# YEAST: PRODUCTS AND DISCOVERY

Proceedings of the 2nd Australian Conference on Yeast: Products and Discovery

28-29 November 2002 Melbourne

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# Welcome to the 2nd Yeast: Products and Discovery Meeting

The photograph is of participants attending the 1st Yeast: Products and Discovery Meeting at Couran Cove, Stradbroke Island, Queensland from 29 June- 1 July 2000

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The organising committee would like to thank the following organisations for their sponsorship and support of this meeting.

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YPD 2002 Conference Dinner

Please note that if you wish to bring a parter/friend to the conference dinner you will need to submit a registration, paying only for the dinner (ie $35).

Pre-Conference Mixer – REMINDER

Carlton & United Breweries have kindly agreed to sponsor a YPD 2002 conference mixer at the CUB Brewhouse in Richmond on the eve of the conference,Wednesday November 27th from 6.00 - 8.00 pm.

The Brewhouse is located on the corner of Nelson and Sth. Audley Streets (Melways ref. Map 2H, C1). There is parking available on Nelson St. immediately in front of the entrance and there is a regular tram service from the city. Tram 109 (to Mont Albert) can be picked up at tram stops anywhere along Collins St in the City, and ask to be dropped off at the first stop after Church St (Abbotsford). Sth. Audley St. is about 200 m past the tram stop (heading in the same direction as the tram). Alternatively a taxi from the City should not cost more than about $12 - $15. We can also arrange transport from the CBD or CSIRO, Parkville (the conference venue) for a limited number of registrants - but please let me know asap if you will require this.

Please let Dr. Paul Chambers whether or not you will attend this mixer so that we can advise CUB on numbers.

Provisional Program (subject to change) * indicates a student presentation

| Session | Chair | Speaker - Title |
|---|---|---|
| **Wednesday November 27, 2002<br>Mixer at the Brewhouse (sponsored by CUB)<br>(6.00-8.00 pm)** | | |
| **Thursday November 28, 2002** | | |
| Welcome<br>(8.45 am) | Ian Macreadie | |
| 1. Genomics, proteomics and metabolomics<br>9.00 am-10.30 am | Paul Chambers | tba |
| | | *Anthony Heinrich - Comparative Proteomics: Identifying novel proteins in the winemaking strain *Saccharomyces cerevisiae* |
| | | *Jeff Eglinton - Using metabolomics to investigate cellular metabolic changes in a glycerol overproducing strain of *saccharomyces cerevisiae* |
| **Tea break and Poster Session** | | |
| 2. New yeast technology<br>11.00 am - 12.30 pm | Rob Learmonth | **Rob Learmonth - Recent advances in laser scanning microscopy** |
| | | *Carlos Rosado - Yeast autophagy: development of novel intracellular pH biosensors |
| | | Mark Prescott - Applications for Fluorescent Proteins in yeast |
| **Lunch break and Poster Session** | | |
| 3. Poster introductions<br>2.00 pm -3.30 pm | | Those with posters will have an opportunity to give a short (5 minute) introduction. |

| | | |
|---|---|---|
| Tea break and Poster Session | | |
| 4. Wine yeast<br>4.00 pm -5.45 pm | Paul Henschke | Graham Fleet – The grape berry as an ecosystem for yeasts and filamentous fungi |
| | | Miguel de Barros Lopes - Investigation into the mechanism of action and biological role of *Saccharomyces cerevisiae* mannoproteins which reduce visible haziness in white wine |
| | | Vladimir Jiranek – Strategies for avoiding problems associated with nitrogen limitation during wine fermentation |
| | | Paul Grbin - Investigation of the formation of volatile phenols and fatty acids by Dekkera and Brettanomyces yeast |
| | | *Kate Howell – Yeast strain dynamics during mixed culture wine fermentation and effect on wine composition |
| | | *Simon Dillon – Can yeast strain affect colour and phenolic content of shiraz wine? |
| **Conference Dinner at the Laureate from 6.30 pm<br>(Wines sponsored by Univ. of Adelaide and Southcorp)** | | |
| **Thursday November 29, 2002** | | |
| 5. Yeast cell and molecular biology<br>9.00 am-10.30 am | Phil Nagley | *Andrew Stephens - Yeast ATP synthase: protein engineering and topology |
| | | *Onisha Patel - Using yeast as a model to demonstrate a new mechanism of sulfa drug action |
| | | Ian Macreadie - Foreign gene expression in yeast |
| Tea break and Poster Session | | |
| 6. Yeast stress | Grant Stanley | Meredith Chandler - |

