Harnessing AWRI's yeast and bacterial research to shape 'next-gen' Chardonnay Part 1: 'Wild' and 'non-conventional' yeast


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Australian Chardonnay is primed for a comeback. After rejection by so-called 'ABC' consumers who preferred 'anything but Chardonnay', there is growing speculation that Australia is reversing the trend, tempting markets with 'CBA', or 'Chardonnay's back again'. But, there is more to Australia's new or 'next-gen' Chardonnays than mere persistence. Fresher, lighter-bodied, less oaky and more textured, the Chardonnays now entering the market are higher in complexity and 'minerality' with hints of so-called 'struck-match-flint' attributes. To help winemakers create such characters, the AWRI has developed a fermentation toolkit. Using the best science available, the toolkit offers better understanding and know-how regarding the application of selected commercial yeast, 'non-conventional' yeast and 'wild' yeast. It also offers expertise regarding malolactic fermentation and nutrients to help winemakers create more refreshing, crisp and livelier styles. The toolkit has been refined through conventional sensory studies as well as consumer group profiling. To win back consumers, however, Australia's 'next-gen' Chardonnays also require appropriate marketing. The AWRI sees an opportunity for 'CBA' styles to transcend all market price points together with a clearer style guide to meet the expectations of target consumer groups.

IS ANYTHING WRONG WITH CHARDONNAY?

In many ways, it is astounding that a variety that has contributed approximately 50% of Australia's annual white winegrape crush for more than a decade has, in recent years, become relatively unpopular. This perhaps speaks to the perceived image problem surrounding Australian Chardonnay alluded to in various press reports, such as those concerning a Treasury Wine Estates (then Foster's Wine Group) meeting to discuss Chardonnay's plight ('It's grape versus grape as Chardonnay takes on Sauvignon Blanc', *The Australian*, 14 October 2009). Memories of overtly 'buttery', 'oaky' and 'flabby' Chardonnay styles from the 1980s and 1990s continue to influence consumers who have been won over by Sauvignon Blanc imported from New Zealand – despite many changes in the styles of Chardonnay produced over the past 20 years.

Current Chardonnay wines that are winning medals and trophies in the wine show circuit are, in fact, the antithesis of wines that turned a generation of consumers into 'anything but Chardonnay' (ABC) drinkers. Moreover, some commentators have suggested that it is the wide range of Chardonnay styles that led to consumer confusion, whereas Sauvignon Blanc wines are more predictable. Success in the premium and super-premium categories will undoubtedly have a flow-on effect, enhancing consumer awareness that 'next-gen' Australian Chardonnay wines are starting to emerge that are 'fresh', 'lighter-bodied' and 'moderately oaked'. Nonetheless, it is critical that Chardonnay wines with these characteristics are available across all market segments so that adventurous Sauvignon Blanc consumers are not disappointed upon revisiting Chardonnay.

With this in mind, the solution for the reinvigoration of Chardonnay will not be to simply turn away from warm-climate fruit – particularly with the spectre of global warming ever-present. In 2004, former AWRI director Terry Lee was quoted as saying: "We have the technology to overcome very warm climates and we are the best at understanding the components responsible for varietal flavour"[1]. Perhaps in light of changing consumer preferences, an adjustment of winemaking approaches to warm-climate fruit is required.

This two-part report aims to outline research conducted over the past decade by the AWRI, focused upon microbial modulation of Chardonnay 'quality' parameters. An outcome of this research is the availability of several tools available for immediate use by winemakers aiming to produce 'next-gen' Chardonnay.

WHAT ARE THE CHARACTERISTICS OF 'NEXT-GEN' CHARDONNAY?

Beyond the rationalisation and survival period currently advised by industry peak bodies[2], a shift to sustainable production at higher price points is the shared goal of the Australian wine industry; therefore, the common goal for all Chardonnay producers must be to increase quality. What are the 'quality' parameters important in defining 'next-gen' Australian Chardonnay?

