



## Reducing SO<sub>2</sub> at crush with the non-*Saccharomyces* yeast BioNature (Lamothe Albeit) in

### Petit Verdot

*Barboursville Vineyards*

Danielle Tessaro/Luca Paschina

### Summary

Many winemakers are looking for alternatives to the use of SO<sub>2</sub> for microbial control. Several strains of non-*Saccharomyces* yeast are now commercially available for bioprotection as well as aroma/flavor enhancement. The purpose of this experiment was to compare the chemistry and microbiology of Petit Verdot after grapes were treated with either 25 ppm SO<sub>2</sub> or 40 g/hL Bionature. There were no notable differences in wine chemistry, volatile acidity, or microbiology in the finished wines. The 2019 vintage was one with very little rainfall or disease pressure. Inoculation with Bionature controlled microbial growth as well as 25 mg/L SO<sub>2</sub> addition at crush.

### Introduction

Many winemakers are looking for alternatives to the use of SO<sub>2</sub> for microbial control. Despite its prowess as an antioxidant and antimicrobial agent in wine, SO<sub>2</sub> may have a negative effect on wine quality such as bleaching color, inactivating thiamine, and favoring production of H<sub>2</sub>S. Furthermore, it may have limited efficacy in musts with very high pH, such as Petit Verdot<sup>1</sup>. One alternative to the use of SO<sub>2</sub> at crush is the use of selected strains of bioprotective non-*Saccharomyces* yeast.

Grapes enter the winery covered in microbes from the vineyard. To date, 52 different species of yeast from 22 different genera have been identified on grapes including *Hanseniospora* (AKA *Klockera*), *Candida*, *Pichia*, *Hansenula*, *Metschnikowia*, *Sporobolomyces*, *Cryptococcus*, *Thodotorula*, and *Aurobasidium*<sup>1</sup>. The cast of characters changes as grapes ripen, with the greatest abundance of microbes present in the last few weeks<sup>1</sup>. Healthy grapes are generally inhospitable environments for any microbe because they are covered in plates of wax that form a cuticle to hold in nutrition and repel water. Microbes cluster around the stomata or next to cracks in the cuticle where seepage from the openings provides both water and nutrients. The overall microbial load on grapes depends on environmental factors such as climate, altitude, variety, age of grapes, disease pressure and vineyard practices. Fog, rain, and fruit damage (like that seen in wet vintages like 2018) quickly transform the microbial desert of grape skins into an oasis. Cells that are present in small numbers quickly multiply when given the chance<sup>1</sup>. For example, *Botrytis* infection can increase the overall abundance of microbes by 1000x. Grapes with sour rot have significantly higher microbial diversity and abundance<sup>2</sup>. Insect pressure will also increase abundance due to increased vectoring from diverse environments<sup>3</sup>. The overall inoculant of non-*Saccharomyces* yeast and bacteria coming into the winery from the

vineyard on the grapes is often larger than the inoculant of selected *Saccharomyces* yeast added at the beginning of fermentation<sup>1</sup>.

Non-*Saccharomyces* yeast have several impacts on the wine, both positive and negative. *Kluyveromyces fragilis* (aka *Hanseniaspora uvarum*) is a common member of the non-*Saccharomyces* yeast community found on grapes<sup>1,4</sup>. This yeast strain is easily identified under a microscope by its lemon shaped cells. It is tolerant to up to 100 mg/L SO<sub>2</sub>, can grow at low temperature (such as that found during cold soak), and can produce both acetic acid and ethyl acetate (which smells like nail polish remover) under aerobic conditions<sup>3</sup>. Other offenders in the non-*Saccharomyces* yeast community include *Pichia guilliemondii*, a film forming yeast prevalent in warm conditions when fermentation is delayed. This yeast can form spores that become resident in barrels and produce ethyl acetate and 4 ethyl phenol (which can smell like band-aid, wet dog, horse sweat)<sup>1-3</sup>.

Many spoilage bacteria also come into the winery on grapes. Sour rot and *Botrytis* increase the prevalence of *Acetobacter*, *Gluconobacter* and *Gluconacetobacter*, all of which produce acetic acid. Several *Lactobacillus* species (*hilgardii*, *plantarum*, *casei*) and *Pediococcus* (*damnosus*) are also residents of mature grapes. These can produce acetic acid, mousy flavor and biogenic amines (which have names like putrescine and cadaverine...). They may also produce polysaccharides that lead to ropy texture<sup>1,3,5</sup>.

