Becoming increasingly osmotically stressful for yeast, leading to higher volatile acidity (acetic acid) in wine
- **Oxidation faults in wine**
  - oxidation compounds increase during grape drying, resulting in higher starting volatile acidity in fruit, oxidation faults in wine (acetic acid, acetaldehyde and ethyl acetate)
  - botrytis development on fruit during drying, leading to further VA in the fruit, flavour changes in wine

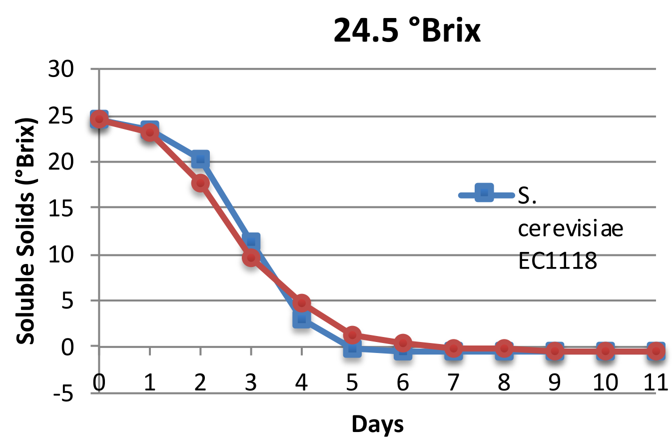
Is there a role for *S. bayanus* isolate in Appassimento wines?
(Dr. Jennifer Kelly)

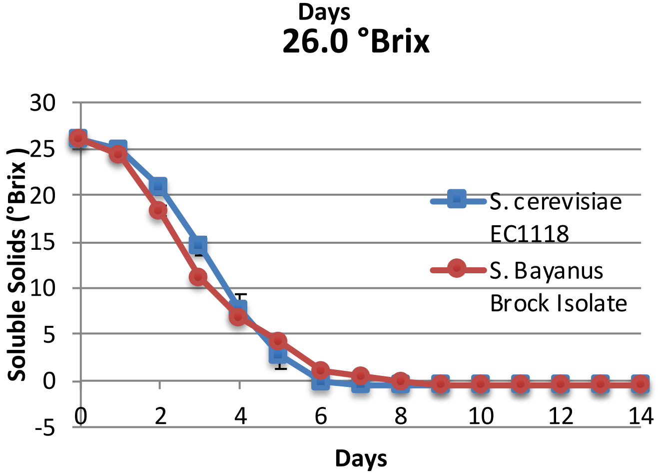
Characterize *S. bayanus* isolate (CN1) for Appassimento winemaking (started in 2013):

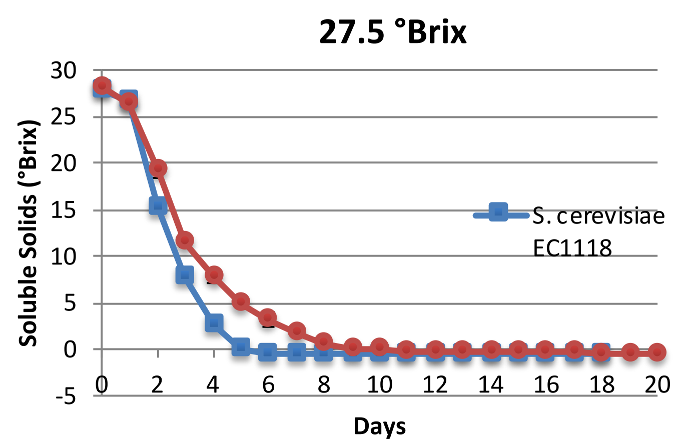
- What are the upper sugar limits of juice that the yeast can ferment to dryness?
- How does it perform vs. *S. cerevisiae* EC1118?
  - Fermentation kinetics, oxidative compounds in finished wine, sensory profile of the wine
- Is there a consumer preference of appassimento wines fermented with the *S. bayanus* isolate CN1 versus the commercially accepted EC1118 yeast?