During last year's 14th Australian Wine Industry Technical Conference 'Next-gen' Chardonnay workshop, this question was asked through a structured

Figure 1. Word-cloud summarising attributes associated with 'next-gen' Chardonnay by participants of the 'Next-gen' Chardonnay workshop held at last year's 14th Australian Wine Industry Technical Conference. Image created using the Wordle web service (www.wordle.net). Font size depicts frequency of term usage.

tasting of benchmark Australian and international Chardonnay wines. Under the tutorship of Nick Stock (Stockwine), Tom Newton (Eileen Hardy, Hardy Wines), Mike Peterkin (Pierro), Kym Schroeter (Bin A, Penfolds Wines) and Virginia Willcock (Heytesbury, Vasse Felix), participants explored wines from ultra-premium single vineyards through to flagship multi-regional wines, searching for the common quality indicators and production techniques underpinning them, which have the potential to reinvigorate Australian Chardonnay.

Amongst workshop participants, it was evident that oak-acid balance was a key differentiator between wines that might be considered classical in style, and those that could be held up as divining 'next-gen'. How much oak flavour is too much? How much acid is too much? These questions were robustly debated and not always agreed upon for each wine tasted, but the general consensus was that 'next-gen' Chardonnay is higher in natural acidity and not overtly oaky. Some participants completed a survey where they were asked to list terms they associated with 'next-gen' Chardonnay, the results of which are summarised in Figure 1. Those terms, or groups of closely-related terms, that were most commonly listed appear as larger words in the 'word-cloud'. Aside from oak- and acid-related terms, workshop participants perceived 'next-gen' Chardonnay to be complex and textural, exhibiting 'primary fruit' characters with some degree of 'struck-match-flint' aroma, and the ubiquitous but ill-defined 'minerality'. Do these attributes or sensory characteristics constitute 'next-gen' Chardonnay quality parameters?

While it is true that expert and novice wine tasters employ different neural networks when tasting wine[3], and the average consumer can be influenced strongly by factors such as branding and context[4], intrinsic sensory attributes of Chardonnay affect consumer preferences[5] and sensory acceptability is a strong foundation for repeat purchase of wine[6]. Consequently, it is possible to develop a sensorially-driven concept of what constitutes quality and then examine the underlying chemical composition of high quality wines – exemplified by studies of Burgundian Chardonnay[7,8,9]. A common theme in consumer sensory research at the AWRI, however, is that a single sensorially-defined concept of quality will not appeal to all consumers[10]. In fact, a recent study of key Sauvignon Blanc aroma compounds and their influence upon consumer preference highlighted that a significant proportion of Adelaide wine consumers prefer wines high in methoxypyrazines[10] in combination with other aroma compounds. It seems reasonable to expect there are consumers out there for so-called classical-style Chardonnay.

A broader approach to finding quality indicators involves multivariate statistical methods applied to compositional and sensory descriptive analysis data, which has proven successful in elucidating critical compounds either driving or masking aromas important in a sample set[11]. Extending this framework with subsequent consumer testing enables preferences to be mapped against sensory attributes and the compositional variables contributing to them – providing potential 'quality' indicators for a larger proportion of the population.

A study conducted at the AWRI[12] applied this approach to a set of 20 unwooded Chardonnay wines that spanned the sensory space observed in a preliminary tasting of 60 wines from various Australian winemaking regions. The goal was to elucidate the 'varietal' character of Australian Chardonnay wines where oak treatment and malolactic fermentation influences were minimised. A large number (n=45) of volatile aroma compounds were accurately quantified using stable isotope dilution (SID) assays, yet only 23 were found to be above their sensory detection thresholds in at least one wine. Of these, fermentation-derived acetate esters and ethyl esters, along with a subset of higher alcohols and volatile fatty-acids, were most predictive of sensory characteristics differentiating the wines – 'floral', 'pineapple' and 'citrus'. Chardonnay and Riesling wines exhibiting these characteristics were preferred by a majority of consumers tested[5]. Reconstitution studies, where different combinations of aroma compounds are added to a relatively neutral wine, demonstrated that a Burgundian Chardonnay of intermediate 'typicality' could be converted to a 'good example' through addition of similar compounds, along with some grape-derived and grape-derived/yeast-modified compounds, such as linalool, Ð-decalactone, 4-vinylphenol and 4-vinylguaiacol[8].

