Taking the stink out of fermentation

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'Rotten egg', 'burnt rubber' or simply 'sulfidic'- these are just some of the more unsavoury terms used to describe so-called 'bottle stink', the bane of winemakers worldwide. One of the culprits responsible for these reductive aromas is hydrogen sulfide (H_2S) – a compound capable of fouling ferments, even at low concentrations. Drawing on non-GM strain development techniques and information gained from our recent comparative wine yeast genomic analyses, we have found a new way to keep H_2S at bay. Three low-H_2S strains - Maurivin Advantage, Platinum and Distinction - have been developed from a commercial wine yeast, Maurivin PDM, which is widely used. Tried and tested successfully during the 2010 vintage, these three yeasts have been commercialised, earning their place in winemakers' 'toolkits'. Alongside judicious nitrogen management, and other strategies, these new strains have the potential to take the stink out of fermentation whilst retaining robust fermentation properties for red and white wines.

A SMELLY PROBLEM

If they were guests at a formal dinner, perhaps their 'character' would cause red-faced embarrassment. Through no fault of their own, these wines have 'gas' – an unsociable and unsavoury problem that is offensive to winemakers and consumers alike.

In the lexicon of wine aromas there is no mincing of words. Their character is 'off' – and likened to 'cabbage', 'garlic', 'burnt rubber', 'rotten egg' or, more imaginatively, the struck flint of a match head (Figure 1).

It is not surprising, therefore, that 'bottle stink' is a matter of concern. It is a common problem, and the majority of winemakers encounter the unwelcome smell at some point in their careers. Like 'gassy' guests at a dinner party, however, few are willing to discuss the problem openly.

Help, however, is at hand. The AWRI has been on a mission to target the central culprits – using non-GM (genetic-modification) technology and comparative genomic analyses – with the aim of identifying factors that play a key role in the creation of these unsavoury aromas. To tackle the problem, our 'prescription' includes judicious nitrogen management during fermentation in combination with 'non-gassy' yeasts that have been developed recently.

UNDERSTANDING THE CULPRIT

It is well documented that volatile sulfur compounds are key contributors to 'bottle stink'. A recent survey at the AWRI examined some 68 Australian red and white wines affected by the problem, representing vintages from 2004 to 2008 as well as a range of varieties and regions. The analysis found detectable H_2S in all 28 white samples and in 33 of 40 red wine samples analysed. Although the concentrations of hydrogen sulfide (H_2S) varied, the survey found it was likely that a proportion of the wines owed their reductive character to H_2S.

There is also no doubt that the presence of unpleasant volatile sulfur compounds in wine is a chronic problem in the global wine industry. Of the many volatile sulfur compounds found in wine, H_2S has received the most attention. Several chemical and biological mechanisms for its formation have also been documented.

Prevention has become a priority. Left untreated, wine can be tainted by H_2S production, leading to lower quality and likely rejection by consumers.

THE YEAST CONNECTION

Laboratory surveys of commercial *Saccharomyces cerevisiae* wine yeast world-wide have shown that almost all strains are capable of producing H_2S. Furthermore, under certain fermentation conditions in grape must, researchers have shown that some strains can produce sensorially-important amounts of H_2S off-flavour. They have also found that the degree of H_2S formation depends on which strain of yeast is used.

On a positive note, not all wine yeast strains produce H_2S; around 1% of naturally-occurring wine strains do not appear to produce the off-flavour at all. However, these strains don't necessarily possess other essential oenological traits, such as fermentation robustness. And there are other factors, too, that contribute to H_2S production: the availability of sulfur compounds, fermentation conditions, nitrogen content and the nutritional environment 'feeding' the yeast.

NITROGEN KNOW-HOW

Many winemakers have come to rely on the addition of nitrogen to prevent H_2S production during fermentation, and routinely add diammonium phosphate (DAP) to their grape juice.

There are restrictions, however, on the amount of DAP that can be added, and today's market trend towards additive-free wine is another factor driving winemakers to seek alternatives. Furthermore, improper use of DAP can dramatically change the wine's style and, in the worst case, produce an 'estery' taint.

There is also no guarantee that adding nitrogen will keep H_2S production under control. A recent AWRI survey of popular commercial yeast strains used in low-nitrogen Chardonnay and Shiraz musts confirmed that several produced significant amounts of H_2S regardless of DAP usage. Nitrogen management, therefore, is part of the puzzle but further research is required. A better strategy involves judicious nitrogen management during fermentation in combination with the 'right' yeast to deliver results.

DE-GASSING YEAST

The AWRI's application of yeast genomics has offered a prime opportunity to identify the 'right' yeast with far greater accuracy. It has also

