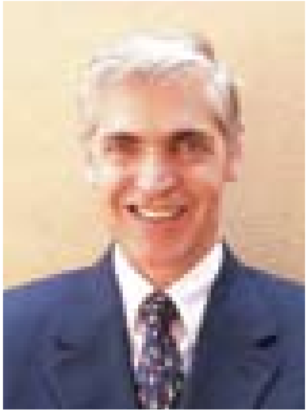
Not all wine yeast are equal



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It may come as a surprise to learn that there are over 200 commercial strains of *Saccharomyces cerevisiae* available for winemakers to work their magic on grape juice. Why so many? Surely one or two reliable workhorse strains should suffice; after all, don't they just make ethanol from sugar? The answer to this is an emphatic no; the more we look at the role(s) of yeast in winemaking, the more we are learning about their influences on appearance, aroma, flavour, mouthfeel and final ethanol concentration. And different yeast are more or less robust and efficient in converting the hostile environment of grape juice into wine. Indeed, not all wine yeasts are equal (Figure 1).

This article highlights some of the characteristics of yeast that are under the microscope in wine research, and looks at how microbiologists are attempting to expand the currently available repertoire of wine yeast to give winemakers even more options. The article also briefly considers what we are learning about some less desirable, wine spoilage yeast.

The essential roles of yeast in development of the sensory properties of wine

Grape juice offers relatively little in the way of a sensory experience, but after wine fermentation it has a myriad of attributes, including intense fruity aromas and flavours, great depth of colour and often, intense smooth or crisp mouth-feel. Wine research is starting to unravel the role that yeast play in this dramatic transformation process. For example, we now know that yeast play an essential role in the evolution of yeast-derived and grape-derived flavour molecules [1-3]. The former are largely responsible for the vinous character of wine and include volatile yeast metabolites, such as esters, higher alcohols, acids, carbonyls and sulfur compounds [1,4]: the latter contribute to the varietal character of wine.

Because of space limitations only two examples will be considered here, and both are of grape-derived flavours. Two important classes of compound exist in grapes as flavourless precursors: cysteine-conjugates [5] and sugar-bound (or glyco-conjugates) [6]. Cysteine-conjugates, which are present in Sauvignon Blanc and related varieties, are hydrolysed during fermentation by the action of yeast carbon-sulfur lyases [2,7,8] (Figure 2). This hydrolysis releases potent volatile thiols, including 4-mercapto-4-methylpentan-2-one (4MMP) and 3-mercaptohexanol (3MH), which elicit aromas of passionfruit, box hedge, grapefruit and blackcurrant. In addition to this, in some strains 3MH is esterified by an alcohol acetyltransferase, encoded by *ATF1*, to produce the more potent 3-mercaptohexylacetate (3MHA) [9]. Recent co-fermentation trials in our laboratory, in which Sauvignon Blanc