With this knowledge, can we then predict the likely chemical composition of a 'next-gen' Australian Chardonnay? Not entirely – some attributes, such as 'minerality' and 'texture', are not clearly defined and, therefore, cannot yet be explained by chemical composition, while 'complexity' by its very definition is not driven by any single compound. Nonetheless, on the basis of compositional studies mentioned above, it is evident that in addition to managing the balance of acidity and oak-flavour, crafting a 'next-gen' Chardonnay style requires consideration of yeast and bacterial influence.

'WILD' FERMENTATION AND 'NON-CONVENTIONAL' YEAST

Traditional methods of Chardonnay production favoured in renowned regions such as Burgundy involve barrel fermentation conducted with 'wild' or indigenous, rather than selected, yeast. Ferments are either allowed to start spontaneously with indigenous yeasts or are inoculated with *pied-du-cuve*, previously prepared from pre-harvested grapes. These spontaneous, or 'wild', fermentations involve a large number of yeast species and strains[13], and are often considered to result in wine with greater complexity compared with those made with inoculation of a single commercial strain[14, 15, 16, 17, 18]. Nonetheless, there is inherent risk in relying upon an unknown population of yeast to complete fermentation, and greater likelihood for fermentation problems or formation of off-flavours.

The considerable international demand for Australian wines, in part, owes its success to controlled fermentation with selected yeast. However, the 'next-gen' Chardonnay workshop revealed a growing trend to ferment Chardonnay with 'wild yeast'; yet, there is scant information on the sensorial role of 'wild' yeast in the scientific literature. As a first step, the AWRI has investigated the relative complexity of wines made with inoculated and 'wild' yeast by dividing Chardonnay juice samples prepared in the winery into two lots: one lot being inoculated with a selected yeast (EC1118 or D47/CY3079) and the other lot being allowed to undergo spontaneous fermentation. After fermentation, the clarified wines were subjected to extensive chemical analysis in order to reveal any differences between each wine pair.

The main differences between inoculated and 'wild ferment' wines were found amongst the volatile yeast metabolites (Figure 2). Principal component (PC) analysis showed that the inoculated wines formed a distinct group that was associated primarily with the esters ethyl hexanoate ('green apple', 'fruit') and 3-methylbutyl acetate ('banana', 'pear'). By comparison, the 'wild' yeast wines showed high variability in volatile compounds that are known to contribute to wine aroma, with higher concentrations of 2-methylpropanol ('spirituous'), 2-methylbutanoic acid ('fruit'), ethyl 2-methylpropanoate ('sweet ethereal fruit'), ethyl decanoate ('grape') and ethyl dodecanoate ('fruit') potentially being sensorially important. 'Wild ferment' wines also tended to

