Yeast selection– back to basics

Offering some fundamental advice on how to select the best yeast for your wine is **Joana Coulon**, manager of microbiology at BioLaffort, and **Alana Seabrook**, technical manager and Laffort Australia

Wine Additives

Introduction

Nowadays, choosing a yeast for fermentation is a simple as flicking through a shiny catalogue or a website and finding all of the desirable attributes. However, not so long ago the choice of commercial yeast was non-existent and winemakers were forced to rely on their own resources to ensure fermentation went through to completion. But now the choices almost seem endless…where to start? What is important? How relevant are all of these so-called desirable attributes in a wine-like environment? The intention of this article is to navigate through the factors around yeast attributes and work out what is key for the winemaker.

Origins

Saccharomyces cerevisiae (*S. cerevisiae*) is arguably a domesticated species and is often found in human environments. It is associated with numerous fermented beverages and can be traced back to 3200BC (Cavalerri *et al.* 2003). Fermentation activities probably due to this microorganism were even detected in neolithic poteries (6000-7000BC) in China (McGovern *et al.* 2004). Nowadays *S. cerevisiae* are found in cellars and on grape berries (Mortimer and Polsinelli 1999) but they are thought to originally inhabit forests on tree bark (Wang *et al.* 2012), transported by insects to colonise highly fermentable ecosystems.

S. cerevisiae vs *S. bayanus*

In past decades, *S. bayanus* was associated with not being able to metabolise galactose (Gal-) and *S. cerevisiae* was Gal+ (Barnett 1992). *S. bayanus* then became a generic term to denominate strains of *S. cerevisiae* that were not able to metabolise galactose. Today, the species *S. bayanus* exists, but no longer refers to the Gal (-) group originally described. Genetically it is very distinct from what we now know as *S. cerevisiae* and not associated with oenology (used primarily for brewing). In 1953 it was observed that what was anciently called '*S. bayanus*' (which belongs, in fact, to the *S. cerevisiae* species) had better fermentation abilities and was often associated with the end of AF (Pinault and Domercq 1953). This is now no longer relevant to oenology (Frezier 1992). Hence the 'Gal-' criteria is not best suited to designate strong fermentation ability strains among *S. cerevisiae*.

Sensory impact

Every yeast strain will possess a different spectrum of enzymatic activities that influence the sensory profile of the wine. Some yeast strains are natural isolates from regions renowned for the production of a particular wine. This means they were identified during a fermentation as being the yeast responsible for the fermentation. Often this is perceived as a way of identifying a yeast strain that will produce the sensory profile desired. But, unfortunately, a strain isolated from a Sangiovese in Chianti may not impart the same sensory profile on an Australian Sangiovese with identical winemaking processes. Moreover, the concept of a 'terroir' strain still remains questionable. Indeed, even though regional strains can be found, the link between the origin of a strain and the organoleptic signature of the corresponding fermented wine is still a debate (Borlin 2014, Knight and Goddard 2015). In addition, these strains would also be tested for alcohol tolerance, fermentation kinetics, YAN demand and temperature sensitivity. This is where crossing yeast strains with ideal attributes becomes important when considering different environments for a particular desired outcome.

Most commercial yeast strains will present an indication of the types of aroma compounds produced and the sensitivities/tolerances. Do they make a big difference sensorially? Absolutely. Depending on the wine in question, particular aroma compounds are critical. For example, in Sauvignon Blanc thiols are key aroma compounds. The volatile form of these compounds is produced by yeast and many yeast will not produce these key thiols. Esters, important for many fruit aromas in both red and white wines, are converted by yeast into a more volatile form making the selection of yeast key. Some aroma compounds are present in grapes and are linked to a sugar'; a yeast may produce enzymes that cleave off this sugar to rendering the