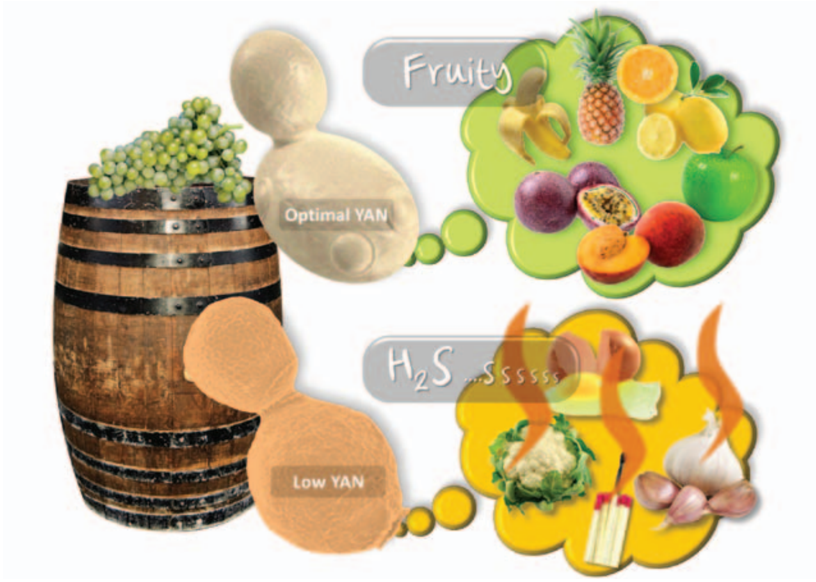

Figure 1. 'Reductive' aromas are often described as 'rotten egg', 'struck-match-flint', 'cabbage' and 'garlic'. These stinky characters can severely compromise wine quality, and hydrogen sulfide (H_2S) is one of the principal offenders; it imparts dreadful off-flavours at very low concentrations. Most of this H_2S gas in wine is a by-product of yeast metabolism, and it is usually produced in grape musts low in yeast assimilable nitrogen (YAN). The AWRI has developed a means of training a commonly-used wine yeast (Maurivin PDM) to release substantially less of the offending gas. As a result, three 'well mannered' wine yeast strains - Advantage, Platinum and Distinction - are now commercially available and represent a readily available new tool for winemakers to minimise off-flavours during fermentation, allowing fruity flavours to be expressed.

opened the door to the development of new non-GM strains, with the right genetic make-up, for reduced H_2S production.

Expertise in wine yeast genomics at the AWRI has played a key role in the advancement of wine science over the past five years. Cracking the complete genetic code of wine yeast strains has changed the way that researchers understand yeast metabolism from the inside out.

In our quest to understand the production of H_2S off-flavour, we singled out two yeast genes – MET10 and MET5 - in the genome of wine yeast. These genes determine the biochemical process leading to H_2S production during fermentation, via a metabolic pathway known as the sulfate reduction sequence (SRS).

Informed by these data, we then adapted a commercially-available wine yeast, Maurivin PDM, to produce less H_2S without compromising its fermentation performance and minimising impact on its many other desirable winemaking characteristics. The result was the isolation of several PDM-derived strains that offered a promising solution to the problem of excessive H_2S formation during fermentation. The next task was to analyse these strains in the lab, testing their performance and examining their genetic make-up to see how they could be used to tackle the problem of H_2S in commercial ferments.

TESTING STRAINS

Six low H_2S-producing strains derived from the parent yeast, Maurivin PDM, were developed, tested and patented. All six strains were tested in low-nitrogen synthetic grape juice and found to produce different amounts of H_2S, and they also produced different amounts of sulfur dioxide (SO_2). The three 'best' isolates were commercialised and marketed as Maurivin Distinction, Maurivin Platinum and Maurivin Advantage. These three strains were tested further in filter-sterilised 2007 Riesling grape juice containing a low concentration of nitrogen (YAN concentration of 145mg/L), and H_2S production was measured under simulated winemaking conditions.

The control strain, PDM, produced 72mg/L H_2S and 32mg/L SO_2, whereas Distinction produced the least H_2S (0.7mg/L) but the most SO_2 (143mg/L). Advantage produced 42mg/L H_2S and 39mg/L SO_2, while Platinum produced 8.1mg/L H_2S and 32mg/L SO_2. Platinum fermented at a similar rate to PDM, Advantage fermented faster and Distinction fermented slightly slower than PDM.

Drawing on the AWRI's expertise in yeast biology and comparative genomics, the next step was to identify the genetic changes that might account for the new, desirable low-H_2S trait in the PDM-derived strains. As predicted, at least one mutation was found in either MET5 or MET10 in all strains tested.

THE MLF FACTOR

During the 2010 vintage, commercial-scale trials were conducted with Maurivin PDM, Distinction, Platinum and Advantage, and it became clear that these strains provide winemakers with new options in their ongoing battle against H_2S and its potential to compromise wine quality.

> "...these strains provide winemakers with new options in their ongoing battle against H_2S and its potential to compromise wine quality."

However, we also found that the lowest H_2S-producing yeasts are not necessarily a cure-all; there is not one 'best yeast' that suits every circumstance. For example, some winemakers will want to induce malolactic fermentation (MLF), but the bacteria that perform this are sensitive to SO_2, the production of which can increase with decreasing H_2S. While the 2010 commercial-scale trials suggest that successful MLF can still be conducted, there is greater risk and it might be advisable to choose a yeast strain that produces some H_2S and non-inhibitory amounts of SO_2.

Thus, Distinction, which produces the lowest amounts of H_2S of the three Maurivin strains but produces considerably higher SO_2 than the PDM parent strain, is probably not the best option. Winemakers seeking to limit H_2S under these circumstances could consider Platinum or Advantage.

LOOKING AHEAD

It is abundantly clear from this study that by having yeast genome sequence data at our disposal, researchers at the AWRI have been able to develop non-GM, low-H_2S strains with a high level of precision, and this was achieved in a very short timeframe. And the outcome of this work? A practical solution to a perennial problem encountered by winemakers. We can, of course, do more if we understand wine yeasts at an even deeper level. For example, we aim next to develop low-VA (volatile acidity) variants from commercial strains.

In the meantime, re-developed strains such as PDM-derived patent-pending Distinction, Platinum and Advantage have much to offer. For example, copper sulfate usage could be reduced if winemakers use new yeast strains to limit H_2S production during fermentation. In addition, popular screwcaps could become even more widely used as the risk of producing 'reductive' aromas during storage is lessened. And, less DAP will be required to keep bottle stink at bay, thereby reducing costs to winemakers and, ultimately, consumers.

Above all, winemakers and consumers can have even greater confidence – knowing their favourite drop is unlikely to 'make a stink'.

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