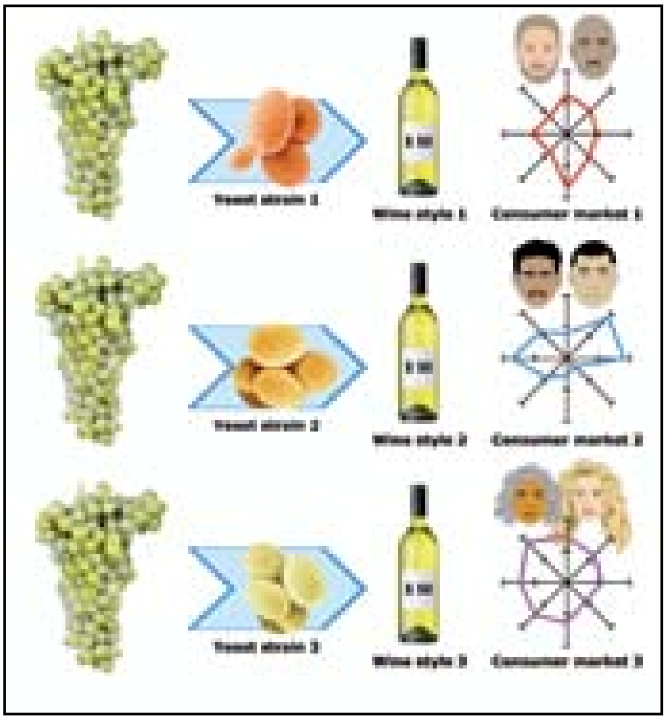

Figure 1. Not all wine yeast are equal. Juice from the same grapes will deliver quite different wines, depending on the choice of yeast. Because there are huge differences in consumer preferences, shaped by such things as ethnicity, gender and age, it is important for winemakers to know which yeast to choose if they want to tailor their wines for particular markets. We are only just beginning to understand the genetics underpinning the way different wine yeast 'shape' wines.

grape juice was inoculated with two wine yeast, demonstrated that one strain hydrolysed the conjugate and the other esterified the product, thereby complementing each other and enhancing Sauvignon Blanc flavours [28]. The non-volatile, glycoconjugates of grapes are especially important in highly aromatic grape varieties (i.e. Muscat and Riesling), but also to some non-aromatic grapes (i.e Chardonnay, Semillon, Sauvignon Blanc). Examples of conjugated compounds are the floral monoterpene alcohols linalool and geraniol, as well as norisoprenoids such as β-damascenone. In the non-aromatic varieties the flavour only develops during fermentation. Wine acidity, which enables a slow chemical hydrolysis of these non-volatile flavour precursors, has long been considered to be an important route of formation for these compounds. However, recent work, demonstrates the existence of at least four other pathways, all involving the contribution of yeast (Figure 3). Glycoconjugate hydrolysis by extracellular yeast glycosides (Pathway II in Figure 2) appears to be quantitatively the most important during fermentation [3].

Colour is another very important sensory attribute of wine and in red wine is largely determined by anthocyanins and anthocyanin derived pigments. Numerous viticultural and winemaking factors are involved in the formation and stabilisation of red wine colour. However, the role of yeast in this has only recently been studied [10,11]. Choice of yeast strain has been found not only to affect the depth of colour but also the red-purple tones of its hue [12]. Work is in progress to understand the biochemical mechanisms so as to provide genetic strategies for tailoring wine colour.

Thus, it is clear that yeast plays a crucial role in shaping the sensory properties of wine, and different yeast impart different characteristics on wine. In this regard wine yeast strain development is an important source of new genetic diversity to increase the options available to winemakers. For example, the production of intra- and inter-specific hybrids from the *Saccharomyces genus,* is enabling the generation of novel yeast with the robust fermentation properties of commercial wine yeast and the diverse sensory properties offered by other species. Hybrids of *S. kudriazevii* and *S. cariocanus* with *S. cerevisiae* generated at The Australian Wine Research Institute (AWRI) are currently undergoing winemaking evaluation by the industry [13].