| | | |
|---|---|---|
| 11.00 am - 12.30 pm | | tba |
| | | Gabriel Perrone - Cellular functions in glutathione homeostasis identified by genome-wide screening / Engineering yeast for glutathione production |
| Lunch break and Poster Session | | |
| 7. Brewing yeast 1.30 pm -3.00 pm | Peter J. Rogers (CUB) | Peter J Rogers (CUB) - New Variations on an age old profession: |
| | | Peter L. Rogers (UNSW) - R&D opportunities in fuel ethanol production |
| | | Dermot O'Donnell - Brewing yeasts-history, types and their applications |
| Tea break (take down posters) | | |
| 8. Medical yeast and disease 3.15 pm. -4.45 pm | Paul Vaughan | Whelan Meyer - Molecular epidemiology of *C. neoformans* revealing a possible link between the old and new world |
| | | **John Warmington - Will Genomics and Proteomics Produce the Next Generation of Antifungal Compounds?** |
| | | Peter Iliades - Modelling *Pneumocystis carinii* drug resistance in yeast |
| 9. ASBMB Student awards<br>YPD - the future 4.45 pm - 5.15 pm | Ian Macreadie<br><br>Paul Henshke | Presentations<br><br>Feedback and Discussion forum |

LIST OF AUTHORS ALPHABETICAL with page number * Bold presenting


1. **Beckhouse A.G**., Higgins V.J., Rogers P. J., Dawes I.W.

   Ergosterol Requirement Of Yeast In The Protection Against Oxidative Stress In Industrial Fermentation

2. Jenny Bellon, Jeff Eglinton, Alan Pollnitz, Cherise Hillier, and Miguel de Barros Lopes

   Hybrid Wine Yeasts With Unique Fermentation Characteristics

3. Shauna L. Brown, **Miguel de Barros Lopes**, Peter B. Høj, Elizabeth J. Waters

   Investigation Into The Mechanism Of Action And Biological Role Of Saccharomyces cerevisiae Mannoproteins Which Reduce Visible Haziness In White Wine

4. **B. A. Butcher** and R. P. Learmonth

   Yeast Membrane Responses To Glucose Are Related To Membrane Protein Expression.

5. **M. Chandler**, G. Stanley, P. Rogers, P. Chambers.

   Gene Array Analysis Of *Saccharomyces cerevisiae* During Ethanol Stress.

6. **Simon Dillon**, Eveline Bartowsky, Peter Høj, Laurent Dulau, and Paul Henschke

Can Yeast Strain Affect Colour And Phenolic Content Of Shiraz Wine?

7. **Jeff Eglinton** and Paul Henschke

   S*accharomyces Bayanus* – An Alternative Wine Yeast?

8. **Jeff Eglinton**, Anthony Heinrich, Alan Pollnitz, Paul Henschke and Miguel de Barros Lopes

   Using Metabolomics To Investigate Cellular Metabolic Changes In A Glycerol Overproducing Strain Of *Saccharomyces Cerevisiae*

9. **G H Fleet**, A L Beh, C. Prakitchaiwattana and G M Heard

   The Grape Berry As An Eco-System For Yeasts And Filamentous Fungi

10. **G.H. Fleet**, A.L. Beh, C. Prakitchaiwattana, S.S. Bae, and G.M. Heard

    The Yeast And Bacterial Ecology Of Wine Grapes

11. **Jennie Gardner,** Andrea Vystavelova, Miguel de Barros Lopes, and Vladimir Jiranek

    Identification Of Genes Contributing To A “High Nitrogen Efficiency” (Hne) Phenotype In Yeast

12. **Gelling, C.L.**, Kornfeld, G , Cook, T.S., Piper, M.D.W., Dawes, I.W

    Kinetic Analysis Of The Activation Of The One-Carbon Regulon Using Microarray Technology

13. **E.M. Goldys**, A. Donnelly, R. Wilkinson

    Rapid Optical Essay For Identification Of Different Strains Of Baker's Yeast *Saccharomyces cerevisiae*.

