Managing Director, Sakkie Pretorius.

AWRI
The Australian Wine Research Institute

The dawn of a new paradigm for wine yeast strain development

Paul J. Chambers, Anthony R. Borneman, Simon A. Schmidt, Jeremy C. Hack, Cristian Varela, Meagan Mercurio, Christopher D. Curtin, Daniel Cozzolino, Maurizio Ugliano, Markus J. Herderich and Isak S. Pretorius
The Australian Wine Research Institute, PO Box 197, Glen Osmond (Adelaide), South Australia 5064, Australia

Deciding on the style of wine a winemaker will produce depends on many factors. A winemaker might choose to produce wine with characters desired by a specific market; a wine that best represents the region of origin; a signature style; or maybe a combination of factors. At the point of decision, how can winemakers be responsive to changes over relatively short timeframes? Current winery technologies provide limited means of changing a wine style from one vintage to the next, but innovative wine yeast research is pointing the way ahead. Delivering substantial changes to wine style might be as simple as choosing the right yeast for the job.

To remain successful in today's crowded and over-supplied global wine market, a wine needs to stand out in its price category and deliver in such a way to ensure repeat purchase. For wine producers considering a new wine style, however, a fast turnaround can be difficult to achieve.

One of the things that would enable winemakers to create different wine styles would be access to a diverse array of wine yeast strains that impart known and predictable characters to wine. Where one yeast strain might be designed to produce lower levels of alcohol and dark-fruit flavours, another might be better suited to releasing the tropical fruit aromas associated with some of the leading Sauvignon Blanc wines; different yeasts to accentuate different characters in wines.

How can we begin to develop such a resource? The starting point is to better understand the inner workings of a wine yeast cell and its metabolism and then use this knowledge to generate novel yeast strains which make products suited to the winemaker's requirements.

Getting to grips with the inner workings of a yeast cell is not easy. Living things are immensely complex, and this applies even to the simplest looking organism. The wine yeast, Saccharomyces cerevisiae, for example, comprises single, ellipsoidal cells that are approximately 0.005-0.01mm 'long' x 0.003–0.007mm 'wide'. It has no distinguishing features other than occasional crater-like scars on the surface, each marking the point of release of a daughter cell following reproduction (Figure 1). However, the content of a book should never be judged from the look of its cover.

Climb inside a yeast cell and you will find a thriving mini metropolis, just like the inside of your cells. All around, there is an intricate array of highly organised scaffold and architectural features supporting compartments known as organelles (little organs: like the organs of our bodies, they are self-contained and have discrete functions). There are transport systems that follow delivery routes laid down in the form of tracks; vehicles carry cargo along these tracks to defined destinations, from one organelle to another. The addressing system that is used to ensure accuracy of delivery is quite amazing, but we will have to tell you about that another time.

Figure 1. An electronmicrograph of a budding cell of the wine yeast Saccharomyces cerevisiae. S. cerevisiae comprises single, ellipsoidal cells. Their sizes vary between 5–10µm in length and 3-7µm in width. Some cells can be seen to have 'extensions' or 'buds', which are the products of asexual reproduction; the buds are 'daughter' cells that will eventually be released from their genetically identical 'mother' cell. Under optimal nutritional and cultural conditions, S. cerevisiae doubles its mass every 90 minutes. It has no distinguishing features other than occasional crater-like scars on the surface of a 'mother' cell; each marking the point of release of a 'daughter' cell following reproduction.

The plans for construction, maintenance and functioning of these intracellular metropolises are written in a set of instructions: the genes, which are housed on chromosomes in a compartment known as the nucleus. There are more than 6000 genes in yeast and each one carries information to build a specific protein. The protein might be an enzyme for making alcohol; a structural protein contributing to cellular architecture; a cargo vessel for carrying materials across the cell; or a transporter for ferrying nutrients into the cell from grape juice.

Chromosomes, and the genes they carry, are faithfully replicated (rather like photocopying plans) each time a cell divides, and a complete copy (known as the genome of a cell) is given to each new daughter cell so that it can build, maintain and operate its own internal mini metropolis, in exactly the same way as its parent.