In addition to outright spoilage, high levels of native flora may also cause nutrient depletion early in fermentations that limit nutrients available to *Saccharomyces*, potentially leading to stuck fermentations<sup>6,7</sup>. In a study of nutrient depletion by non-*Saccharomyces* yeast species, Medina et al (2012)<sup>6</sup> found that *Metchnikowia*, a non-*Saccharomyces* yeast strain present in potentially high numbers on grapes<sup>8</sup>, consumed YAN quickly within the first few days of fermentation. Mimicking what may be occurring in fermentations with cold soaking or delayed inoculation, sequential inoculation with *Metchnikowia* followed by *Saccharomyces* led to stuck fermentations that could be resolved with nutrient addition. In the same study, *Hanseniaspora*, another prevalent member of the grape microbiota, did not show large YAN depletion (90% of the YAN remained 3 days after inoculation with this species), however it did deplete thiamine, an essential vitamin for *Saccharomyces*. Excessive use of SO<sub>2</sub>, as would occur in vintages with high microbial load, also leads to reduction in thiamine, further increasing the potential for stuck fermentations.

Despite the risks, there are also some benefits to having a rich microbial community early in fermentation. Several non-*Saccharomyces* yeast species have been shown to produce positive compounds that add complexity to wine aroma such as esters, higher alcohols, glycerol, succinic acid and thiols. Proteases produced by non-*Saccharomyces* yeast have been shown to break down cells and add nutrients, ultimately making a more protein stable wine. Some produce glycosidases that help unmask aromas compounds that are bound to sugar molecules. Others produce enzymes to break down polysaccharides that would otherwise

inhibit clarification and filtration. *Lachanacea thermotolerans* has been shown to consume acetic acid, reducing volatile acidity<sup>1,8,9</sup>. It is likely these are some of the mechanisms that occasionally lead winemakers to employ ambient fermentations.

Many winemaking decisions affect the abundance and diversity of the microbial community present at the beginning of fermentation. Mechanical harvesting and long transport times, especially at warm temperatures, can lead to a high microbial load<sup>8,10</sup>. As soon as the grapes are crushed, nutrients are released to feed the organisms that are present. *Klockera (Hanseniaspora)* is often the most abundant species on the grapes, and remains prevalent until alcohol levels rise above 4-7% and oxygen is used up<sup>4,11</sup>. The low pH environment of the juice, rising alcohol, rising temperatures, and presence of phenolics tend to inhibit spoilage organisms in early fermentation. Harvesting wet grapes, prolonged cold soak, cool fermentation conditions, low inoculant of yeast, and lack of clarification (for white wines) can all lead to higher counts of yeast and bacteria in the fermentation<sup>4,12</sup>

In wet vintages such as 2018, the prevalence of damaged berries and wet grapes likely increased the inoculant of non-*Saccharomyces* microbes in fermentation and may have contributed to overall higher volatile acidity in wines that year. One approach to microbial management is to use higher than normal levels of SO<sub>2</sub>. Though SO<sub>2</sub> has efficacy against some microbial spoilage, many of these microbes (such as *Hanseniaspora*) have high tolerance to it. Much of the SO<sub>2</sub> added at crush is lost as it binds to grape solids that are prevalent in red wine fermentations, making it less effective. High SO<sub>2</sub> additions can also bind thiamine and slow down or halt fermentation, and may even select for SO<sub>2</sub> tolerant microbes that will cause spoilage during aging<sup>13,14</sup>. Still, fermentations that have some SO<sub>2</sub> added at crush do tend to have faster onset of fermentation (leading to lower potential for spoilage) and steadier kinetics (Egli et al 1998).

Another addition to the toolkit for the prevention of microbial spoilage is to use a selected strain of bioprotective non-*Saccharomyces* yeast. Several strains of bioprotective yeast have been developed as commercially available products that inhibit the activity of spoilage organisms without depleting nutrients, undergoing fermentation, or inhibiting *Saccharomyces*. These strains are generally tolerant to SO<sub>2</sub>, so they can be used as an additional antimicrobial measure. Different strains use different mechanisms for bioprotection. One strain of *Torulaspora delbrueckii* has been shown to produce a killer toxin, TdKT, that uses β-glycanase and chitinase activity to attack the cell walls of spoilage yeast<sup>15</sup>. Not all strains of *T. delbrueckii* tested had this activity, and if they did, it was not active against all of the spoilage yeasts tested. Several strains of *Metschnikowia pulcherrima* have been shown to have broad and effective antimicrobial action against spoilage microbes. These employ pulcherriminic acid, which depletes iron from the surrounding medium to cause nutrient limitation. In addition, the presence of *M. pulcherrima* changed the gene expression of *S. cerevisiae* such that the fermentation yeast produced 40% less acetic acid and 12% more glycerol<sup>16</sup>.