14. **Paul R. Grbin**, Mathew Reynolds, Peter Dillon, and Stephanie G. Lambert

Investigation Of The Formaton Of Volatile Phenols And Fatty Acds By *Dekkera* And *Brettanomyces* Yeast

15. **Anthony Heinrich**, Jelle Lahnstein, Kris Ferguson, Graeme Currie, Vladimir Jiranek and Miguel de Barros Lopes.

    Comparative Proteomics: Identifying Novel Proteins In The Winemaking Strain *Saccharomyces cerevisiae***.**

16. **L.Helfenbaum**, C. McNees, C. Dyke, A. Lim, N. Amin, S. Shahin, R.Wickramasinga and M-J. Gething

    Physiological Regulation of the Unfolded Protein Response in Yeast

17. **V.J. Higgins**, T. Oliver, P.J. Rogers and I.W. Dawes

    Talking To Yeast: Application Of Genome-Wide Expression Analysis To Communicate Conditions Important For Successful Industrial Fermentations

18. **K.S. Howell**, E.J. Bartowsky , G.H. Fleet and P.A. Henschke

    Yeast Strain Dynamics During Mixed Culture Wine Fermentation And Effect On Wine Composition

19. **P. Iliades**, J. Berglez, S. Meshnick, I.G. Macreadie

    Modelling *Pneumocystis carinii* drug resistance in yeast.

20. **Shanchita Khan**, Sharon Guffogg, Masego Tsimako and Kenneth Watson

    UVB sensitivity of mesophilic yeasts and psychrophilic Antarctic yeasts at different stages of growth

21. **R. P. Learmonth**

    Recent Advances In Laser Scanning Microscopy.

22. **I.G.Macreadie**

    Foreign Gene Expression In S*accharomyces cerevisae*

23. Florante Jon Meneses[1,2] And Vladimir Jiranek[1]

    Physiological And Genetic Characterisation Of Brewing Strains Of *Saccharomyces cerevisiae* Displa Constitutive Utilisation Of Maltose And Sucrose.

24. **Wieland Meyer** & the IberoAmerican Cryptococcal Study Group.

    Molecular Epidemiology Of *C. Neoformans* Revealing A Possible Link Between The Old And The New World

25. **I.Mohammed**, G. A. Stanley, L. Serwecinska, P. J. Rogers, and P. J. Chambers[1].

    The Transcriptional Response of Ethanol-Stressed Yeast in the Presence and Absence of Acetaldehyde

26. **Gabriel G. Perrone**, Chris M. Grant and Ian W. Dawes

    Cellular Functions In Glutathione Homeostasis Identified By Genome-Wide Screening / Engineering Yeast For Glutathione Production

27. Kate Poole, Jennie Gardner, Miguel de Barros Lopes, and **Vladimir Jiranek**.

    Strategies For Avoiding Problems Associated With Nitrogen Limitation During Wine Fermentation

28. Jeff Potter

    Beer Brewers or Yeast Farmers? - Managing Yeast in the Modern Brewery

29. **Peter Rogers**

    R&D Opportunities In Fuel Ethanol Production

30. **C. Rosado**, D. Mijaljica, L. Samara, P. Gavin, R.J. Devenish & M. Prescott

    Studying mitochondrial turnover by autophagy in *S. cerevisiae* using of a fluorescent protein biosensor

31. **Andrew N. Stephens**, Rodney J. Devenish and Phillip Nagley

    Cysteine Scanning Mutagenesis Of Yeast Mitochondrial $F_1f_o$-Atp Synthase Subunit 8.

32. **Diego Torrea** and Carmen Ancín

    Biogenic Amines In Wines Obtained With Different Yeast Strains: Relationship With Their Precursor Amino Acids

33. **Diego Torrea,** Pablo Fraile, Teresa Garde and Carmen Ancín

    Production Of Volatile Metabolites During Fermentation Of Grape Must Inoculated With Two Different Nitrogen Requiring Yeast Strains

34. Frank Vriesekoop, Andrew Barber and Neville Pamment

    The Acetaldehyde Effect: An Overview of Recent Research in the Biochemical Engineering Laboratory