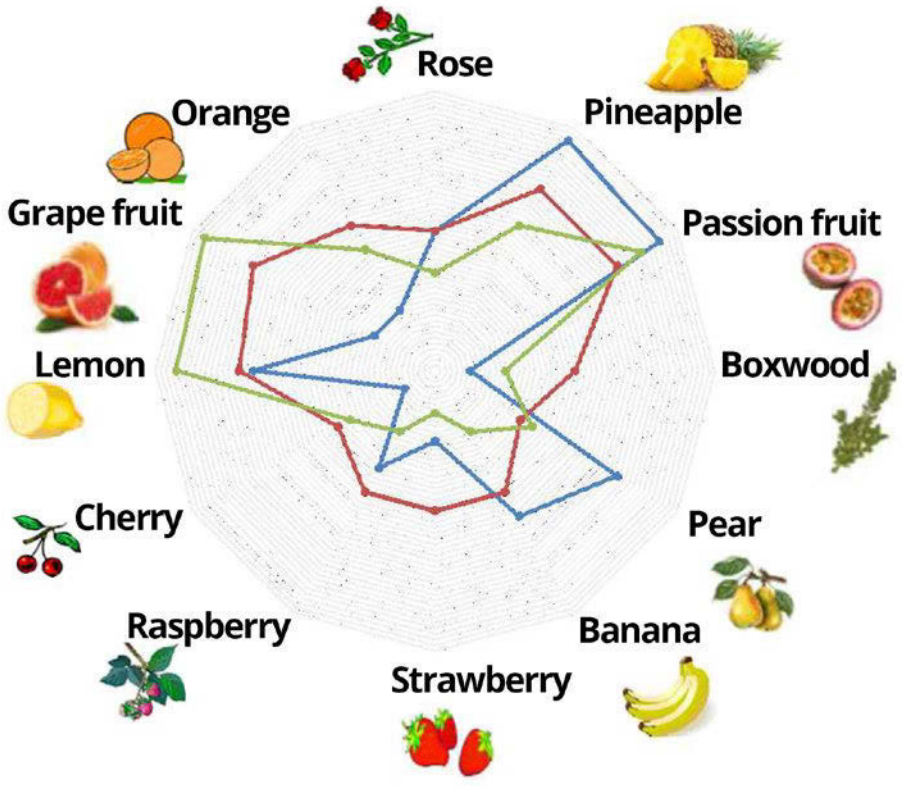

Figure 1. The aroma profile of three different yeast strains.

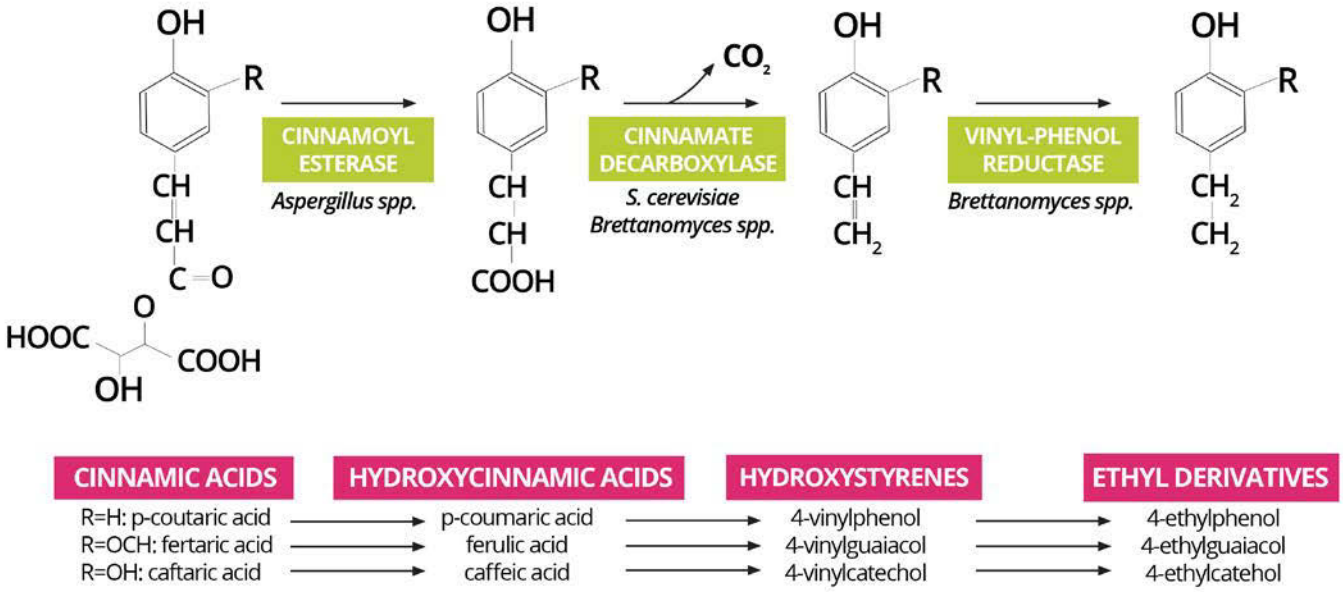

Figure 2. Conversion of cinnamic acids to Ethyl derivates (Sourced from BioLaffort, France).

aroma volatile. Understanding that these enzymes have temperature sensitivities and that the aroma compounds themselves are volatile and may be susceptible to oxidation is critical. Figure 1 demonstrates the aroma profiles of three different yeast strains in the same wine. Understanding the desired profile is ideal when choosing a yeast strain, but really the only way to know what sensory profile a yeast strain will produce is by trialing it on the desired grapes in those particular conditions.