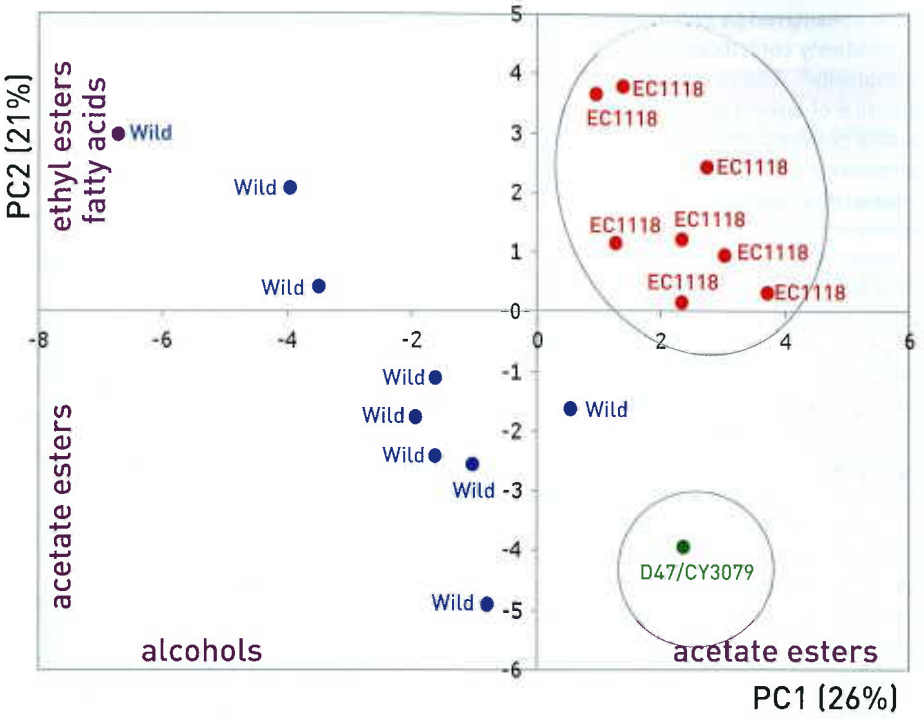

Figure 2. Principal component plot illustrating separation of Chardonnay wines made with inoculated (EC1118 and D47/CY3079) and 'wild' fermentations on the basis of volatile aroma compound concentrations (adapted from Varela *et al.* 2009[18]).

contain higher concentrations of H_2S. In addition, both wine types contained some of the volatile compounds that have been identified as being associated with sensory descriptors of unwooded Australian Chardonnay wines. They include the alcohol, 2-methylbutanol, the ethyl ester, ethyl hexanoate, the acetate ester, 3-methylbutyl acetate, and the acids, hexanoic and octanoic acids[11, 12].

Consequently, this work has led to a list of target aroma compounds that appear to be associated with flavour-diverse and complex 'wild fermentation' wines. Ongoing research at the AWRI aims to develop inoculation strategies that impart 'wild-yeast' character, or complexity, while minimising the associated risks of spontaneous fermentation. Several approaches have been investigated including non-*Saccharomyces* species[19], non-*Saccharomyces cerevisiae* species[20] and *Saccharomyces* hybrids[21, 22].

Non-*Saccharomyces* yeasts

The so-called 'non-*Saccharomyces*' yeasts refer to those species that are invariably found in grape juice and are thought to contribute strongly to 'wild yeast' ferments. These species are also well known to play a role in the early stages of conventional fermentation when yeast starter cultures are used since sulfite does not completely suppress their activity. The potential of some of these strains and species to modulate wine flavour has been recognised for some time, and they might represent a more reliable means for adding flavour diversity and complexity to wine. Early studies on the potential of non-*Saccharomyces* yeasts at the AWRI involved screening their growth and aroma characteristics[23] and carrying out co-fermentation trials with selected species in Chardonnay[19, 24].

Because non-*Saccharomyces* have poor tolerance to ethanol, they are typically used in co-fermentation with a robust *Saccharomyces cerevisiae* wine yeast to make wine. In general, non-*Saccharomyces* can substantially modulate both the aroma and flavour of wine. For example, wines made with the *Candida stellata* strains AWRI 861 and AWRI 1159 were found to contain more glycerol and less ethanol than the reference wine, and aroma/taste profiles were different from *S. cerevisiae* and from each other (Figure 3, see page 18). Both the *C. stellata* 861 and the *S. cerevisiae* 838 wines had in common 'fruity ester', 'pineapple' and 'citrus' aroma attributes, whereas the *C. stellata* 861 wine, in addition, contained a substantially greater intensity of 'floral/rose' and 'banana ester' attributes. In contrast, the wine made with *C. stellata* 1159 exhibited higher acidity, and was rated highest in 'cooked apricot' aroma, 'volatile acidity' and 'sulfidic'. Surprisingly, when this yeast was used in a winery setting, the wine's 'negative' attributes