35. **Michelle E. Walker,** Jennie Gardner, Andrea Vystavelova, Vladimir Jiranek

    Development Of Heterothallic Wine Yeast Strains For Oenological Research

## ABSTRACTS

Ergosterol Requirement Of Yeast In The Protection Against Oxidative Stress In Industrial Fermentations

**Beckhouse A.G**.[1,2], Higgins V.J.[1,2], Rogers P. J.[3], Dawes I.W.[1,2]

[1]Clive and Vera Ramaciotti Centre for Gene Function Analysis and [2]School of Biotechnology and Biomolecular Sciences, UNSW, Sydney, NSW.
[3]Carlton & United Breweries Ltd, Abbotsford, VIC, Australia.

Oxygen is an essential component for ergosterol and unsaturated fatty acid biosynthesis in yeast, but it is also responsible for producing reactive oxygen species (ROS). The damage that ROS can cause to the DNA, proteins and cell membranes can affect fermentation performance and the flavour stability during and after an industrial scale lager brew. Northern analysis was performed on the early stages of a large-scale lager fermentation to monitor the expression of a gene involved in ergosterol biosynthesis (*ERG10*) and a gene involved in the response to oxidative stress (*TRR1*). This was undertaken to elucidate the relationship between ergosterol biosynthesis and oxidative stress during a full scale lager fermentation. The expression profiles show that both genes were highly induced in the initial period of the fermentation. Mutants defective in ergosterol biosynthesis, but able to produce intermediates in the pathway were used to test if ergosterol enhanced resistance to oxidative stress. Spot test evidence indicates that these mutants are highly sensitive to oxidative stress. The observed increase in *TRR1* expression and therefore oxidative stress in the initial hours of fermentation was the result of hypersensitivity of cells lacking ergosterol. As *ERG10* expression increased, cellular ergosterol levels increased and the oxidative stress response was relieved as the cells are better able to cope with the ROS. This highlights the need to optimise oxygenation during the initial stages of lager fermentation to maximise ergosterol biosynthesis and reduce oxidative stress on the yeast.

# HYBRID WINE YEASTS WITH UNIQUE FERMENTATION CHARACTERISTICS

**Jenny Bellon** [1], Jeff Eglinton [1], Alan Pollnitz [1], Cherise Hillier [2], and Miguel de Barros Lopes [1]

[1]The Australian Wine Research Institute, South Australia, 5064, Australia, [2]Flinders University, Bedford Park, South Australia, 5042, Australia

In order to produce novel yeasts that impart diverse flavours to wine, interspecific hybrids have been made between commercial wine strains of *S. cerevisiae* and other *Saccharomyces* sensu stricto species using rare mating. One of these strains, a wine yeast X *S. paradoxus* hybrid (CPH1), has been analysed in some detail. CPH1 shares properties of both parents, including osmotic and ethanol tolerance from the wine yeast parent, and improved growth at low temperature from the *S. paradoxus* parent. In grape juice, the hybrids fermented at the same rate as the commercial wine yeast parent, whereas the *S. paradoxus* parent was unable to ferment grape juice at all. The genome of CPH1 appears to be generally stable during fermentation, although some evidence for transposon instability has been observed. Metabolome analysis indicates that the hybrid yeast produces wines with altered composition compared to the commercial wine yeast. Preliminary sensory data supports these findings. These results show that producing interspecific hybrids between commercial wine yeasts with species that are less suited for grape juice fermentation is a potential strategy for developing novel winemaking strains.

Hybridisation experiments between these two species demonstrate that mating efficiency within and between the species is equivalent, suggesting that no interspecific barriers to conjugation exist. Also, interspecific rare matings were detected with all of the described *Saccharomyces* sensu stricto species, and the hybrid yeasts. These results, together with the characterisation of natural interspecific hybrids and tribrids, indicate that the existence of chimeric genomes could be widespread, and that introgression takes place within the *Saccharomyces* sensu stricto complex.

# INVESTIGATION INTO THE MECHANISM OF ACTION AND BIOLOGICAL ROLE OF *Saccharomyces cerevisiae* MANNOPROTEINS WHICH REDUCE VISIBLE HAZINESS IN WHITE WINE

Shauna L. Brown[1,2,3], **Miguel de Barros Lopes**[1,2], Peter B. Høj[1,2], Elizabeth J. Waters[1]

[1] The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA, 5064, Australia. [2] Department of Horticulture, Viticulture and Oenology, The University of Adelaide, Waite Campus, Glen Osmond, SA, 5064, Australia.