Appassimento wine production
Grapes dried up to 27.5 Brix
EC1118 vs *S. bayanus* isolate



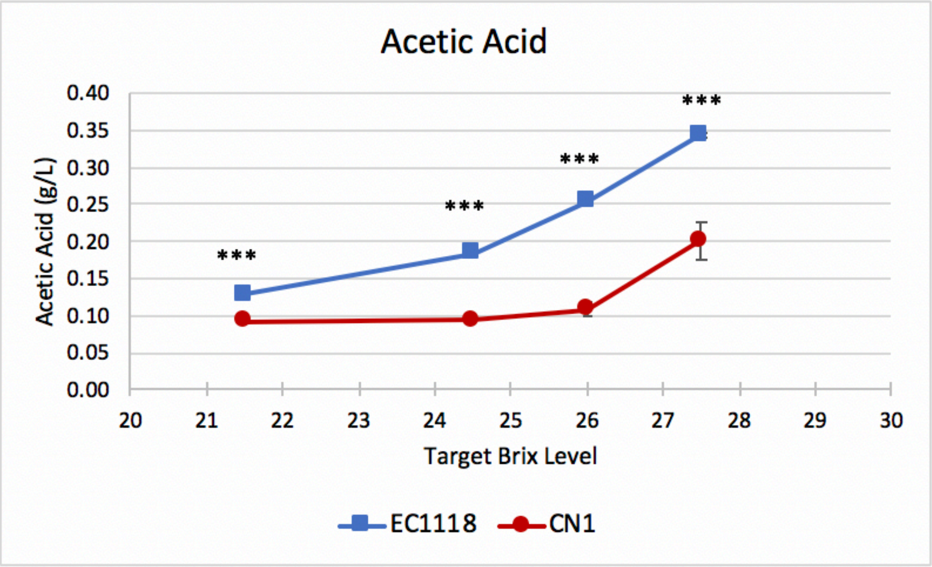


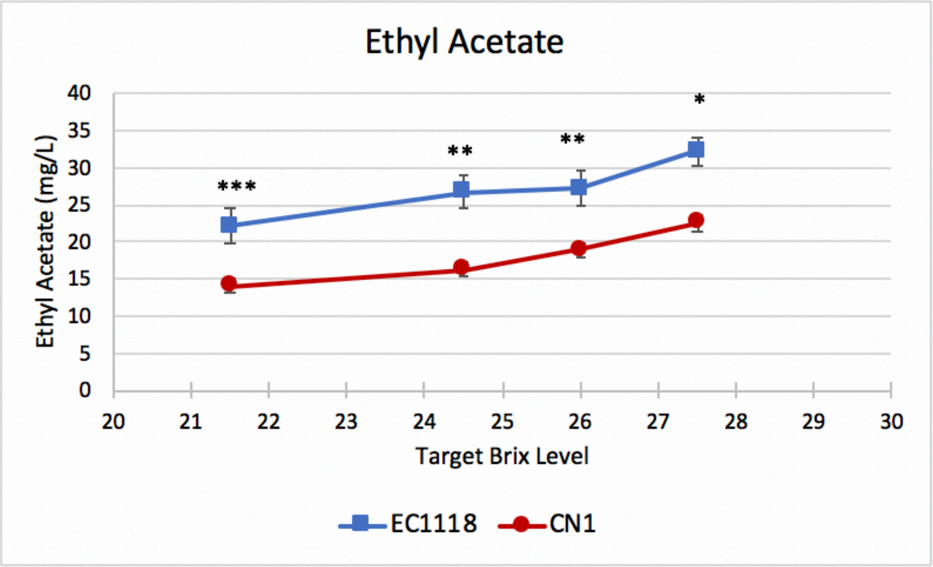