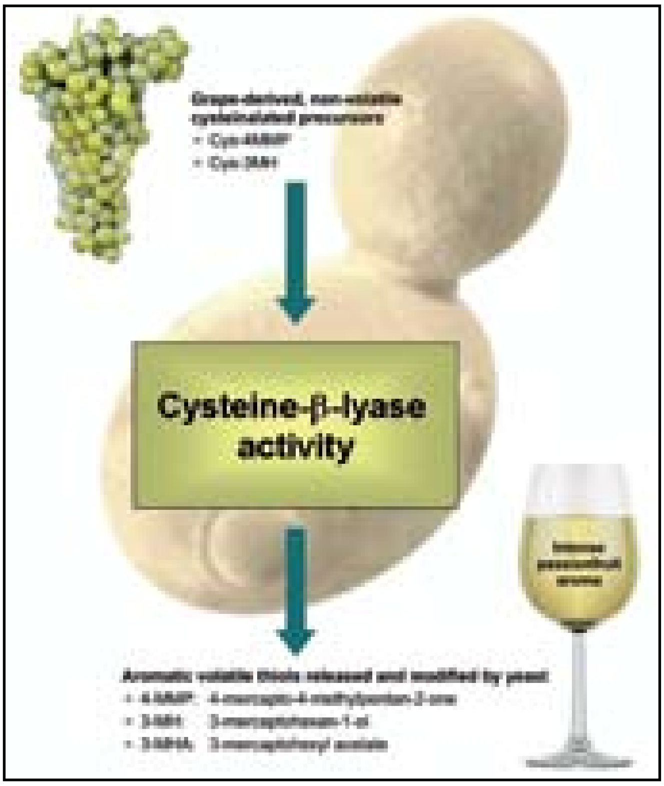

Figure 2. Release of volatile thiols by wine yeast. Overexpression of a cysteine-β-lyase in yeast results in a Sauvignon Blanc with, for example, enhanced passionfruit aroma [28].

Using yeast to control the alcohol content of wine

In winemaking, more mature grapes generally deliver greater intensity and complexity in flavour. However, lengthy maturation, particularly in warm climates, translates into high sugar levels in grapes and this, in turn, leads to wines with high levels of ethanol. High alcohol levels can compromise wine flavour and excessive consumption of alcohol is associated with health issues. Therefore, consumer and market demands are driving research to develop strategies to reduce the ethanol content of wine, without compromising wine quality.

Physical processes to remove sugar from grape juice or ethanol from wine can be costly and compromise wine quality. Alternatively, it is possible to persuade wine yeast to make less alcohol by diverting their metabolism away from ethanol production, and genetic engineering can be used to achieve this end. For example, recombinant yeast carrying the *Aspergillus niger* gene, encoding glucose oxidase (β-D-glucose:oxygen oxidoreductase) [14], and over-expression of *S. cerevisiae* glycerol-3-phosphate dehydrogenase genes (*GPD1* [15] and *GPD2* [16]), have been shown to deliver reduced levels of ethanol. However, none of the above changes have yet been successfully introduced into wine yeast without significant impacts on wine quality, and GMO yeast are not yet used in European or Australian winemaking.

Thus, adaptive evolution, a non-GMO approach, is being developed at AWRI to generate 'low-ethanol' yeast. While not yet ready for publication, results to date are very promising.

Stress tolerance and robust wine yeast

Wine yeast encounter numerous stresses from the moment they are inoculated into grape juice and throughout the subsequent fermentation. For example, grape juice is acidic (typically pH 3.0 – 3.5), high in sugar (200–270 g/L), and deficient in many critical nutrients (nitrogen, lipids and sterols), and fermentations may be performed at cooler than optimal temperatures (e.g. 18°C rather than 30°C). Fermentation to dryness (when all sugar has been consumed) in such an environment is a challenge, the yeast response to which is the subject of intense investigation [17-19].

Commercially available wine yeast exhibit great diversity in degrees of robustness [20] and unfortunately the most resilient strains do not necessarily deliver optimal sensory characteristics to the wine. Generating yeast capable of making top quality wine and performing consistently and reliably in grape juice, is one of the many challenges in wine yeast development. Due to the relative genetic complexity of stress tolerance and issues associated with using GMOs in the wine industry, the most promising strain improvement strategies involve the use of non-GM approaches, particularly adaptive evolution [21-24].

Systems biology based approaches to studying wine yeast – an eye to the future

One of the key challenges in wine science is to obtain a thorough understanding of the biology of yeast and to apply this knowledge to develop novel strains with improved winemaking features.

The use of 'omic methodologies and systems biology based approaches will transform wine yeast strain development. Systems biology describes the process of combining 'omic and computational methodologies to create models of complex biological systems. Implementation of 'omics approaches in wine science will enable accurate 'mapping' and definition of fermentation. By combining this with mathematical modelling of metabolic flux, scientists will be better able to manipulate yeast to the advantage of winemakers.

One key technology currently being implemented for the study of wine yeast performance is the use of 'gene deletion library' approaches. This technique enables genome-wide screening to identify genes associated with specific activities, replacing traditional random mutagenesis approaches with an ordered array of strains, each with a different, defined mutation. This library can then be systematically screened for strains that display altered winemaking characteristics. However, the currently available deletion libraries are in laboratory strain backgrounds and lab strains are not well suited to winemaking. Hence, researchers at AWRI are working in collaboration with Prof Charlie Boone (University of Toronto, Canada) to construct a deletion library in a wine yeast background.

