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Biographies

Ian Macreadie has worked with yeast genetics and molecular biology for 33 years, at Monash University, University of Texas Health Science Centre at Dallas, and at CSIRO. He is currently the Chief Scientific Officer of Sienna Cancer Diagnostics and Editor of Microbiology Australia.

Professor Ralph Martins career in Alzheimer's disease over 24 years has resulted in over 200 publications. He established the McCusker Alzheimer's Research Foundation and the Centre of Excellence for Alzheimer's Disease Research and Care. In 2004, he was appointed inaugural Chair in Ageing and Alzheimer's at Edith Cowan University. He is a senior editor for the *Journal of Alzheimer's Disease* and editorial board member for *CNS and Neurological Disease.* Martins is a board member of three research foundations and Alzhyme, a biotech company developing anti-amyloid drugs, which has taken a lead compound from concept to successful animal trials in four years. He is the WA Australian of the Year for 2010.

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Generating interspecific wine yeast hybrids for funky wines

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When we think of *Saccharomyces cerevisiae*, fermentation immediately comes to mind, but this is not the only trait that makes this yeast the organism of choice for bread, beer and wine production. The winemaking industry, for example, requires robust strains, capable of converting sugar to ethanol in challenging conditions; high osmotic stress and low pH in the initial grape must, followed by high ethanol concentration at the later stages of fermentation. Winemakers also look for ways of using fermentation to introduce aroma and flavour diversity to their wines as a means of improving style and for product differentiation. Choice of wine yeast from the plethora of strains available to winemakers is one way of achieving this, particularly with the new breed of interspecific hybrid yeast strains currently being generated.

Economies of scale, the risk of indigenous microorganisms of the grape must spoiling a wine and the desire to produce consistency in wines between vintages have led to the modern winemaking practice of using an inoculum of a single robust yeast strain, typically of *S. cerevisiae,* for each fermentation. While wines made using this practice produce consistently sound fermentations, there are indications that the contributions of many different microorganisms in spontaneous fermentations deliver greater diversity of flavour profiles and better palate structure to wine. Many winemakers would like to produce more complex wines, but don't want to risk their fermentations to an unknown yeast population that might cause spoilage.

One strategy used to generate robust wine yeast strains that produce a wider range of cellular metabolites, and hence a more complex wine profile, has been to cross-existing, commercial *S. cerevisiae* strains with other, non-*cerevisiae,* members of the *Saccharomyces* genus. *Saccharomyces* species are diverse, but they share a common mating system and, therefore, some species can mate with each other, and this is a natural, non-GM (non-genetically modified), process. In fact, a small number of available commercial lager, wine and cider yeast strains, originally isolated from natural environments, have been identified as interspecific hybrids between members of the *Saccharomyces* senu stricto complex, a group of closely related species within the *Saccharomyces* genus [1-5].

Several interspecific wine yeasts have been generated at the Australian Wine Research Institute over recent years, and some of these are now marketed internationally. These include AWRI 1503 *(S. cerevisiae x S. kudriavzevii)* and AWRI Fusion [6] *(S. cerevisiae x S. cariocanus*), which produce wines in industrial-scale fermentations that are described by winemakers as having desirable 'funky' yeast characteristics [7]. These characteristics bring complexity and novelty to wines and are of great interest to consumers in many market segments. Thus the potential is great. The following describes how interspecific hybrids are generated and work that is in progress on strains that are almost ready for industry-scale trials.

The generation of *Saccharomyces* interspecific wine yeast hybrids

Saccharomyces spp are typical eukaryotes and, as such, engage in sex. They have two different mating haploid states, 'a' and 'α' and mating occurs between haploid cells of opposite mating types, with the resultant diploid being 'a/α'. At a very low frequency, mating type switching occurs in diploid cells rendering them 'a/a' or 'α/α'. When this occurs, the diploid cell can mate with a cell of the opposite mating type to generate offspring with higher than the diploid complement of chromosomes; this is referred to as 'rare-mating'.