S. bayanus isolated fermented to dryness up to 27.5 Brix

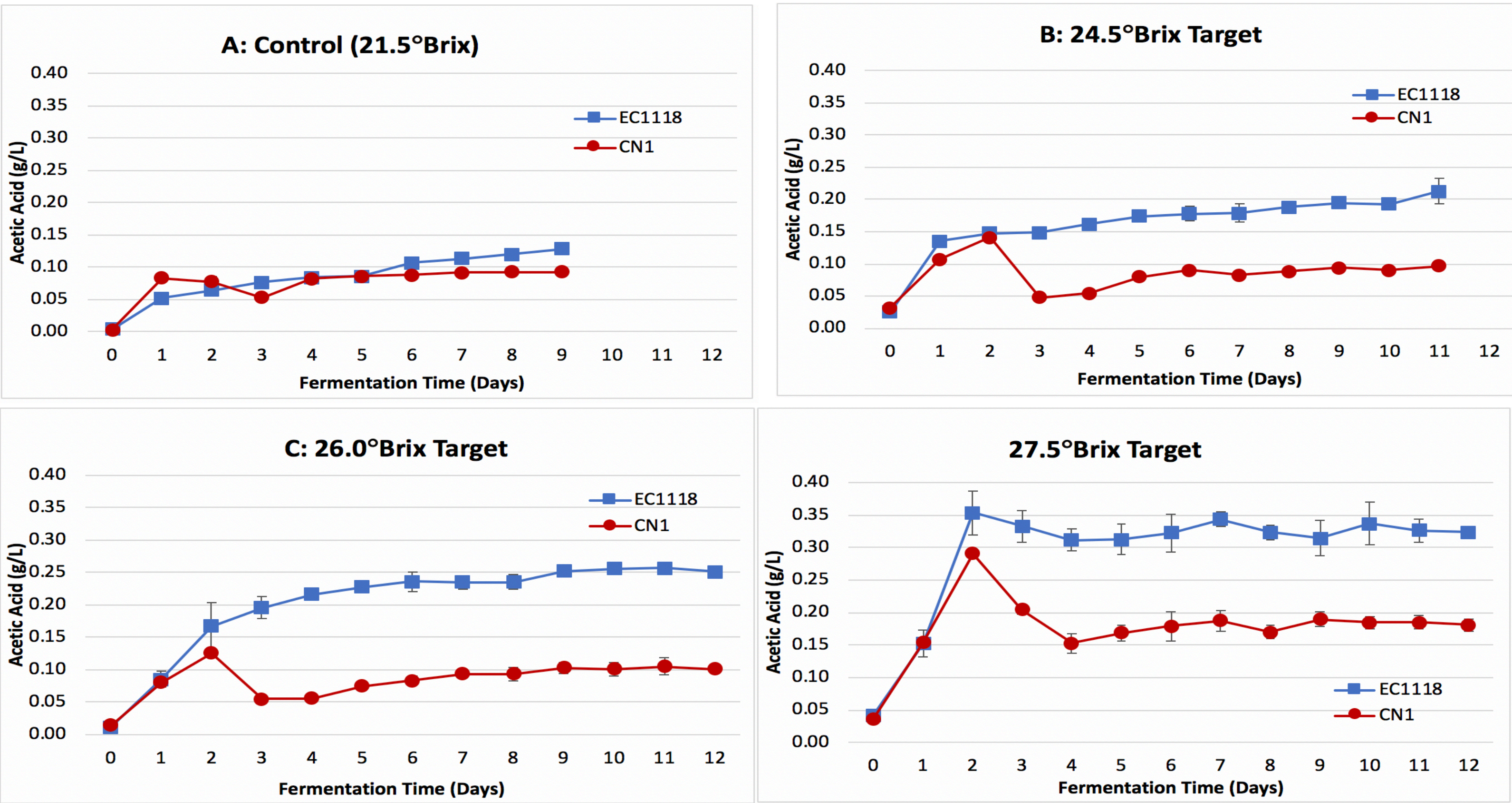
S. bayanus isolate results in lower acetic acid and ethyl acetate in wines





