Breeding for success: yeast strain development at the AWRI

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For more than 55 years, the AWRI has been at the forefront of developing yeast strains, resulting in smoother fermentation processes for winemakers and world-class wines. This article explains how the AWRI is investigating 'alternative varieties' of yeast through breeding and selection programs to generate improved strains.

In the developed world, we take for granted ready access to high quality foods in quantities that are more than adequate to sustain us. The foods we consume are not simply gifts of nature; they come to us as the products of extensive plant and animal selective breeding and the application of genetics. The tomato plants that we grow in our gardens today barely resemble their ancestor, which came from South America more than 500 years ago. The diversity that has been spawned from this one species is enormous (fruits that are yellow, black or red; large, small or tiny; sweet or tangy; and a myriad of different shapes).

In the wine world, domesticated grapevines provide us with large quantities of high quality fruit and, as for tomatoes, there are many different varieties producing grapes for dry or sweet, red or white, table, sparkling or dessert wine. Vines and the rootstocks they are grafted on have been selected for their disease resistance and tolerance to different environmental constraints and climes.

What of the micro-organisms that are used to make wine? How has domestication shaped them? Do we see the same level of variation and extreme 'types' as is evident in domesticated plants and animals? The continual improvement sought in viticulture through exploration of alternative varieties and selection of the best clones has a parallel in microbiology. Do we have the best wine yeast available for Australian winemaking conditions and objectives?

Since its inception, the AWRI has been isolating, selecting, and developing yeast

strains, with the aim to provide winemakers with options to differentiate their wine styles while conducting risk-free fermentations. In this article, we describe how we go about 'exploring alternative varieties' of yeast, and 'selecting the best clones' – a task made challenging by the microscopic form of our target for domestication.

CONVENTIONAL YEAST – NATURAL ISOLATES

The conventional, and still the most widely practised procedure for developing starter cultures is to isolate the dominant strain from freshly fermented wine, typically one that has produced a wine with outstanding sensory characteristics. This is no different to the process that has yielded many of our agricultural crops, where practitioners with a keen eye for novelty can find the 'next big thing', a 'sport', that is more interesting than the monoculture surrounding it. In the context of yeast strain development, how does this work?

The domestication of yeast was pioneered by microbiologists such as E. Hansen and A. Kuhle, building on the foundations put in place by Louis Pasteur. From the earliest days, scientists observed differences in fermentation characteristics and in flavours imparted by different yeast strains. These observations led to the notion of 'pure culture inoculation technology' in order to exploit the special characteristics of selected strains. Through the work of John Fornachon and Bryce Rankine this technology became reality across the Australian wine industry during the late 1950s and early 1960s, although a number of large wineries had been using European strains for many decades. In essence, the AWRI supplied selected strains, optimised procedures for producing starter cultures and promoted procedures such as adding SO_2 to minimise the impact of indigenous yeasts and bacteria.

Today, the AWRI yeast culture collection houses more than 1500 natural winery and vineyard isolates, many of which belong to the predominant wine yeast species *Saccharomyces cerevisiae*, although other closely related species, such as *Saccharomyces bayanus,* are also represented. The winemaking properties of strains from this collection can be evaluated, ideally in a panel of juices/musts for desirable fermentation and sensory attributes. While a slow and labour-intensive process, conventional selection has yielded several strains that have been used by Australian winemakers.

Until the early 1990s, strains were provided by the AWRI as live cultures on agar slopes, whereas popular strains are now produced in active dried form under contract by commercial yeast producers; 12 active dry yeast products arising from AWRI research are commercially available. From about 1943, Dr John Fornachon, in the capacity as senior research officer of the Australian Wine Board, provided a small number of yeast cultures from his laboratory located at the Waite Agricultural Research Institute. A number of these cultures are listed in Table 1 (see page 38), including the flor yeast J7, which is still in demand globally. By 1953, Dr Bryce Rankine had selected some 42 strains from 98 cultures that had been obtained from local wineries, and from commercial and imported cultures (AWRI publication #XIV). These strains were selected primarily on the basis of strong growth in grape juice, efficient production of ethanol and low residual sugar, and production of volatile and total acids, glycerol, acetaldehyde and responses to temperature. The 'Port Yeast' AWRI 350 consistently stood out as exhibiting superior tablewine-making properties, and is still available in the form of an active dried preparation (Maurivin AWRI 350). Winemaking properties of popular AWRI yeast are described in detail on AWRI's website (www.awri.com.au).