were considered by some winemakers to positively contribute to Chardonnay complexity[21]. The common Burgundian practice of ageing on lees in barrel is widely believed, by some experienced winemakers, to integrate these initially undesirable flavours and to enrich the wine's complexity.

Yeast AWRI 863 is another AWRI non-*Saccharomyces* selection with potential to build flavour complexity in Chardonnay. In a barrel-fermented Chardonnay trial (2004), the wine had a distinct 'tropical fruit' aroma with 'pineapple', 'banana' and 'bubblegum'. The palate was especially noteworthy with a dry 'mineral' character, tight acid, creamy and rich palate with good oak integration, similar in style to some 'wild ferment' wines. Wines were low in volatile acidity (VA) and, sometimes, alcohol, higher in succinic acid while pH and TA were typical of *S. cerevisiae* wines.

These results demonstrate that, in addition to producing wine with different chemical composition, non-*Saccharomyces* can produce wines with a different sensory profile from those of the widely used *S. cerevisiae* strains. Non-*Saccharomyces* wines are envisaged to be useful blending components for lifting aroma and improving palate structure. The potential of non-*Saccharomyces* yeasts has recently been reviewed by Jolly and colleagues[17], Ugliano and Henschke[25] and Ciani and colleagues[26].

Several selections of non-*Saccharomyces* yeasts are now commercially available as active dried yeast preparations. Examples include various combinations of *Kluyveromyces thermotolerans, Torulaspora delbrueckii* and *S. cerevisiae* that have been produced by Chr. Hansen and Lallemand (www.chr-hansen.com; www.lallemand.com[27]).