Protein haze is a common problem in beer and wine making. In white wine, the pathogenesis related proteins from grape berries can aggregate into light scattering particles which form haze. The presence of haze is a quality defect, and so, bentonite, an aluminium silicate clay, is frequently used to remove haze-forming proteins before wines are bottled. This treatment, essentially an ion-exchange process, also removes flavour compounds and may result in a lower quality wine. A potential alternative to bentonite fining may be the use of mannoproteins that reduce the particle size of the protein aggregates (haze protective factors). Two mannoproteins exhibiting such haze protective activity (Hpf1p and Hpf2p) have been isolated from a winemaking strain of *Saccharomyces cerevisiae* (EC1118). Hpf1p has a homologue in *S. cerevisiae*, Hpf1'p. Characterisation of Hpf1p and Hpf2p indicated that these proteins were highly mannosylated and were localised in the cell wall. Deletion and overexpression strains of the HPF genes have been constructed in the laboratory yeast S288c to determine the role of these proteins in haze protective activity and the function of these proteins in yeast. Material isolated from ferment supernatants of *Δhpf* strains have a lower haze protective activity than the wild type yeast and material from HPF1 and HPF1' overexpressor strains have increased haze protective activity. The HPF genes have been tagged with a 6xHis epitope to aid purification and localisation. A *Δhpf* strain was more tolerant to cold and ethanol stress and had decreased mating efficiency. Further experiments to establish the function of these proteins are currently being performed.

# YEAST MEMBRANE RESPONSES TO GLUCOSE ARE RELATED TO MEMBRANE PROTEIN EXPRESSION.

**B. A. Butcher** and R. P. Learmonth

Centre for Rural and Environmental Biotechnology, Department of Biological and Physical Sciences, University of Southern Queensland, Toowoomba 4350.

During growth and fermentation, yeasts experience frequent changes in cellular environments, including varying nutrient and metabolite concentrations and ethanol accumulation. Yeasts adapt rapidly to these changes, with the primary mechanism of response being plasma membrane fluidity modulation. We assessed membrane fluidity modulation in *Saccharomyces cerevisiae* strain FY1679-28c and the deletion mutants of the $\Delta^9$ acyl-CoA desaturase (ole) and the membrane associated stress protein Hsp30. We assessed responses to glucose concentrations above (0.5% w/v) and below (0.1%) the threshold for catabolite repression, and to control for osmotic effects, to the same concentrations of sorbitol. Real time progressive fluidity changes were determined by fluorescence spectroscopy measuring polarization anisotropy and Generalized Polarization (GP) of 6-lauroyl-2-dimethylamino naphthalene (laurdan) labelled cells.

We found that while the membrane fluidity responses to sorbitol controls were negligible in all strains, the deletion mutants showed altered responses to glucose. The parent strain FY1679-28c showed a negligible response in anisotropy to glucose, but a small rise in GP after addition of 0.5% glucose. The hsp30 and ole mutants both showed decreases in anisotropy after addition of 0.1% or 0.5% glucose. However under the same conditions both mutants showed a pronounced increase in GP on addition of either concentration of glucose.

Thus we found that membrane responses in the protein expression mutants were different to the parent strain, however we found conflicting data. Both GP and anisotropy are considered to be inversely related to membrane fluidity, i.e. the higher the value the lower the fluidity. Thus it would be expected that variation in anisotropy should be reflected by a similar change in GP. Further studies will be directed to elucidate the underlying mechanism for the inconsistencies observed.

# GENE ARRAY ANALYSIS OF *Saccharomyces cerevisiae* DURING ETHANOL STRESS.

**M. Chandler**, G. Stanley, P. Rogers[2], P. Chambers.

*School of Life Sciences and Technology, Victoria University of Technology, Werribee Campus (WOO8), P.O. Box 14428, Melbourne City, MC, Victoria, Australia, 8001, [2]BrewTech, Carlton and United Breweries Limited, 4-6 Southampton Cresent, Abbotsford, Victoria, Australia, 3067.*

Brewing yeast performance is often compromised during alcoholic fermentations due to by-product inhibition. Endogenous ethanol is arguably the product with the greatest impact on yeast performance, acting as a potent chemical stress on yeast cells. This stress eventually inhibits yeast growth and reduces cell viability, therefore limiting alcohol concentrations in the final product and increasing fermentation turnover times. The reduced cell growth rate and viability, as well as an increased growth lag period, are characteristic signs of cell stress. This is often accompanied at a molecular level by the induction of stress response genes.