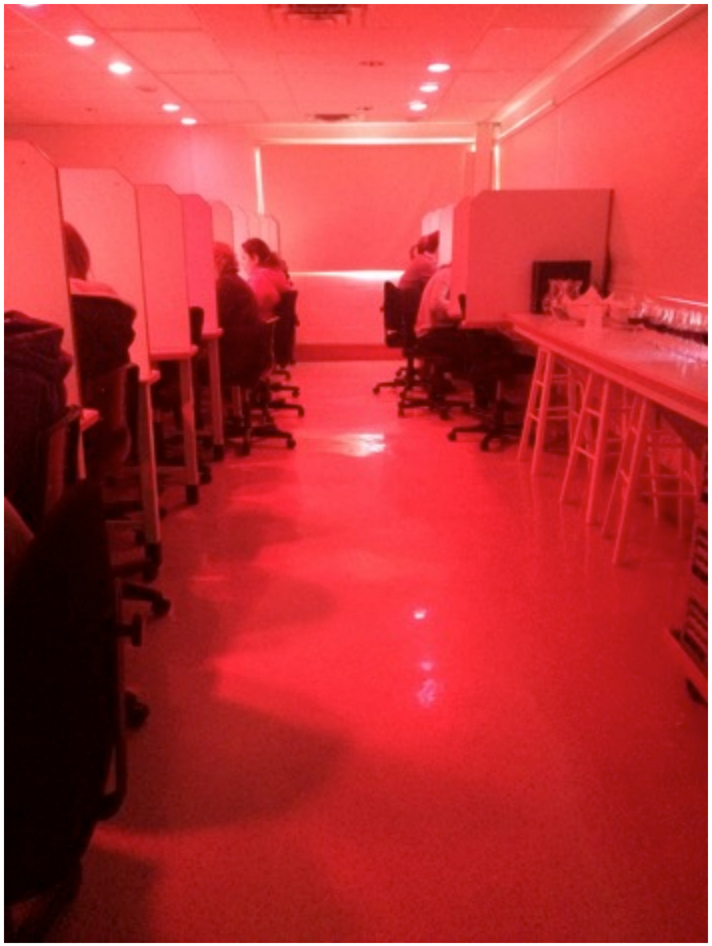
S. bayanus isolate produces lower acetic acid and ethyl acetate in appassimento wines in comparison to EC1118, similar to what was observed in Icewine (40-60% less acetic acid over Brix range tested, 30-35% less ethyl acetate)

Acetic Acid Production/consumption: Comparison of EC1118 to *S. bayanus* isolate



S. bayanus isolate starts to consume acetic acid that it generates in the fermentation (where uptake rate becomes greater than release rate, dropping the [acetic acid] in the fermenting wine)

Summary of sensory analysis of wines (presented in detail in Inglis CCOVI Lecture 2016)



S. bayanus in Appassimento Wine

- Shifted the sensory profile of the wine towards **increased black fruit flavour and aroma**
- **Reduced sourness and astringency** vs. *S. cerevisiae* EC1118 commercial yeast
- Has demonstrated its feasibility for industry use
- Consumer Preference????

Consumer Preference Summary

	p-value	*S. cerevisiae* EC1118 Appassimento wine 27.5 Brix	*S. bayanus* CN1 Appassimento wine 27.5 Brix
Overall Liking	0.16	6.2	6.4

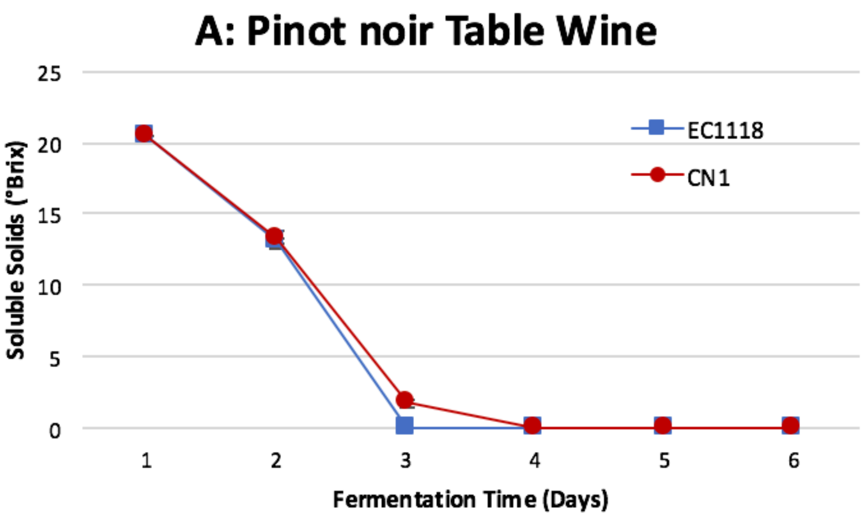
There was no significant difference in consumer preference between wines fermented with EC1118 and *S.bayanus* CN1