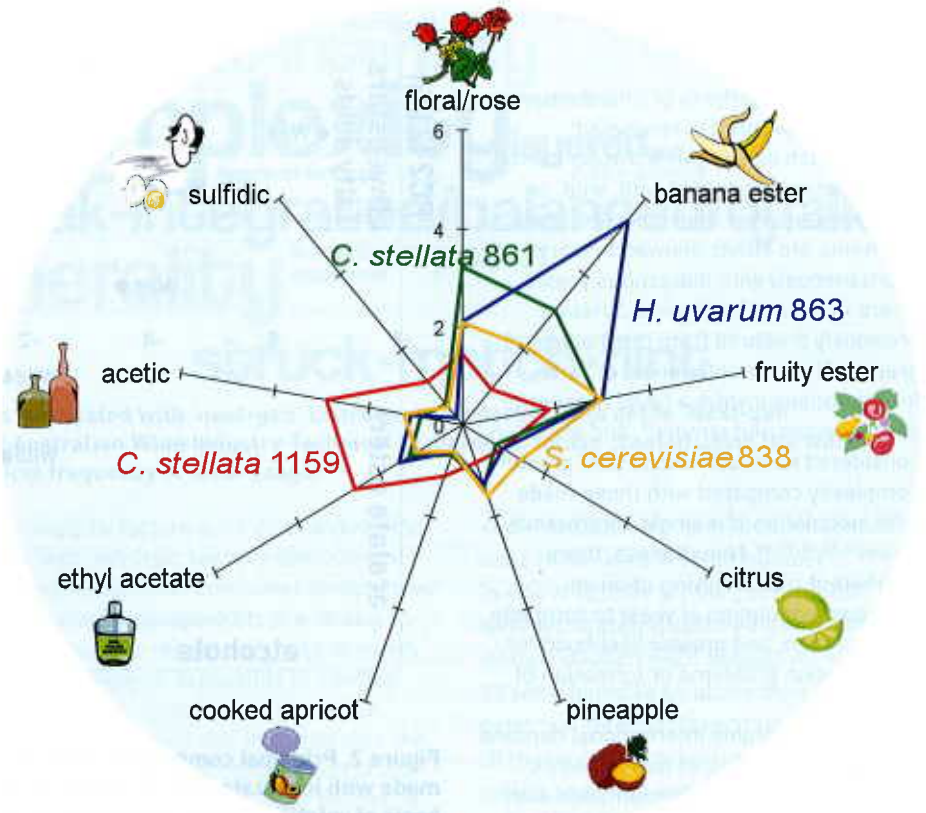

Figure 3. Aroma profiles of Chardonnay wines made by fermentation with experimental strains of non-conventional yeast and compared with a conventional strain of *Saccharomyces cerevisiae*. (Source: Henschke et al. 2002[24]).

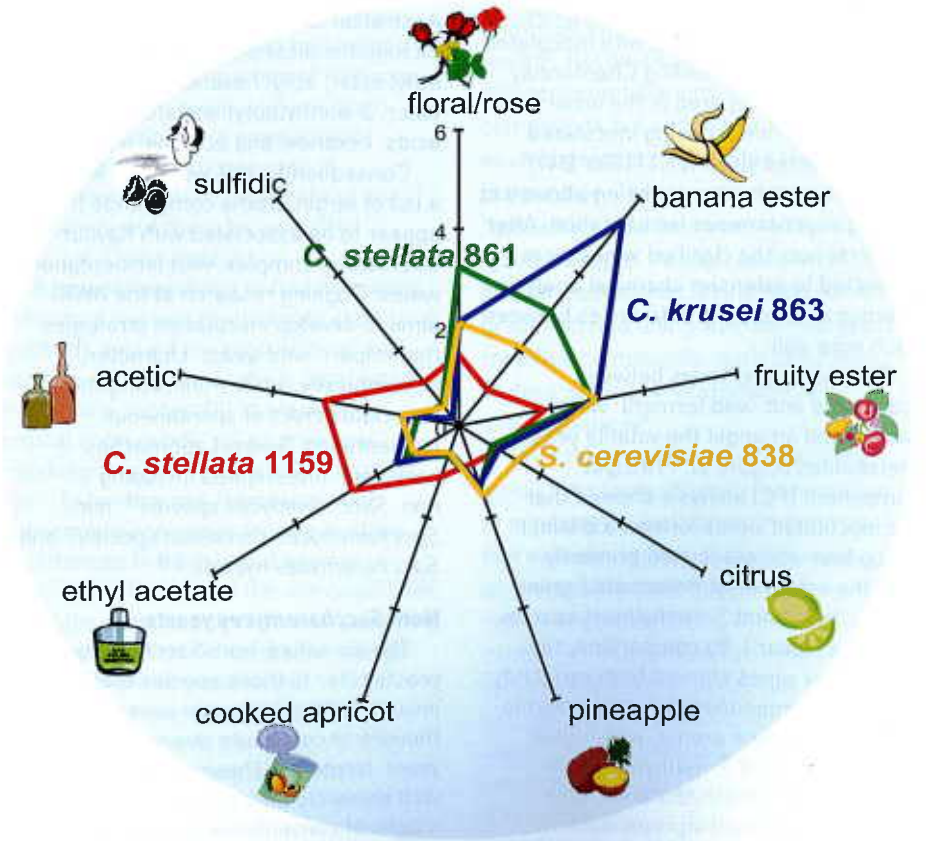

Figure 4. Aroma profiles of Chardonnay wines made by fermentation with *Saccharomyces bayanus* AWRI 1176 and AWRI 1375 and *Saccharomyces cerevisiae* AWRI 838 at 18°C. (Source: Fogarty 1998[55])