Killer Character

Yeasts that have a 'killer factor' are deemed to be positive in oenology, as their presence suggests they can outcompete other yeast strains and species by producing a killer toxin. A yeast may contain virus like particles that allow:

- K(+) Killer toxin production
- R(+) Immunity factor production

If a yeast contains both it is able to produce a killer factor and an immunity factor; it is a killer strain, whilst K(-)/R(+) is a neutral strain and K(-)/R(-) is a sensitive strain. However, the killer factors produced by yeast would appear not to be relevant to winemaking conditions due to the pH of must/wine and the presence of polyphenolic compounds in red wines (Guiterrez *et al.* 2001).

So, is this really relevant to oenology or is it a marketing tool? Research suggests that killer factors are likely inhibited in wine-like conditions thereby making them less critical factors in selecting a yeast strain.

Ability to produce vinyl phenols – The POF character

Saccharomyces cerevisiae strains may be characterised as either POF+ (ability to produce vinyl phenols), or POF- (not able to produce vinyl phenols). This means that they can produce vinyl-phenols from hydroxycinnamic acids which are naturally present in grapes (Figure 2). This is of concern as some yeasts are able to convert vinyl phenols into ethyl-phenols. Of winemaking concern is the production of 4-ethyl-phenol and 4-ethyl guaiacol by *Brettanomyces bruxellensis* which can be detrimental to wine quality. Other yeast species are able to produces these compounds, but *B. bruxellensis* is very good at it and able to tolerate winemaking conditions that many other species are not able to tolerate.

How important is choosing a POF- or POF+ yeast? In white wine vinyl phenols have a detrimental sensory impact at 750ug/L (1:1 ratio of 4-vinyl phenol + 4-vinyl guaiacol) (Chatonnet *et al.* 1993). Choosing a yeast with POF- character becomes even more relevant in white wines when commercial enzyme preparations with cinnamate esterase activity are present, as *Aspergillus* spp produces enzymes that convert cinnamic acids into hydroxycinnamic acids. If these precursors are present, they will also serve as a substrate for ethyl phenol production should *B. bruxellensis* be allowed to grow. But the primary concern in white wines is the detrimental sensory effect of the vinyl phenols which can not only taint but mask varietal aromas (Chatonnet *et al.* 1993).

In red wines the POF+ character is strongly inhibited by phenolic acids present, making it more critical to white wine production (Chatonnet *et al.* 1993). In red wine the major risk is the presence of *B. bruxellensis*. Whilst *Pichia* spp has been known to produce ethyl phenols pre-fermentation (Barata *et al.* 2006), its spoilage potential is only a fraction of that of *B. bruxellensis*. If this is allowed to proliferate due to low levels of molecular SO_2, the presence of residual glucose/fructose, or lack of sterility in bottle, the taint will likely form. It is unlikely that using a POF+ strain will increase the amount of substrate. Ultimately control of *B. bruxellensis* is key (Malfeito-Ferreira 2018) to preventing the production of ethyl phenols.

S. cerevisiae and SO_2 production and consumption

All strains of *S. cerevisiae* both consume and produce SO_2. How much they consume and produce is strain dependent and also relies on must condition and composition. Some will consume more than they produce whilst others will produce more than they consume. The starting concentration of SO_2 will impact the final amount present at the end of alcoholic fermentation. Work conducted by BioLaffort France, based on standardised fermentation conditions

set up by Peltier *et al.* (2018) demonstrated the maximum and minimum levels of SO_2 post fermentation on five different must, 34 strains each in triplicate (Table 1). It means that the influence of yeast strain alone can alter the levels of SO_2 post fermentation from a 23% decrease in total SO_2 to a maximum of 77% additional TSO_2 from the starting amount of TSO_2 in the must.

Apart from initial SO_2 added in the vineyard or at picking, the main precursor of SO_2 is sulfates (Jiranek *et al.* 1995). Production of methionine and cystine is regulated by the input of sulfates and output of SO_2. In the presence of amino acids, SO_2 consumed by yeast will go on to form cystine and methionine, important aroma precursors.