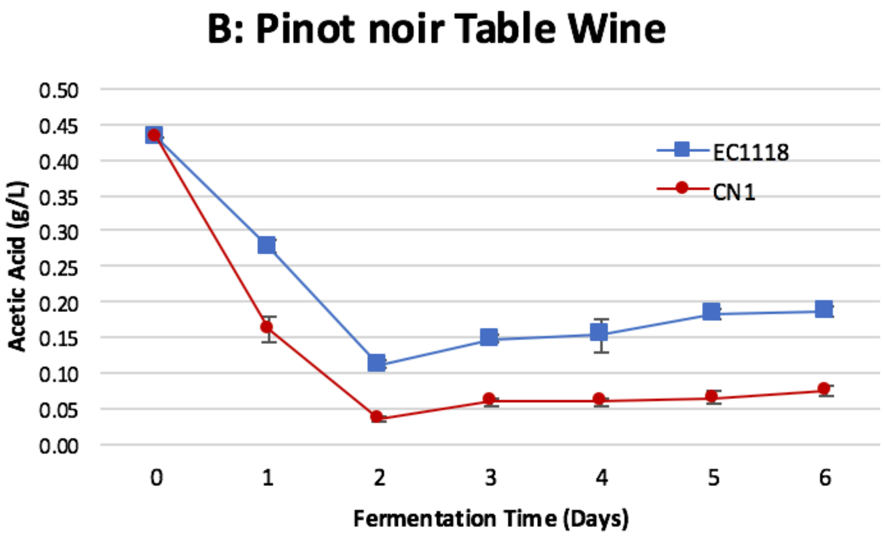
Next: Application of *S. bayanus* to juice with sour rot Pinot noir red wine

- Pinot noir notorious for breakdown at harvest
- Sometimes due to sour rot infection where acetic acid builds up in fruit from microbial sour rot complex
  - Wendy McFadden Smith (Ontario, Canada)
- With the acetic acid consumption observed for the *S. bayanus* isolate during appassimento wine production and table wine production, is there application to fruit with sour rot infection?
- We tested this with sour rot infected Pinot noir fruit.

Red Wine Production from Sour Rot Infected Fruit

EC1118 vs *S. bayanus* isolate (S. van Dyk, 2018)





S. bayanus isolate consumes acetic acid to a greater degree than commercial *S. cerevisiae* EC1118, dropping acetic acid 85% from starting juice to only 0.07 g/L in wine

Next: Application of *S. bayanus* to sparkling wine for base wine fermentation when juice has sour rot

- Base wines in traditional sparkling wines made mainly with Pinot noir and Chardonnay in Niagara
- Riesling also used in Niagara for charmat method sparkling wines, some using it in traditional method
- Riesling and Pinot noir can suffer from sour rot infections

**stay tuned for upcoming talk by Dr. Belinda Kemp on sour rot and impact on aroma and flavour compounds

Question arose:

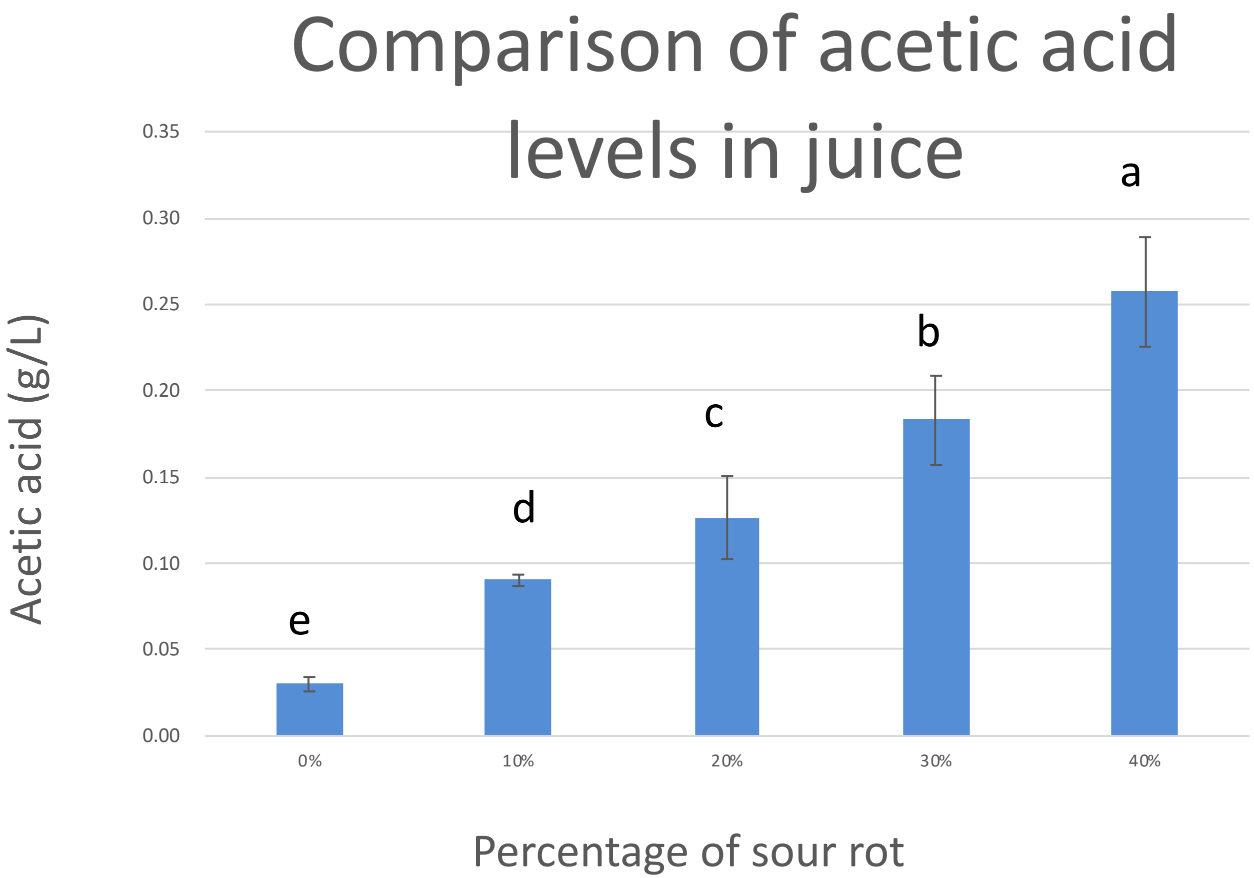
Can our *S. bayanus* isolate ferment acidic juice for sparkling production and reduce acetic acid from sour rot infections?

Pinot noir juice composition for sparkling base wine

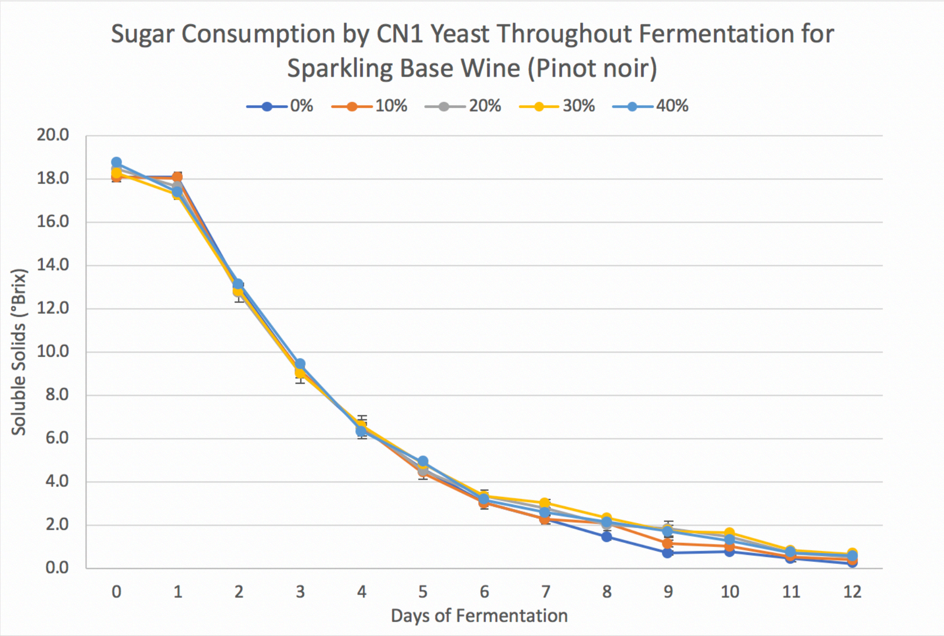
Sour rot: 0-40% v/v juice (S. Liying, B. Kemp, D. Inglis)