Saccharomyces bayanus

A previous AWRI report[28] detailed the winemaking potential of the yeast species *Saccharomyces bayanus*. This yeast, although being genetically closely related to *Saccharomyces cerevisiae*, is a cryotolerant yeast (can ferment below 10°C) and has interesting winemaking properties that are not apparent with *S. cerevisiae*. Nevertheless, it can be used as a direct replacement for *S. cerevisiae* strains, although in practice it is slightly less tolerant to ethanol in highly clarified, high-Brix juices so good nutritional conditions can be beneficial. Figure 4 shows the sensory profiles of Chardonnay wines made with an industry benchmark wine

yeast (AWRI 838) and the complexity garnered by fermentation with *S. bayanus* AWRI 1176 or AWRI 1375.

The AWRI selections of *S. bayanus* produce a profile of aroma- and flavour-active minor fermentation products differing from that of typical *S. cerevisiae* wine yeasts. These strains produce higher concentrations of the higher alcohols 2-phenylethanol (old-rose), iso-butanol, and iso-amyl alcohol ('fusel-like'/'whiskey' aromas), which might contribute to the characteristic 'savoury' nature of *S. bayanus* wines. Of the volatile esters, 2-phenylethyl acetate ('floral' aroma) is typically produced in greater amount by *S. bayanus* yeast strains than by *S. cerevisiae* strains, as is iso-amyl acetate ('banana' aroma) at low fermentation temperature, although an elevated 'banana' aroma in Chardonnay wines made at low temperature with AWRI isolates has not been observed. Ethyl lactate and diethyl succinate are also generally more abundant in wines made with *S. bayanus*. Chemical analysis of aroma- and flavour-active compounds cannot yet give a complete picture of the sensory properties of a wine. Quantitative sensory descriptive analysis is a powerful tool for discriminating between wines made using different yeasts. Chardonnay wines made with *S. bayanus* are generally characterised by more complex aromas and a less dominant fruity fermentation bouquet (Table 1[20]). Some of the different aromas that are characteristic of AWRI 1176 and AWRI 1375 are 'cooked orange peel', 'apricot', 'honey', 'yeasty' and 'nutty', which are considered by some experienced judges as positive contributors to wine aroma dependent on the wine style. The mouth-feel of wines made with *S. bayanus* AWRI 1176 or AWRI 1375 consistently appears to be fuller than that of wines made with control strains of *S. cerevisiae*, often showing greater weight and texture with lower apparent acidity and greater fruit persistence[29].

Table 1.Aroma descriptors for Chardonnay wines made using *Saccharomyces cerevisiae* and *Saccharomyces bayanus*.

Yeast	Aroma description
S. cerevisiae AWRI 838	*estery, pineapple, peach,* floral, ethyl acetate
S. bayanus AWRI 1176	*estery,* pineapple, peach, floral, melon, *apricot, honey, nutty*
S. bayanus AWRI 1375	*estery,* pineapple, citrus/lime, *floral, apricot, honey, nutty*

Descriptors that were considered more important are shown in italics.

Saccharomyces interspecific hybrids

Although the genetic diversity of *S. cerevisiae* wine yeast and, hence, flavour diversity is considered significant, the *Saccharomyces* genus contains a group of closely related species (so-called *sensu stricto* group) that appear to have interesting flavour properties but are mostly incapable of fermenting grape juice to dryness. These species can form hybrids and, through this process of 'genome shuffling', the flavour properties of the non-*S. cerevisiae* species can be combined with the robust fermentation properties of *S. cerevisiae*.

Several hybrids have been bred at the AWRI and winemaking characteristics of selected strains studied in Chardonnay on a winery scale, 1000 to 20,000L stainless steel tanks and barrel (old) fermentation trials. The *Saccharomyces* species under consideration, which included *S. paradoxus*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii* and *S. mikatae*, were crossed with a commercial *S. cerevisiae* wine yeast strain[22]. Hybrids were selected on their ability to ferment efficiently and to tolerate 14% ethanol. These new strains, which were produced by natural mating, are not considered GMOs and can be immediately used in commercial wine production.