Yeasts also produce SO_2 binding compounds, that is compounds that bind to free SO_2, rendering the SO_2 bound as opposed to in the free molecular form. The higher the amount of SO_2 binding compounds present the more SO_2 will be required to achieve a desired molecular SO_2. Low consuming SO_2 strains (which consume less SO_2 than they produce) usually can be correlated to high levels of SO_2 binding compounds (Table 2) (data sourced from Biolaffort R&D).

What does all this mean in terms of yeast selection? Every yeast strain commercially available and spontaneously found in nature will consume SO_2 and produce SO_2. The amount of SO_2 produced will depend on how much is in the must initially, the strain selected and the quantity of sulfates (precursors) present in the must.

Table 1. Maximum and minimum levels of total SO2 using 34 strains in triplicate on 5 must (derived from Peltier *et al*. 2018).

	Total SO2 (mg/L or ppm)		
	must	Minimum at the end of AF	Maximum at the end of AF
Cabernet Sauvignon 2015	35	39	56
Merlot 2014	37	38	53
Merlot 2015	46	39	55
Sauvignon Blanc 2014	34	38	61
Sauvignon Blanc 2015	67	48	85

Table 2. Amount of SO2 A) Produced and; B) Required to add to achive 35 ppm of FSO2 (CL35 value) with a low and high SO2 consuming strain (BioLaffort R&D).

	Resulting SO_2 (and CL35*)	
	Initial total SO_2: 30 ppm	Initial total SO_2: 70 ppm
Low SO_2 consuming strain	+ 39 ppm TSO_2 (150 ppm SO_2 CL35)	+ 45 ppm TSO_2 (181 ppm SO_2 CL35)
High SO_2 consuming yeast strain	+3 ppm TSO_2 (100 ppm SO_2 CL35)	+3 ppm TSO_2 (141 ppm SO_2 CL35)

*CL35 is the amount of SO_2 required to archive 35 ppm FSO_2

Yeast assimilable nitrogen (YAN) demand

When choosing the right yeast strain, often YAN demand is a factor. What does this mean? A yeast strain with a high YAN demand indicates a strain that will produce more biomass (more yeast cells) with a given amount of nitrogen (Figure 3). Conversely a low nitrogen-demanding strain will produce less biomass with the same level of nitrogen. This has downstream implications; if there is a higher number of cells in a ferment, they will likely need more YAN to support them through the fermentation. The two critical points in fermentation for YAN addition is in the first 24 hours of inoculation for biomass production, and a third of the way through ferment when maximum population has been achieved (this is dependent on how much sugar is in the must and the nitrogen demand of the strain) to sustain the population through alcoholic fermentation.

H_2S production

Hydrogen sulfide (H_2S) production by yeast can not only mask fruit aromas, but its production can hinder the formation of key aroma compounds (Mestres *et al.* 2000). In the absence of key amino acids, the SO_2 or sulfates taken up by the yeast will be converted to H_2S and released (Figure 4). In the presence of key amino acids, the H_2S formed by yeast can go down the cystine and methionine pathway, important aroma precursors. The timing of and type of YAN supplementation is critical to managing H_2S (Mendes-Ferreira *et al.* 2010).

Affinity for fructose

In perfect ripening conditions, the ratio of the fermentable sugars glucose and fructose is 1:1. As grapes head towards over-ripeness the ratio can change to favour fructose over glucose (Kliewer 1967, Shiraishi 2000). *Saccharomyces cerevisiae* metabolises glucose more easily than fructose (Guillaume *et al.* 2007). Because glucose is the preferred sugar by yeast, fructose is often the main sugar left in a stuck or sluggish fermentation. A higher fructose-to-glucose concentration in stuck wines is the consequence and not the cause of a stuck fermentation. The limiting factor is the transportation of sugar into the cell regulated by a gene called *HXT3* (Luyten *et al.* 2002), and in the presence of ethanol it is even harder for yeast to take up fructose (Berthels *et al.* 2007).

Yeasts that have a better chance of taking up fructose have been identified to have a particular form of the HXT3 transporter that has a higher affinity for fructose (Guillaume *et al.* 2007). It is linked to an alternative form of the *HXT3* gene, encoding for the corresponding transporter. Not all yeast strains have this, hence why it is important to choose a robust strain with both high tolerance to alcohol and affinity towards fructose when dealing with high alcohol and/or stuck fermentations.

But despite some yeast strains containing both forms of *HXT3*, the yeast will always take up glucose as a preference.

However, having the alternative form helps to better assimilate fructose. Within the Laffort range BO213 contains two alleles of the *HXT3* gene enhancing affinity for fructose and is, in itself, tolerant to 18% v/v alcohol. Other strains do not have this allelic form present at all and would struggle coping with high levels of sugar as well as alcohol.