To generate novel interspecific yeast strains for the wine industry it is important to maintain essential oenological traits such as robustness and fermentation efficiency. With few exceptions, these traits are found only in strains of *S. cerevisiae.* Thus, interspecific wine yeast hybrids should always have *S. cerevisiae* as one of the parents. To ensure maximum probability of carrying wine relevant traits, the cross described in the work below was carried out with a diploid wine yeast and haploid non-*S. cerevisiae.*

Hybrid progeny were produced by a rare mating [8] between a commercial *S. cerevisiae* wine yeast strain (AWRI 838),

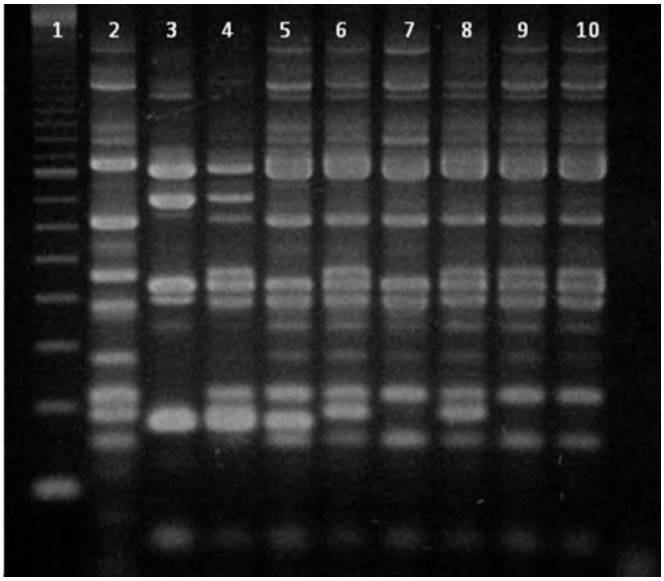

Figure 1. Lane 1: DNA ladder, Lane 2: AWRI 838, Lane 3: 52-153, Lane 4: DNA from both parents, Lanes 5–10: Hybrid strains H1-H6.

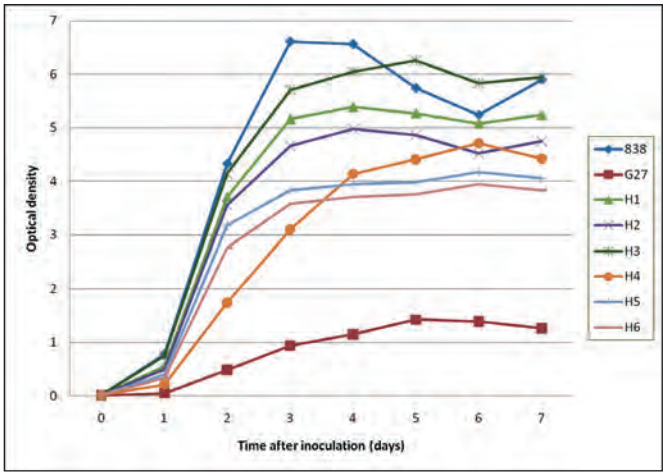

Figure 2A. Cell growth during fermentation as measured by optical density at 600 nm.

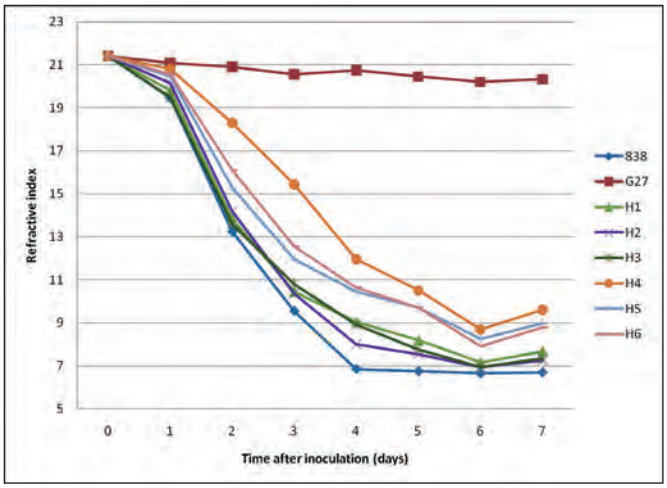

Figure 2B. Sugar utilisation during fermentation as measured by refractive index.

mutagenised with ethidium bromide to render it mitochondrial deficient [9] and spores from a *Saccharomyces paradoxus* strain (52-153). Plates with glycerol as the carbohydrate source and 14% v/w ethanol, (inhibitory to the *S. paradoxus* strain), allowed selection of hybrid mitochondrial sufficient and ethanol tolerant colonies. Confirmation of the hybrid nuclear genome of each strain was by multiplex PCR using both Transposon primers [10] and Intron primers [11] (Figure 1). Lanes 2 (AWRI 838) and 3 (52-153) display parental, strain-specific, fingerprints. Amplification from a mix of both parental DNA in Lane 4 gives an indication of the expected hybrid banding fingerprint. Bands specific to each parent are observed in all hybrid fingerprints (Lanes 5–10), although there are noticeable differences between the hybrid fingerprints. However, the absence of bands specific to the *S. cerevisiae* parent in hybrids H1, H3, H5 and H6 (Lanes 5, 7, 9 & 10) indicates genomic instability.