Of the non-*S. cerevisiae* species studied only two are found naturally in grape juice and capable of complete fermentation. As mentioned above, *S. bayanus* is a cryotolerant yeast and is usually detected in musts from cool winemaking regions, such as Alsace and Adelaide Hills. The two AWRI selected strains (AWRI 1176 and AWRI 1375) have been extensively characterised in Chardonnay and found to possess useful winemaking properties at normal winemaking temperatures[29]. However, while they are capable of completing fermentation, they are less robust than many *S. cerevisiae* strains. *S. paradoxus* is sometimes found in grape must, and being cryotolerant it has some properties in common with *S. bayanus* but having low vigour, it generally fails to complete fermentation[30, 25].

All of the hybrid yeast have medium to high vigour fermentation properties similar to *S. cerevisiae* AWRI 838, and produce low residual sugar in Chardonnay. Basic wine chemical parameters are also similar to those produced by AWRI 838. The aroma profile of *S. cerevisiae x S. paradoxus* hybrid AWRI 1501 in Chardonnay was described as low in ester with 'nuts' and 'figs'[21]. The aroma profile of *S. cerevisiae x S. cariocanus* hybrid AWRI 1502 (Maurivin AWRI Fusion) was described as 'peach', 'pear', 'nectarine', 'violets', 'lemon fruit/zest', 'creamy', 'mineral' and 'matchstick' based on wines made in a barrel- and tank-fermented Chardonnay trial. The palate was described as complex, full flavoured, tight, slightly sour, leaner, phenolic and having flavour persistence[21]. In barrel-fermented Chardonnay trials (2004/2007), the aromas produced by the hybrid *S. cerevisiae x S. kudriavzevii* AWRI 1503 (Maurivin AWRI 1503) were described as 'apricot', 'peach', 'floral', 'tropical', 'banana', 'citrus', 'herbal', 'biscuit', 'cashew', 'nutty' and 'waxy', and the palate showed a creamy texture with complex, lively acid, with a late mineral character[21]. The *S. cerevisiae x S. bayanus* hybrid AWRI 1505 combines the robust fermentation properties of its parent *S. cerevisiae* strain and a blend of the flavour properties of both parent yeasts. In a recent winery trial of tank-fermented Chardonnay, this hybrid produced a wine described as having aromas of 'pineapple', 'tropical fruit', 'floral', 'lemon rind' and 'ester', combined with an estery, savoury, mineral palate and rich, creamy mouth-feel[21]. All of these hybrid yeasts produce a great diversity of flavours, many of which are not typical of *S. cerevisiae* strains, and appear to be well-suited for building flavour complexity in white wines, such as Chardonnay.

DISCUSSION

Chardonnay wines succeeding in the wine show circuit over recent years epitomise a trend towards increased levels of natural acidity coupled with more subtle use of oak—'next-gen' Chardonnay. If these wines are the style leaders around which a positive marketing campaign to lure consumers back to Chardonnay can be constructed, it is essential that wines consistent with these styles exist across market segments. Some of the techniques used to build complexity in super-premium wines are not readily transferrable

to wines sold at lower price points, necessitating innovation.

Within the first part of this article, we have outlined various 'non-conventional' microbial tools and practices applicable to modulating Chardonnay aroma and flavour, whilst retaining control of the fermentation process. If 'complexity' is a key target, then harnessing yeast and bacterial diversity to deliver wine expressing different flavours and aromas can augment viticultural efforts to maximise fruit quality. Part 2 of this article, which will be published in the March/April issue of the *Wine & Viticulture Journal*, will expand upon the microbial toolkit available for shaping 'next-gen' Chardonnay styles, exploring applications of 'conventional', commercially-available yeast products, the importance of considering nutritional management, and judicious application of malolactic fermentation.

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