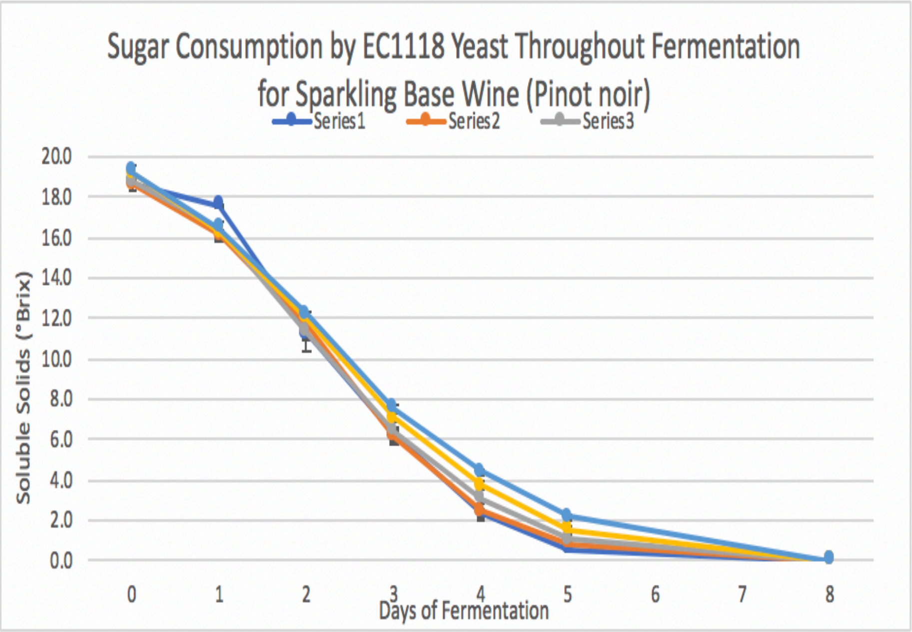
Juice Parameter	0% sour rot	10% sour rot	20% sour rot	30% sour rot	40% sour rot
Brix	17.5 ± 0.1	17.7 ± 0.1	17.7 ± 0	17.8 ± 0.1	18.0 ± 0.1
pH	3.05 ± 0.02	3.08 ± 0.02	3.08 ± 0.01	3.09 ± 0.03	3.07 ± 0.01
Titratable Acidity (g/L tartaric acid)	11.8 ± 0.1	12.0 ± 0.2	12.3 ± 0.3	12.8 ± 0.1	13.2 ± 0.1
Yeast Assimilable Nitrogen (mg N/L)	151 ± 3	146 ± 3	141 ± 3	134 ± 3	129 ± 4
Acetic Acid (g/L)	0.03 ± 0.01	0.09 ± 0.01	0.13 ± 0.02	0.18 ± 0.01	0.26 ± 0.02

Acetic Acid 0-40% v/v sour rot juice 2019 harvest



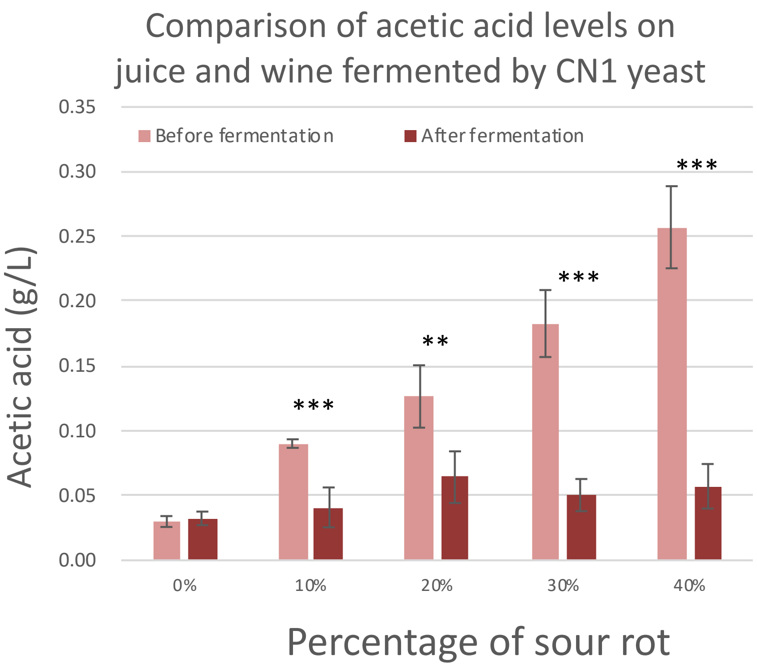
Sparkling base-wine fermentation when fruit has sour rot: 0-40% CN1 vs EC-1118