Our investigations into the stress response of *Saccharomyces cerevisiae* have used time-course gene arrays to identify genes up regulated in response to ethanol stress during the lag phase adaptation period. Yeast cells were grown in a defined medium (unstressed controls) and in a defined medium with the addition of 5% ethanol (stressed). cDNA templates were prepared from equal numbers of unstressed and stressed lag phase cells collected at intervals over a four hour time course. Gene array results revealed many genes with altered expression profiles. Of these genes a large number were associated with known general stress responses. In addition a number of genes involved in energy metabolism were expressed. This may reflect a possible pseudo-starvation state in yeast during the early stages of ethanol stress. A wide range of other genes were also up regulated in response to ethanol. The potential roles of these genes are currently being explored.

# CAN YEAST STRAIN AFFECT COLOUR AND PHENOLIC CONTENT OF SHIRAZ WINE?

**Simon Dillon**[1], Eveline Bartowsky[1], Peter Høj[1], Laurent Dulau[2], and Paul Henschke[1]

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[2]Formerly Lallemand SA, 31023 Toulouse Cedex 1, France

Red wine colour is the result of many factors, which include grape variety, and viticulture, fermentation and maturation conditions. Yeast strains that enhance the content of anthocyanins and other desirable phenolic compounds during alcoholic fermentation of grape must may be useful for improving the colour and flavour of red wine made from low phenolic musts.

The effect of yeast strain on the accumulation of phenolic compounds (anthocyanins, polymeric phenols, and late eluting pigments) in Shiraz wine was determined by spectrophotometric and HPLC analyses. Musts, prepared from mature Shiraz grapes sourced from the Clare Valley, were fermented with 17 commercial *Saccharomyces cerevisiae* strains in triplicate 1 kg portions at 25º C with cap plunging four times daily. Musts from Langhorne Creek and Adelaide Hills were also fermented with six strains selected from the original 17.

Based on wine colour density measurements of Clare Valley wines, the 17 strains fell into three distinct categories that varied up to 38%. Two yeast strains (B and Q) gave the lowest colour density wines and another two strains (F and K) gave the highest. Wine colour density comparisons for six yeast strains over the three viticultural regions, showed a similar ranking for each strain. Yeast strain B consistently demonstrated an ability to produce low colour wines whereas strain F consistently generated wines with high colour. HPLC analysis of all the wines showed that yeast strain differences were evident in the amounts of the major colouring components (malvidin-3-glucoside and late eluting pigments). Strain B wines tended to contain less of these components whereas strain F wines tended to contain more.

These results indicate that yeast strain can have a significant influence on the final content of phenolics in wines. Strain selection may provide a useful tool for the optimisation of wine colour which is a significant contributor to wine quality.