Do these *Saccharomyces* interspecific wine yeast hybrids have what it takes to make wine?

Laboratory-scale fermentations were used to determine the suitability of each hybrid for inclusion into an industrial wine

Table 1 Standard deviation of O.D. and R.I. measurements taken during fermentation.

	Standard deviation															
	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7	
	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.	O.D.	R.I.
838	0.001	0	0.009	0.071	0.006	0.071	0.014	0.071	0.226	0.071	0.150	0.212	0.255	0.035	0.495	0.212
H1	0.004	0	0.030	0.283	0.541	0.636	1.782	1.485	1.796	1.626	1.598	1.556	1.216	0.318	1.117	0.424
H2	0.001	0	0.008	0.071	0.134	0.000	0.424	0.778	0.849	1.556	0.679	0.778	0.764	0.106	0.849	0.141
H3	0.001	0	0.004	0.000	0.011	0.000	0.042	0.000	0.071	0.141	0.057	0.495	0.212	0.035	0.453	0.212
H4	0.001	0	0.003	0.000	0.039	0.000	0.000	0.636	0.735	0.495	0.778	0.849	0.919	0.495	1.131	0.990
H5	0.003	0	0.001	0.071	0.021	0.000	0.184	0.071	0.085	0.071	0.085	0.000	0.240	0.106	0.198	0.071
H6	0.003	0	0.018	0.071	0.004	0.141	0.000	0.071	0.014	0.071	0.057	0.141	0.156	0.141	0.099	0.071

yeast trial. Hybrid strains were screened by growth in chardonnay grape juice, (glucose 114 g/L, fructose 111 g/L, yeast assimilable nitrogen 322 mg/L, ammonia 96 mg/L, pH 3.17). Duplicate ferments in conical flasks, fitted with water traps, were carried out at 22°C, shaking at 150 rpm. Cell growth was assessed by optical density measurements (Figure 2A) and sugar utilisation by refractive index measurements (Figure 2B), with standard deviation values (Table 1) providing an indication of fermentation consistency.

The higher level of robustness of the *S. cerevisiae* wine yeast parent relative to all hybrids was apparent as this strain grew fastest and had the highest final cell density. The drop in cell density after Day 4 did not affect the success of the fermentation as sugar utilisation was completed by this time (R.I. 6.8). The *S. paradoxus* parent strain, 52-153, displayed poor growth in grape juice and was unable to carry out fermentation. Hybrids H1, H2 and H3 grew at a moderate rate and did not reach the cell density of their wine yeast parent, but the rate of sugar utilisation only diminished slightly when compared to their wine yeast parent, and both strains were able to complete fermentation within six days. Hybrid H1 appears to ferment at roughly the same rate as H2 and H3, but this strain was inconsistent with growth and sugar utilisation replicates varying widely (Table 1). Hybrid strains H5 and H6 grew slowly, producing sluggish fermentations, and were not able to complete fermentation. Of the six hybrids, H4 had the slowest growth rate and, even though cell density eventually peaked at a level higher than strains H5 and H6 by Day 4, this strain displayed poor utilisation of sugars.

In the search for new commercial wine strains, growth rate in grape juice is of paramount importance. However, very fast growing strains can generate heat, leading to increased temperatures in the fermenting must, resulting in loss of volatile compounds that could otherwise confer positive flavours and aromas to the wine. The wine yeast parent of the hybrids, AWRI 838, is considered by winemakers to be one such rapidly growing yeast. Hybrids with slower growth rates than the parent strain, but still able to complete fermentation in a timely manner, may be the preferred option by many winemakers. Hybrid strains H4, H5 and H6 were not able to complete fermentation and will not be considered for further trials. Similarly, inconsistent growth and sugar utilisation of H1 led to it being rejected. Of the progeny screened for this study, H2 and H3 were chosen for further large-scale winemaking trials to determine their performance and stability in an industrial setting.

If successful in industry-scale winemaking trials, these new hybrid strains will complement other novel *Saccharomyces* interspecific wine yeast strains generated by this project enabling winemakers to reach the 'funky' niches of the marketplace.

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Biography

Jenny Bellon is a scientist at the Australian Wine Research Institute. Her research interests are the generation of *Saccharomyces* interspecific yeast hybrids produced for the Australian wine industry, and the study of genomic changes that led to their stability.