Following establishment of The Australian Wine Research Institute in 1955, a greater range of yeast cultures became available and, by 1968, more than 20 strains had been comprehensively characterised for making different styles of wine (see Table 1; AWRI publication #55). Evaluations were carried out systematically with at least several clarified grape musts, initially in laboratory-scale ferments followed by pilot-scale confirmatory trials. The key criteria were time to complete fermentation and basic wine analysis, particularly alcohol production/residual sugar, volatile acidity, SO_2 and hydrogen sulfide and sensory score. Other tests included yeast cell counts, flocculation and clarification, redox potential, SO_2 tolerance, higher alcohol, malic acid consumption and SO_2 binding compounds.

The highly robust strain AWRI 729 became widely used and was produced initially as compressed yeast, and later as dried yeast by Mauri Bros and Thompson in 1964; the dried form proved unreliable for production reasons. Four strains from these early isolates have become major red and white table wine production strains in Australia and overseas. Availability in active dried form since the late 1980s, in addition to live cultures, has dramatically facilitated their widespread use.

NON-CONVENTIONAL YEASTS – NATURAL ISOLATES

In addition to *Saccharomyces cerevisiae*, other species of *Saccharomyces* and non-*Saccharomyces* yeasts participate in wine fermentation. In recent years, these species have been extensively characterised for their potential winemaking application as starter cultures. Of the non-*S. cerevisiae* species, *S. bayanus*, which is most commonly found in ferments in very cool climates (e.g., Alsace and New Zealand), has winemaking characteristics not been found amongst *S. cerevisiae* strains, such as lower growth temperature optimum, high glycerol and succinic acid production, and sensory attributes that are more savoury in character (AWRI publication #647). The distinctive aromatic profile, together with improved mouthfeel properties, has led to the application of this yeast species in barrel fermented, lees stirred wines, in addition to conventionally made wines. The AWRI was first to commercialise this species for winemaking, providing winemakers with novel flavour profile wines and greater blending options.

Non-*Saccharomyces* yeasts are characterised by lower tolerance to ethanol and, hence, their inability to complete fermentation. Consequently, these yeasts are used in combination with *S. cerevisiae*. A growing range of cultures are available from commercial yeast suppliers. Several AWRI strains have been evaluated extensively and, when used in combination with a *S. cerevisiae* strain, can produce flavour profiles quite different to those of conventional yeast. In particular, these strains provide some of the characteristics of 'wild yeast' ferment, but in a more reliable manner (Soden *et al.* 2000, AWRI publication #1020). Non-*Saccharomyces* AWRI strains are only available as live cultures and, so, must be propagated within the winery.

MIXED CULTURE YEAST – COMBINATIONS OF NATURAL ISOLATES

On the basis that 'wild' fermentations contain many strains of each yeast species, we have also investigated the outcome of co-fermentation with multiple *S. cerevisiae* strains – a practice some winemakers have applied with active dried yeast for many years. This work concluded that, by using the same combination of yeast strains in a co-fermentation and in different monoculture fermentations, co-fermentation did indeed produce wines that were different to those produced by blending monoculture wines (AWRI publication #688). Moreover, co-fermentation of Sauvignon Blanc with two or more strains revealed considerable enhancement of the aroma attributes arising from poly-functional thiols that impart 'passionfruit' and 'box tree' aromas (AWRI publication #1199). The judicious blending of yeast strains in the right proportion has led to the development of mixed or blended yeast cultures, which are used to enhance the

Table 1. Characteristics of conventional and several non-conventional yeast isolates for winemaking evaluated by the AWRI*
***Due to the large number of yeast strains evaluated since the 1940s, this table represents some of the yeast strains provided by the AWRI to the Australian wine industry. **Prior to 1955, cultures were supplied from CSIRO and the Waite Agricultural Research Institute**