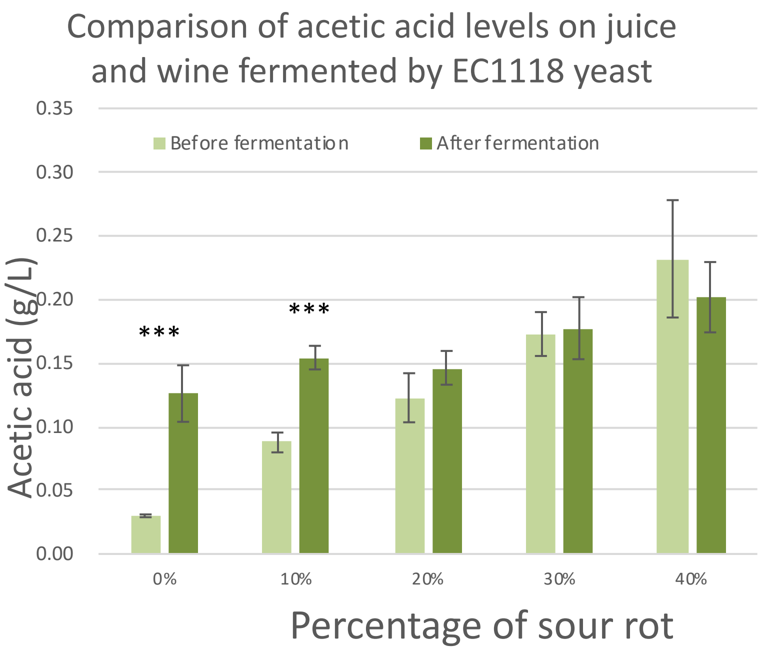




S. bayanus fermented the sparkling juice to dryness, took ~4 days longer than EC1118

S. bayanus isolate (CN1) consumes the acetic acid during fermentation CN1 vs EC-1118





S. bayanus isolate consumes acetic acid, reducing the value from the starting juice by up to 80% to only 0.055 g/L in wine. *S. bayanus* does not add acetic acid to the base wine with 0% sour rot.

Next Steps: Producing an Active Dried Yeast for commercial trials

- Dehydration trial is underway with a yeast manufacturer (Lallemand) to see if we can produce an "active dried yeast" (ADY) to facilitate testing the yeast on a commercial scale for fermentation performance
- If the ADY trial is successful, our funding will support an appassimento trial this fall with industry partner Pillitteri

Summary

- We isolated a fermenting yeast from Riesling Icewine grapes with potential for wine production with a regional signature
- The yeast was identified as a strain of *S. bayanus* , named CN1
- A main distinguishing characteristic of the yeast isolate (CN1) is that it produces low acetic acid and ethyl acetate
- It can also consume acetic acid from starting juice so it may have application for grape varieties that are prone to sour rot infection

Summary

- **Icewine:** using 38-40°Brix juice, CN1 can only produce ~8-9% v/v ethanol but reduces acetic acid by ~30% in comparison to EC1118
- **Appassimento wines:** CN1 ferments juice up to 27.5 Brix to dryness, producing wines with 14% v/v ethanol. It produces less acetic acid and ethyl acetate in wine in comparison to EC1118 over a range of starting Brix in juice
  - 30-60% less acetic acid
  - 30-35% less ethyl acetate
- **Sour rot grapes for red wine:** CN1 reduced acetic acid during fermentation of sour rot infected Pinot noir by 85%, dropping the juice value from 0.45 g/L to only 0.07 g/L acetic acid in wine.
- **Sour rot grapes in sparkling base-wine:** CN1 fermented Pinot noir juice used for sparkling wine production to dryness, requiring ~4 additional days in comparison to EC1118. It reduced acetic acid from 0.25 g/L in the juice to 0.05 g/L in base wine, reducing acetic acid by 80%. CN1 did not add any acetic acid into the control base wine made from 0% sour rot fruit.

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- Pillitteri Estates Winery
- Lallemand

Thank you!

Questions?

Cheers!

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