Some of the instructions from the yeast genome direct the production of alcohol from sugars, enabling the yeast cell to extract energy from sugars in the grape juice. Other genes encode the instructions to make enzymes that will release flavour and aroma molecules (e.g. esters and thiols) into wine. Others encode instructions on how to build new cellular components, compartments and even whole new cells.

All of these activities of a yeast cell are critical to the organism for its survival and growth; and to the winemaker for turning grape juice into 'bottled sunshine'. Therefore, one of the goals of a wine scientist is to better understand the complexity of the internal workings of wine yeast so that we can develop more informed ways of working with yeast and generate new and improved strains for the winemaker.

How do we start to unravel and reshape this complexity? How can we reshape it in a controlled, informed way to develop novel yeast strains that will deliver wine styles as identified by the winemaker? Traditionally, when attempting to understand the inner workings of an organism, biologists have isolated small components of cells and then attempted to understand what these parts do by studying them in the laboratory.

For example, they might isolate a protein, such as an enzyme, and use this to learn about the chemistry of the reactions that the enzyme drives. With modern molecular biology techniques, we are also able to characterise the gene carrying the instructions to produce this enzyme; learn, for example, how the gene is expressed (i.e. how the instructions in the gene are used to make the enzyme) and what regulates its expression.

Gradually a picture emerges on the biochemistry of the enzyme being studied, but what about its role in a living yeast cell, where it will be produced along with thousands of other proteins, many of which might impact on its production or activity? The more we are learning about the parts that make up a cell, the more we appreciate the complexity of cell physiology and biochemistry.

Fortunately, a new set of technologies has emerged to help us look at the inner workings of cells in a more complete, holistic way. For example, we can now have an inventory of the protein composition of cells, known as the proteome. No longer are we restricted to following one or small numbers of proteins in an experiment; state-of-the-art analytical chemistry instrumentation is being applied in the field of proteomics to afford us greater access to the building blocks of the architecture, machinery and infrastructure of the intracellular metropolis.

At another level, and of particular relevance to winemakers, there is the metabolome; access to which requires similar tools and skills as are used for proteomics. A metabolome is the cell's full complement of metabolites, the small molecule of a cell, including organic acids, fatty acids, amino acids, small sugars, alcohols, thiols and more; chemicals that shape the flavour, aroma, mouthfeel and colour of wine.

In a wine context, one might argue that wine is essentially the metabolomic footprint of wine yeast grown on grape juice. Using this paradigm immediately suggests new ways of approaching wine research. For example, where we might previously have targeted 40–50 known metabolites in fermentation product analysis, we instead broaden the net to capture as many metabolites as possible, and we do not restrict analysis to known compounds, thereby increasing the chance of discovery.

Metabolomics will undoubtedly enable a more complete description and understanding of wine composition and quality, and how these are sculpted by yeast. When tested alongside complementary genomic and proteomic analyses of wine yeast, a 'complete picture' of the complex make-up of the cell is within reach.