# *SACCHAROMYCES BAYANUS* – AN ALTERNATIVE WINE YEAST?

**Jeff Eglinton** and Paul Henschke

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Yeasts are an important source of aroma and flavour compounds during the production of alcoholic beverages. Although *Saccharomyces cerevisiae* remains the yeast of choice for pure culture inoculation during winemaking, non-*S. cerevisiae* yeasts that form part of the natural microflora of the vineyard and winery can have a significant impact on the aroma and flavour of wine. We are studying several of these 'alternative' yeasts to determine their potential for use commercially in a controlled fashion to enhance aroma and flavour and, hence, product value. In particular, *Saccharomyces bayanus* has demonstrated potential as a wine yeast, either individually or in conjunction with commercial wine yeasts, based on the ability of strains to produce wines with some desirable chemical, sensory and visual properties. Two strains of *S. bayanus* isolated by us, AWRI 1176 and AWRI 1375, can completely dominate and ferment standard grape juices and musts to dryness, although they are also amenable to co-fermentation with *S. cerevisiae*. Wines made with 1176 or 1375 are characterised by a different chemical profile when compared to those made with a control yeast, including a lower concentration of acetic acid and ethanol (a variable property), and a higher concentration of glycerol and succinic acid. Each of these compounds can contribute to wine quality. The aroma of wines made using 1176 or 1375 often shows complex herbaceous, stewed/dried fruit, savoury and nutty characters, in addition to the estery/fruity aromas that are typical of wines made with *S. cerevisiae*. AWRI 1176 and 1375 produce less of the aroma-active compounds, isoamyl acetate and ethyl hexanoate, but much more of 'rose'/'floral' compounds 2-phenylethanol and 2-phenylethyl acetate. The palate of wines made with *S. bayanus* is usually different, with a distinct fullness that is typically not evident in wines made with control strains of *S. cerevisiae*. The palate can also consist of more 'developed' and 'richer' flavours than control wines, particularly on the middle palate. The metabolome of AWRI 1375 has been determined in Chardonnay ferments to help elucidate those compounds that are responsible for the novel aromas and flavours.

# USING METABOLOMICS TO INVESTIGATE CELLULAR METABOLIC CHANGES IN A GLYCEROL OVERPRODUCING STRAIN OF *Saccharomyces cerevisiae*

**Jeff Eglinton**, Anthony Heinrich, Alan Pollnitz, Paul Henschke and Miguel de Barros Lopes.

The Australian Wine Research Institute, PO Box 197, Glen Osmond SA 5064, Australia. Email:Jeff.Eglinton@awri.com.au

Glycerol is a major fermentation product of *Saccharomyces cerevisiae* and contributes to the sensory character of wine. Diverting sugar to glycerol production during fermentation has the potential to decrease the alcohol content of wine, which is a desirable outcome from a consumer perspective. Strains of *S. cerevisiae* that overproduce glycerol, as a result of overexpressing the glycerol 3-phosphate dehydrogenase gene *GPD2*, produce less ethanol but accumulate considerably more acetic acid than the wild-type. By deleting an aldehyde dehydrogenase gene, *ALD6*, acetic acid accumulation was reduced to wild-type levels in a strain that was overexpressing *GPD2*. The growth rate and fermentation rate, which are important properties for winemaking yeasts and basic phenotypic variables, were similar for the modified and non-modified strain. Measurement of the yeast metabolome is a recent development in functional genomics and can be a useful tool for studying genes of unknown function. We have used metabolomics differently, to understand better the effect of modification of two genes of well-defined function, *GPD2* and *ALD6*, in fermenting *S. cerevisiae* cells by analysis of the mutant metabolome using GC-MS. This approach proved useful for investigating the effect of the genotypes on whole-cell metabolic networks, rather than the isolated pathways involved in glycerol and acetic acid biosynthesis, with the result that, whilst the predicted metabolic effects occurred, some unexpected metabolic changes were observed. Analysis of the metabolome of the strains when grown in a defined medium revealed that the accumulation of some fermentation products, including acids, esters, aldehydes and higher alcohols (many of which are flavour-active in wine) was different for the strains. Modification of *GPD2* and *ALD6* expression represents a potentially effective strategy to add novel aroma and flavour diversity to fermented beverages, in addition to decreasing the ethanol concentration. The implication for the use of these modifications in commercial beverage production requires further investigation in commercial yeast strains.

# THE GRAPE BERRY AS AN ECO-SYSTEM FOR YEASTS AND FILAMENTOUS FUNGI

**G H Fleet,** A L Beh, C. Prakitchaiwattana and G M Heard

Food Science and Technology, School of Chemical Sciences, The University of New South Wales, Sydney, New South Wales, Australia, 2052

The surface of the grape berry represents a phyllospheric habitat for yeasts and filamentous fungi. The biodiversity of this yeast-fungal ecology has varying impacts on the efficiency and quality of wine production. The yeasts play a significant role in the alcoholic fermentation of wines, but they also act as natural agents for the biocontrol of grape spoilage fungi in the vineyard. Pre-harvest spoilage fungi have a significant impact on grape quality and viticultural practices that could affect yeast ecology. This presentation examines how vineyard grapes become contaminated with yeasts and fungi, followed by a critical evaluation of those factors which affect the survival, growth and biodiversity of this microflora from the time of vine bud burst, through flowering, fruit formation, berry maturation until harvesting. Factors considered include the chemical composition and physical structure of the grape surface as a substrate for microbial colonisation, spatial distribution and development of microorganisms, climatic and geographical influences, berry damage, and viticultural practices such as the application of pesticides and fungicides. Microbial adaptation to the phyllospheric habitat through the production of hydrolytic enzymes, tolerance of the extremes of temperature, irradiation and dehydration, production of adhesive factors and species interactions are considered. Methods for the cultural and molecular analysis of the species and strain diversity of grape yeasts and fungi impact on the reliability of ecological data, and will be critically reviewed.