Wine spoilage yeast

Wine, by virtue of its high acid and ethanol content, is intrinsically stable to attack by most microbes. Thus, in contrast with other foods and beverages, wine is made in sanitary but essentially non-sterile conditions. As a consequence, although *S. cerevisiae* is the key and predominant wine yeast, the eventual product is often the result of several yeast species, and strains of those species, interacting with one another [25]. Two schools of thought have emerged when it comes to the involvement of 'wild' *Saccharomyces* and non-*Saccharomyces* yeast in winemaking; that they are a natural and desirable element that adds to the complexity of wine or conversely that they pose a significant risk of adverse outcomes and lead to product spoilage.

Yeast spoilage in wine leads to undesirable aromas and flavours, haze and deposit formation, carbonation and bottle explosion. Wine spoilage due to post-bottling refermentation can in some cases be caused by growth of the production strain of *S. cerevisiae* that was inoculated into the grape must; thus, the definition of wine spoilage yeast is entirely functional. That said, few yeast species have been isolated from wine [26], as the ecosystem from grape to wine is highly selective. Undoubtedly, one of the most internationally widespread and economically important spoilage yeast is *Dekkera bruxellensis,* and this is the subject of a separate article in this issue of Microbiology Australia (see Curtin, Grbin & Henschke).

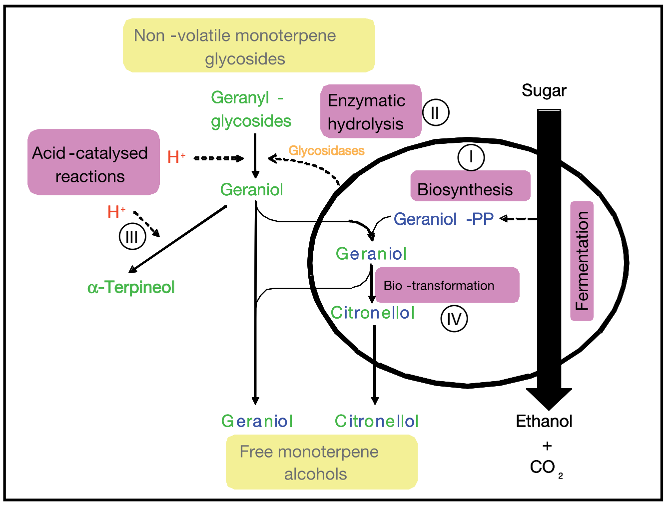

Figure 3 Pathways for terpene formation: geraniol synthesis: The *de novo* biosynthesis of monoterpenes by some yeast strains via the sterol pathway (I) [27]; the direct yeast-driven hydrolysis of juice glycosides, catalysed by the various yeast glycosidases (II); the acid-catalysed transformation of terpenes released by yeast via glycoside hydrolysis (III); and the yeast-driven transformation of terpenes present in the juice or released by yeast via glycoside hydrolysis (IV).

Closing statement

S. cerevisiae has been at the forefront of scientific research for many decades, not only because it is an excellent model organism for fundamental research, but also because of its importance in the food and beverage industries. Nowhere in science is the nexus between applied and fundamental research as evident as it is in yeast research; originating as it did in studies on brewing and wine yeast. Now the latest innovations in this field are being returned to their industrial roots so that the oldest of biotechnologies can benefit from the newest techniques in the biological sciences. In the context of what is covered in this paper, winemakers are set to benefit from recent developments, because they will ultimately have improved options to create wine. Not all wine yeast are equal and the differences between them are likely to widen – and this is great news for winemakers.

Acknowledgements

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Nominations are to be in the hands of the General Manager, Chris Daly before 5pm Friday 8 June 2007. Please use the Nomination for the Position of Vice President, Scientific Affairs form, which is set out below. The form can also be obtained from the National Office by emailing Chris Daly, General Manager at chris@theasm.com.au

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Proposer (FASM / MASM / SASM / Honorary Life Member)

Name: ______________________________

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