Species	AWRI #	Details (origin/usage)	Availability from the AWRI**
S. cerevisiae race capensis	81	J7 Flor yeast, isolated by J.C.M. Fornachon	1940s – 2010s
S. cerevisiae	134	Australian port yeast, sometimes used in Australian wineries	Mid-1940s – 1970s
S. cerevisiae (formerly *S. fructuum*)	138	Champagne yeast, used in some Australian wineries	Mid-1940s – 1980s
S. cerevisiae	161	Tokay yeast #144, Division of Food Technology, University of California, Berkeley	Mid-1940s – 1970s
S. cerevisiae	162	#146 University of California, Berkeley, 1946. Champagne (flocculating) yeast	Mid-1940s – 1970s
S. cerevisiae	170	Local isolate from starter culture, 1947. Agglomerating yeast	Mid-1940s – 1970s
Kluyveromyces thermotolerans (formerly *S. veronae*)	173	Isolated locally from fermenting Sauternes containing 700 ppm SO_2	Mid-1940s – 1970s
S. cerevisiae	183	#127 C.C. Morges, Swiss wine yeast from Lausanne	Mid-1940s – 1970s
S. species	205	#162 frigo vin 42, Swiss wine yeast from Lausanne, cold tolerant	Mid-1940s – 1970s
S. cerevisiae	213	Isolate from starter tank, 1949. High acid producer. Used in some Australian wineries	1950s – 1970s
S. cerevisiae	228	Local isolate from Tokay grapes, Stonyfell vineyards, 1949	1950s – 1970s
S. cerevisiae	275	French champagne yeast, NSW Department of Agriculture, 1949	1950s – 1970s
S. cerevisiae (formerly *S. chevalier*)	317	'Médoc rouge', University of Bordeaux, 1950. Formerly used in some Australian wineries	1950s – 1970s
Kluyveromyces thermotolerans (formerly *S. veronae*)	318	'St. Emilion rouge', University of Bordeaux, 1950	1950s – 1970s
S. cerevisiae	348	Pasteur Institute, Tunis, 1950. Sulfite tolerant	1950s – 1970s
S. cerevisiae	350	'Port Yeast' isolate from Thomas Hardy and Sons, 1950. Moderate vigour, low H_2S and SO_2. Used in Australian wineries	1950s – 2010s / ADWY since 1987
S. species	719	IZ-710, Instituto Zimotecnico, Piracicaba, Brazil	1960s – 1970s
S. cerevisiae (formerly *S. oviformis* and later *S. bayanus*)	723	Isolate from Angove's, Remark, 1959. High ethanol tolerance, low H_2S	1960s – 1970s
S. cerevisiae	727	UCD#522 Montrachet strain, University of California, Davis, 1964. Used in California wineries	Mid-1960s – 1970s
S. cerevisiae	729	Epernay strain via Lindemans, used as commercial compressed or dried yeast starter (Mauri Bros and Thompson) in Australian wineries. Widely used, high vigour wine yeast	Mid-1960s – 1980s; compressed yeast 1964; ADWY late 1960s – 1980s
S. pastorianus (formerly *S. carlsbergensis*)	731	Lager yeast, C.B.S. 1513, Delft, Holland, 1965	Mid-1960s – 1970s
S. species	741	Weinforschunginstitut, Trier, 1968, wine yeast, SO_2 formation	1970s
S. species	785	Geisenheim, 1974, non-foaming, low H_2S yeast	Mid-1970s
S. cerevisiae	786	Geisenheim, 1974, high vigour wine yeast	Mid-1970s
S. cerevisiae	796	Isolated in South Africa by the Oenological and Viticultural Research Institute. Deposited 1975. Widely used for red and white wines	Mid-1970s – 2010s; ADWY from 1987
S. cerevisiae	834	Sparkling wine yeast	1980s – 1990s
S. cerevisiae	835	Neutral yeast	1980s – 2010s
Kluyveromyces thermotolerans (formerly *Candida stellata*)	861	UNSW 3007, isolated from Hunter Valley must by the University of New South Wales. Used in co-fermentation with S. cerevisiae	Mid-1990s – 2010s
Hanseniaspora uvarum (formerly *Candida krusei/Issatch-enkia orientalis*)	863	UNSW 8010, isolated from Hunter Valley must by the University of New South Wales. Used in co-fermentation with S. cerevisiae	Mid-1990s – 2010s
S. cerevisiae	1017	R2, isolated from Cht. Rahoul, France by Petaluma Pty Ltd	1980s – 2010s; ADWY from 1987
Torulaspora delbrueckii (formerly *S. rosei*)	1034	R21,Te Kawata, N.Z. High osmotolerance, used in sweet wine production, particularly botrytised style	1980s-2010s
S. cerevisiae	1081	Y92E, neutral yeast, isolated in France	1980s-2010s
S. bayanus	1176	Isolated from cold-stored Chardonnay by The Australian Wine Research Institute	2000s-2010s; ADWY 2007 – 2011
S. bayanus	1375	Isolated from cold-stored Chardonnay by The Australian Wine Research Institute	2000s-2010s; ADWY from 2011
S. cerevisiae x *S. paradoxus*	1501	Hybrid yeast bred by The Australian Wine Research Institute	2005 – 2010s
S. cerevisiae x *S. cariocanus*	1502	Maurivin 'Fusion'. Hybrid yeast bred by The Australian Wine Research Institute	2005 – 2010s; ADWY from 2007
S. cerevisiae x *S. kudriavzevii*	1503	Hybrid yeast bred by The Australian Wine Research Institute	2005 – 2010s; ADWY from 2005
S. cerevisiae x *S. mikatae*	1504	Hybrid yeast bred by The Australian Wine Research Institute	2005 – 2010s
S. cerevisiae x *S. bayanus*	1505	Hybrid yeast bred by The Australian Wine Research Institute	2005 – 2010s

flavour attributes of white wines, especially Sauvignon Blanc. Two AWRI-developed yeast blends have been commercialised by Anchor Yeast as Alchemy I and Alchemy II.