Despite these promising results, it is important to keep in mind these bioprotectors cannot make up for poor cellar practices. James Osborne's lab at Oregon State University conducted a series of experiments testing the ability of commercially available non-*Saccharomyces* yeast to reduce growth of *Hanseniaspora uvarum* and reduce the spoilage compounds made by this microbe (acetic acid and ethyl acetate). They used a model grape juice to grow mono-cultures and co-cultures of *H. uvarum* with several commercially available non-*Saccharomyces* yeast marketed as bioprotective (three *Methchnikowia*, three *Lachanacea*, and four *Torulaspota*). Each strain of bioprotective yeast decreased the level of acetic acid, and the number of *H. uvarum* cells present relative to mono-cultures of just *H. uvarum*. A single strain (*M. fructicola*) was further tested in Pinot noir must with and without cold soaking, at three levels of inoculation with *H. uvarum*. The number of *H. uvarum* cells decreased in cultures when *H. uvarum* was inoculated at low levels ( $1 \times 10^3$  cells/mL) but not high levels ( $1 \times 10^6$  cells/mL). At all levels, inoculation with *M. fructicola* decreased ethyl acetate and acetic acid levels relative to control, but high levels of *H. uvarum* still led to high levels of ethyl acetate (20-30 mg/L) and acetic acid (175-200 mg/L) produced during cold soak. After fermentation was complete, there were no significant differences in ethyl acetate or acetic acid between "no cold soak" control and cold soaked wines treated with *M. fructicola*. The take-home message here seems to be that though non-*Saccharomyces* yeast can help, they cannot overcome high levels of *H. uvarum* inoculant caused by damaged grapes or long cold soaking<sup>17</sup>.

Several strains of non-*Saccharomyces* yeast are now commercially available for bioprotection as well as aroma/flavor enhancement (Table 1). BioNature from Lamothe-Abiet is a selected strain of *Metchnikowia pulcherrima* marketed for bioprotection as well as increased aromatic complexity. This strain was selected from 77 isolates for its low fermentation kinetics, low production of H<sub>2</sub>S, strong inhibition of *Brettanomyces bruxellensis*, *Hanseniaspora uvarum*, and *Candida apicola*. It can be used instead of or in conjunction with sulfur dioxide (It is resistant to up to 50 mg/L of SO<sub>2</sub>)<sup>1</sup>. The purpose of this experiment was to compare the chemistry and microbiology of Petit Verdot after grapes were treated with either 25 ppm SO<sub>2</sub> or 40 g/hL Bionature.

### Methods

Fruit was hand harvested from the same vineyard rows on Sept 13 and destemmed the same day. The BioNature treatment received 40 g/hL BioNature sprinkled onto the grapes at crush while the control received 25 ppm SO<sub>2</sub>. Destemmed grapes were transferred to fermentation tanks by a must pump. Grapes were held in tank with CO<sub>2</sub> gassing without pumping over for two days, then both tanks were inoculated with 25 g/hL FX-10 rehydrated in 25 g/hL GoFerm and 20 g Fermaid O. Fermentations received two pumpovers of 1.5 volumes of the tank per day for the duration of fermentation. Nutrients (20 g/hL Fermaid K and 20 g/hL DAP) were added at 1/3 Brix depletion. Wine was allowed to macerate for 2 weeks after the completion of fermentation with daily CO<sub>2</sub> gassing. Both tanks of wine were drained and

pressed on the same day with the free run only used for the experiment. Wines were inoculated for malolactic fermentation, which completed in tank prior to treatment with SO<sub>2</sub> and transfer to barrels for aging.

Table 1: Partial list of commercially available non-*Saccharomyces* yeast

Supplier	Trade Name	Scientific name	Marketed for...
Scottlabs	Biodiva	<i>Torulaspora delbrueckii</i>	Complexity (increased aromas, esters, mouthfeel)
Scottlabs	Flavia	<i>Metchnikowia pulcherrima</i>	Revealing enzymes to increase flavor
Scottlabs	Gaia	<i>Metchnikowia fructicola</i>	Bioprotection during cold soak, aromatic expression
AEB	Primaflora	<i>Torulaspora delbrueckii</i>	Bioprotection, limit spoilage
Laffort	Egide	<i>Metchnikowia pulcherrima</i> & <i>Torulaspora delbrueckii</i>	Bioprotection (transport, settling, cold soak)
Lamothe Abiet	BIO-Nature	<i>Metchnikowia pulcherrima</i>	Bioprotection, increased aromatic complexity, low SO <sub>2</sub>