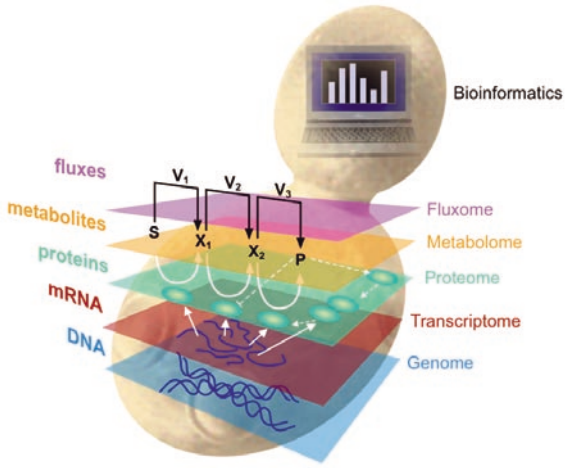

Figure 2. Yeast systems biology and 'omics research.
'Omics research is revolutionising the way we do wine research. No longer are we restricted to following a few metabolites (e.g. glycerol, acetic acid, ethanol, etc.) in an experimental fermentation. Now, we can follow hundreds of metabolites (many of which we will not know the identity of) in same experiment; this is metabolomics. In a similar vein, genomics is concerned with characterising the genome (i.e. all of the genes and other genetic material in an organism), and proteomics attempts to identify the full set of proteins in a cell. Other levels of 'omics enquiry that are pertinent to wine research include transcriptomics and fluxomics. The first of these determines the transcript (also known as messenger RNA or mRNA) composition of a cell. An mRNA is a copy of the of the instructions carried in a gene; the copy is made in the nucleus, where the gene resides, and is transported out into the cytoplasm where it will be read and translated to build the protein that the gene encodes. Fluxomics, like metabolomics, is concerned with metabolism, but in this case the focus is on fluctuations in metabolites and what controls their production. If we want to generate new yeasts strains that, for example, produce particular flavours and/or less ethanol, fluxomics will enable us to identify key steps in metabolism to target, which can then be either up- or down-regulated, to get the desired outcome during fermentation. Bioinformatics provides the computational tools to handle and integrate the massive 'omics datasets that come out of these experimental approaches. Computers are then used to design new strains of yeast with improved winemaking properties.

How do we make sense of the massive amounts of data that will be accumulated from these various 'omics (e.g., genomics, proteomics, metabolomics) investigations? The emerging field of systems biology promises to take us that last step; it utilises data from 'omics level investigations and uses computing and mathematical tools to bring it all together (Figure 2).

Once we have robust mathematical models of yeast cellular functions, we will be able to design and trial the performance of prototype novel yeast strains *in silico* (i.e. using computer models) rather than going through time-consuming costly fermentations. Engineers in other industries have used this approach for decades; cars, aeroplanes, ships, etc., are typically designed and evaluated computationally, and only those predicted to deliver improved performance outcomes get to see the light of day. Computer-designed yeast, tailored to winemakers' specifications and tested *in silico*, will give Australian winemakers the competitive edge they need when faced with increasingly over-crowded markets.

A major problem confronting scientists attempting to access the 'big science' necessary to do systems biology is that it requires a broad range of expertise and state-of-the-art (and very expensive) resources. These things are not available in any single laboratory, requiring researchers to work in large consortia, collaborating to achieve a common goal.

How can we take Australian wine research into this arena? Fortunately, the Australian Government has funded the development of four service delivery platforms: Genomics Australia, Proteomics Australia, Metabolomics Australia (part of which is housed at the AWRI) and Bioinformatics Australia (which provides computing infrastructure). The activities of these four platforms, whilst largely autonomous, are overseen by Bioplatforms Australia.

In a coup for the Australian wine sector, Bioplatforms Australia has adopted wine yeast fermentation as a model to demonstrate how we can do systems biology in this country. The project, which commenced in January 2009, brings together scientists from the AWRI and the other platforms. The model fermentation will utilise wine yeast AWRI1631 for which the genome was characterised (and published) in 2008. To ensure reproducibility, a synthetic white winegrape juice will be used for the early stage of the project, and findings from this work will be tested at a later stage in grape juice fermentations.

This initiative puts Australia at the forefront of international wine research, and puts wine research at the forefront of Australian science.

SUMMARY

It is not yet possible in the winery, to shape wine composition according to, for example, specific preferences of potentially lucrative consumer markets. However, by adopting new 'omics technologies and recent developments in systems biology, we can unravel the complex biology of wine yeast. This will lead to a much greater understanding of the genes and metabolic pathways that drive such things as ethanol production and flavour development. From there, we can begin to use *in silico* approaches to systematically tailor wine yeast to deliver wines of the dreams of winemakers and consumers alike.

ACKNOWLEDGEMENTS

The Australian Wine Research Institute, a member of the Wine Innovation Cluster in Adelaide, is supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. Systems biology research at the AWRI is performed using resources provided as part of the *National Collaborative Research Infrastructure Strategy* (NCRIS), an initiative of the Australian Government, in addition to funds from the South Australian Government. AWRI's collaborating partners within this NCRIS-funded initiative, which is overseen by Bioplatforms Australia, are Genomics Australia, Proteomics Australia, Metabolomics Australia (of which the Microbial Metabolomics unit is housed at the AWRI) and Bioinformatics Australia. The authors wish to thank Rae Blair for editorial assistance.