DOMESTICATION BY IMPROVING PRE-EXISTING YEAST STRAINS

The key to successful domestication of any species involves harnessing natural genetic diversity through targetted breeding. Plant breeders have, for several decades, augmented this process by taking an already useful variety, and exposing it to a mutagenic agent, such as ultra-violet light, to 'speed up' the normal rate of mutation, thereby generating a mass of new variants. Amongst these, a savvy citrus breeder, for example, may find a mandarin that is sweeter, juicier, and has fewer seeds (http://newsroom.ucr.edu/news_item.html?action=page&id=2602). Mutation breeding, as it is commonly known, can also be applied to wine yeast. The challenge is that while a plant breeder can walk through their plot of mutation breeding progeny and look for individual plants that have grown taller, faster, produced more fruit, or fruit of an unusual colouring, the

yeast mutation breeder is confronted with billions of yeast cells that all look the same.

A yeast researcher actively working on how and why yeast produce H_2S, for example, might know a few tricks – on certain types of agar plates you can see colonies that are likely to produce less H_2S than its parent or siblings. On the back of the AWRI's yeast research program, an array of 'tricks' can be applied to mutation breeding, and we have used these to develop commercial wine yeast strains. Maurivin 'Platinum', 'Distinction' and 'Advantage' were the first commercialised variants found amongst a billion mutants derived from the widely used Maurivin PDM wine strain (AWRI publication #1120). We are currently working with similar procedures to isolate strains that produce less volatile acidity during fermentation, compared with available commercial wine strains.

DOMESTICATION BY GENERATING *SACCHAROMYCES* INTER-SPECIFIC HYBRID YEAST

In nature, inter-specific hybridisation of closely related species is common, and tends to occur in narrow geographic regions where closely related species meet. Plant, bird, reptile and fish hybrids are commonly found in such regions. Plant species hybridise more easily than animals, and this has been used to advantage by plant breeders in modern times to generate a plethora of new varieties of grains, fruits and flowering plants with improved seed or fruit size, or with disease resistance traits.

However, some plants cultivated today are the result of ancient hybridisation events; the tangelo (thought to have originated in South East Asia around 3000 years ago) is a hybrid between a mandarin, orange and a grapefruit, while most ancient and modern wheat varieties are hybrids between different species of grasses.

In a similar vein, cross species matings occur between *Saccharomyces* yeast to form inter-specific hybrids and a small number of industrial yeast strains have been identified as such. For example, the lager yeast, *Saccharomyces pastorianus*, is a stable hybrid of *S. cerevisiae* and *Saccharomyces eubayanus*; it probably arose in cool brewing cellars in Europe in the 17th century. Another example is the wine yeast VIN7 (Anchor), which is a hybrid between *S. cerevisiae* and *Saccharomyces kudriavzevii*.

For the past decade, the AWRI wine yeast breeding program has taken advantage of inter-specific hybridisation to generate novel yeast that bring more complexity to wine than can be achieved with *S. cerevisiae* strains. In order to develop a more complex spectrum of flavours, in addition to those found among *S. cerevisiae* strains, other *Saccharomyces* species have been hybridised with selected *S. cerevisiae* wine yeast. Two such hybrids, AWRI 1502, which is a cross between *S. cerevisiae* and *Saccharomyces cariocanus*, and AWRI 1503, a *S. cerevisiae* x *Saccharomyces kudriavzevii* cross, have performed well in winery trials, international tasting workshops (Australian Wine Industry Technical Conferences and the 2012 International Cool Climate Symposium) and wine shows (AWRI publication #1020). These hybrids have been recently commercialised by AB Mauri.

CLOSING REMARKS

Domestication generates an amazing diversity of 'types', often with extremes that are not found in nature, and all within single species. Wine yeasts have been with us for millennia, but it is only in recent years that attempts have been made to use breeding and selection programs to generate improved strains. As a result of this, winemakers have available to them a much greater choice of strains with which to reveal and enhance the mostly cryptic flavours present in grapes before fermentation. Through this work, the AWRI is enabling winemakers to enhance vineyard, regional and vintage characteristics.

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