Conclusions

It is evident that there is a lot of information to assess before picking a yeast strain for a particular wine.

For white wines:

- aromatic characteristics determined by enzymatic activities are important in the wine style; of relevance to white winemaking are thiol production, ester production and terpene release
- choosing a POF- strain and using an enzyme preparation purified from cinnamate esterase activity to minimise the formation of vinyl phenols before they reach a critical level that has a detrimental sensory impact
- alcohol, pH and temperature tolerances should be taken into account
- starting SO_2 levels and yeast strain production of SO_2
- understanding YAN and correct supplementation to ensure support for biomass production based on starting YAN, potential alcohol and nitrogen requirements of the yeast strain.

For red wines:

- aromatic characteristics determined by enzymatic activities are important for wine style
- choosing a POF- strain not critical as red wine phenolics inhibit this reaction
- use of an enzyme preparation purified from cinnamate esterase activity is key to minimising the amount of precursors available to *B. bruxellensis;* limiting the proliferation of *B. bruxellensis* post alcoholic fermentation will minimise ethyl-phenol production.
- alcohol, pH and temperature tolerances should be taken into account
- starting SO_2 levels and yeast strain production of SO_2. SO_2 binding is even more critical here as there are more SO_2 binding compounds naturally present in red must
- understanding YAN and correct supplementation to ensure support for biomass production based on starting YAN, potential alcohol and nitrogen requirements of the yeast strain.

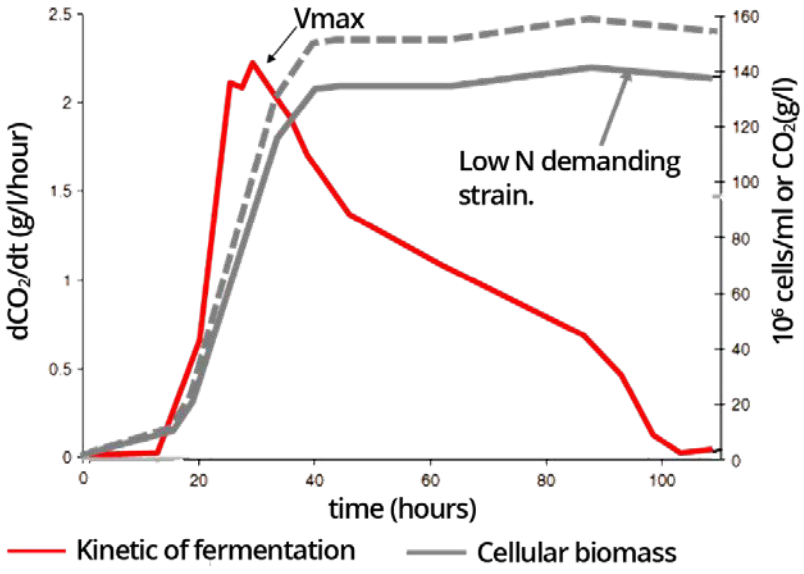

Figure 3. Graph demonstrating the difference between a high nitrogen demanding strain and a low nitrogen demanding strain in terms of cell biomass and fermentation kinetics (personal communication Marina Bely, University of Bordeaux).

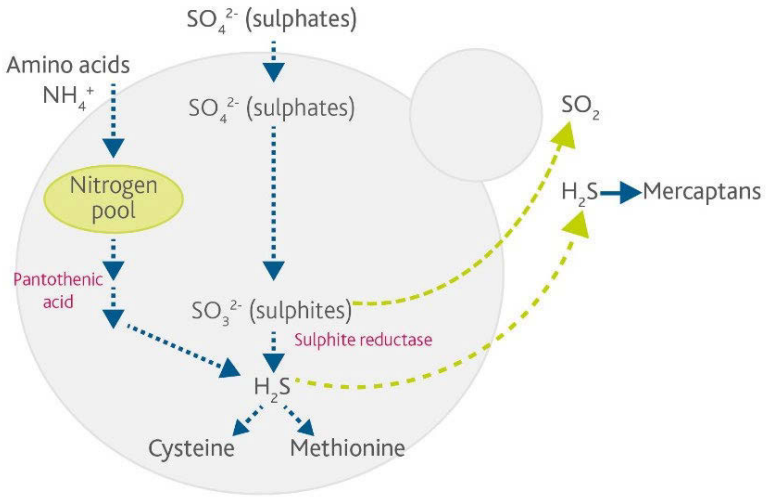

Figure 4. Schematic representation of the SO2 pathway in and out of the yeast cell.

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