FURTHER READING

Bartowsky, E.J.; Bellon, J.R.; Borneman, A.R.; Chambers, P.J.; Cordente, A.G.; Costello, P.; Curtin, C.; Forgan, A.; Henschke, P.A.; Kutyna, D.; McCarthy, J.; Macintyre, O.J.; Schmidt, S.A.; Tran, T.; Swiegers, J.H.; Ugliano, M.; Varela, C.; Willmott, R. and Pretorius, I.S. (2007) Not all wine yeasts are equal. Microbiology Australia 28:55-58.

Borneman, A.R.; Chambers, P.J. and Pretorius, I.S. (2007) Yeast Systems Biology: modelling the winemaker's art. Trends in Biotechnology 25:349-355.

Borneman, A.R.; Forgan, A.H.; Chambers, P.J. and Pretorius, I.S. (2008) Unravelling the genetic blueprint of wine yeast. Australian and New Zealand Wine Industry Journal 23:21-23.

Borneman, A.R.; Forgan, A.; Chambers, P.J. and Pretorius, I.S. (2008) Comparative genome analysis of a Saccharomyces cerevisiae wine strain. FEMS Yeast Research 8:1185-1195.

Borneman, A.R.; Chambers, P.J. and Pretorius, I.S. (2009) Systems biology as a platform for wine yeast strain development. In: König, H.; Unden, G. and Frölich, J. (eds.), Biology of microorganisms on grapes, in must and wine. Springer, Heidelberg, Germany. Chapter 22:395-414.

Borneman, A.R.; Chambers, P.J. and Pretorius, I.S. (2009) The way forward: modelling the winemaker's art using yeast systems biology. In: Romano, P. and Fleet, G.H. (eds.), Yeast in Wine Fermentation. Springer, Heidelberg, Germany (in press).

Chambers, P.J.; Bellon, J.R.; Schmidt, S.A.; Varela, C. and Pretorius, I.S. (2009) Non-genetic engineering approaches to isolating and generating novel yeasts for industrial applications. In: Kunze, G. and Satyanarayana, T. (eds.), Yeast Biotechnology: Diversity and Applications. Springer, Berlin, Germany (in press).

King, E.S.; Swiegers, J.H.; Travis, B.; Francis, I.L.; Bastian, S. and Pretorius, I.S. (2008) Co-inoculated fermentations using Saccharomyces yeasts affect the volatile aroma composition and sensory properties of Vitis vinifera L. cv. Sauvignon Blanc wines. Journal of Agricultural and Food Chemistry 56:10829-10837.

Swiegers, J.H.; Capone, D.; Elsey, G.; Sefton, M.A.; Francis, I.L. and Pretorius, I.S. (2007) Engineering volatile thiol release in Saccharomyces cerevisiae for improved wine aroma. Yeast 24:561-574.

Swiegers, J.H.; Francis, I.L.; Herderich, M.J. and Pretorius, I.S. (2006) Meeting consumer expectations through management in vineyard and winery: The choice of yeast for fermentation offers great potential to adjust the aroma of Sauvignon Blanc wine. Australian and New Zealand Wine Industry Journal 21:34-42.

Swiegers, J.H.; Saerens, S.M.G. and Pretorius, I.S. (2008) The development of yeast strains as tools to adjust the flavour of fermented beverages to market specifications. In: Frenkel, D.H. and Belanger, F. (eds.), Biotechnology in Flavour Production. Blackwell Publishing, Oxford, UK. Chapter 1:1-55.

Swiegers J.H.; Kievit, R.L.; Siebert, T.E.; Lattey, K.; Bramley, B.R.; Francis, I.L.; King, E.S. and Pretorius, I.S. (2009) The influence of yeast on the aroma of Sauvignon Blanc wine. Food Microbiology 26:204-211.

Verstrepen, K.J.; Chambers, P. and Pretorius, I.S. (2006) The development of superior yeast strains for the food and beverage industries: challenges, opportunities and potential benefits. In: Querol, A. and Fleet, G.H. (eds.), The Yeast Handbook, Yeasts in Food and Beverages. Springer-Verlag (Heidelberg), Germany. Chapter 13:399-444.