## Results

The juice chemistry was very similar between lots (Table 2), as was finished wine chemistry (Table 3). There were no notable differences in volatile acidity or color between lots. Microbial load was also similar in the finished wine (Table 4) with no detectable *Brettanomyces* in either lot. Both lots had measurable *Lactobacillus* and *Pediococcus* populations. Use of SO<sub>2</sub> vs. non-*Saccharomyces* yeast led to no notable change in phenolics (Table 5). The 2019 vintage was one with very little rainfall or disease pressure. In this case, inoculation with Bionature controlled microbial growth as well as 25 mg/L SO<sub>2</sub> addition at crush.

Table 2: Juice chemistry for two treatments of Petit Verdot (in-house data)

	Brix (deg)	pH	Titrateable Acidity (g/L)	YAN (mg/L)
Control	22.8	3.81	5.5	198
BioNature	22.8	3.7	6.5	198

Table 3: Wine chemistry for two treatments of Petit Verdot (ICV Labs)

	VA (g/L)	pH	TA (g/L)	Alcohol (%)	f SO <sub>2</sub> (ppm)
Control	0.61	3.68	5.39	13.6	21
BioNature	0.65	3.68	5.39	13.73	16

Figure 1: Color intensity for two treatments of Petit Verdot (ICV Labs)

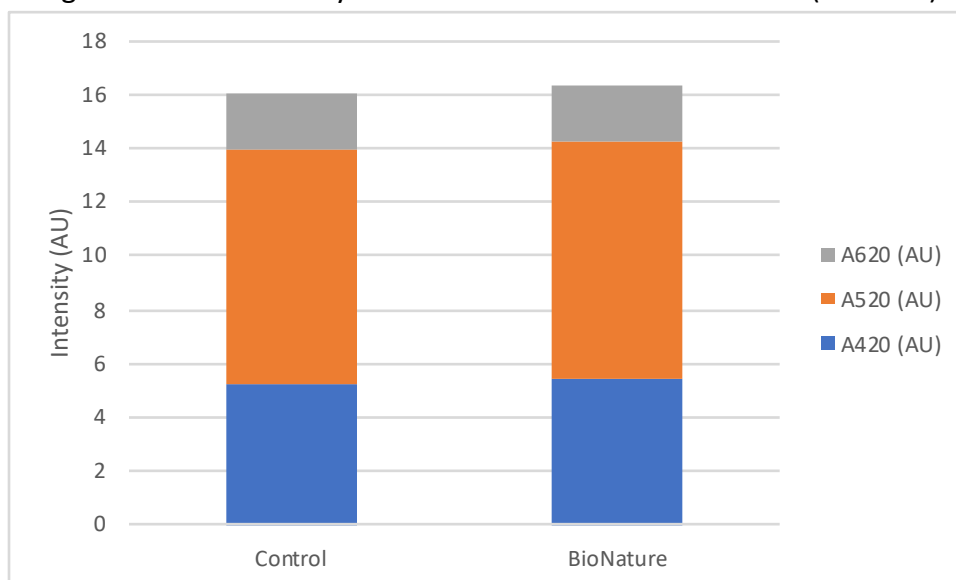


Table 4: Microbiology for two treatments of Petit Verdot (ETS Labs)

	Control	BioNature
<i>Saccharomyces cerevisiae</i>	$1.9 \times 10^4$	$6.8 \times 10^3$
<i>Oenococcus oeni</i>	$>1 \times 10^7$	$>1 \times 10^7$
<i>Acetic Acid Bacteria</i>	$3.08 \times 10^5$	$5.97 \times 10^5$
<i>Brettanomyces bruxellensis</i>	0	0
<i>L. brevis/hilgardii/fermentum</i>	170	200
<i>Lactobacillus kunkeei</i>	10	10
<i>Lactobacillus plantarum/casei/mali</i>	$6.02 \times 10^3$	$5.15 \times 10^3$
<i>Pediococcus Species</i>	$2.83 \times 10^4$	$1.7 \times 10^4$
<i>Zygosaccharomyces Species</i>	0	0

Table 5: Rapid Phenolic Analysis of two treatments of Petit Verdot (mg/L) (ETS Labs)

	Catechin	Total Anthocyanins	Polymeric Anthocyanins	Tannin	Index	
					Catechin/tannin	Polymeric anthocyanin:tannin
BioNature	37	643	59	735	0.05	0.08
SO <sub>2</sub>	39	675	60	732	0.053	0.082

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