# THE YEAST AND BACTERIAL ECOLOGY OF WINE GRAPES

**G.H. Fleet**, A.L. Beh, C. Prakitchaiwattana, S.S. Bae, and G.M. Heard

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Grapes are a primary source of yeasts and bacteria associated with wine fermentations. The biodiversity of this ecology has varying impacts on the efficiency and quality of wine production, as well as biocontrol of spoilage fungi on grapes in the vineyard. Grape cultivar, vineyard geography and climate, and viticultural practices such as insecticide and fungicide applications impact on this microbial ecology, but further research is needed to determine the specificity of these influences. Using a combination of cultural and molecular methods, including denaturing gradient gel electrophoresis of ribosomal DNA subunits, the populations of yeast and bacterial species on four grape varieties were monitored in several Australian vineyards from the time of bud burst, through flowering, and various stages of grape berry maturity, to harvest. The impact of berry damage on microbial ecology was also examined. Yeast-like fungi, especially *Aureobasidium pullulans* and the bioinsecticide bacterium *Bacillus thuringiensis* predominated on grapes until the later stages of maturity, along with lesser populations of *Rhodotorula*, *Cryptococcus* and *Sporobolomyces* spp. The latter yeasts were not detected by DGGE, but only recovered by cultural methods. At 1-2 weeks before harvest, significant populations ($10^4$-$10^5$ cfu/g) of *Kloeckera*/*Hanseniaspora* and *Metschnikowia* spp had developed, along with increased diversity of bacterial species. Damaged grape berries had higher populations and species diversity than undamaged berries. The principal wine yeast, *Saccharomyces cerevisiae*, was not found on most grapes. The yeast ecology of the grapes is being correlated with grape cultivar, berry maturity and use of pesticides.

# IDENTIFICATION OF GENES CONTRIBUTING TO A “HIGH NITROGEN EFFICIENCY” (HNE) PHENOTYPE IN YEAST

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A key issue of industrial wine fermentation is the availability of assimilable nitrogen. When nitrogen is limiting sugar transport systems are inactivated and biomass formation is restricted. Consequently fermentation may fail to complete. The development of a highly nitrogen efficient wine yeast which catabolises more sugar with a reduced degree of nitrogen utilization would be of great benefit to the wine industry.

This study aims to identify genes that when altered confer nitrogen efficiency. HNE strains were selected from a tailored fermentation screen of insertional yeast mutants generated by transposon mutagenesis (Ross MacDonald *et al.,* 1997, *PNAS, USA* **94:**190-195). Six genes have been identified that when modified increase glucose consumption by between 3 and 15% in a chemically defined grape juice with limiting nitrogen (75mg FAN/L).

To determine the impact of these gene modifications on other attributes of oenological importance apart from nitrogen efficiency, selected genes have been deleted in a modified wine yeast strain (C911D, M. Walker, this laboratory). The most promising strain so far has a deletion of *NGR1* (Negative Growth Regulator) enabling this strain to complete fermentation in a significantly reduced time (73-83% of the parental).

Work is underway to evaluate the impact of such deletions upon the aroma and flavor of wine fermentation and to determine the mechanism by which such deletions confer a nitrogen efficient phenotype.

# KINETIC ANALYSIS OF THE ACTIVATION OF THE ONE-CARBON REGULON USING MICROARRAY TECHNOLOGY

**Gelling, C.L**.[1], Kornfeld, G.[1,2] , Cook, T.S.[1], Piper, M.D.W[3]., Dawes, I.W[1,2].

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The advent of microarray technology has provided an excellent method for identifying the members of transcriptional regulons. Kinetic analysis is one approach to this task that is particularly useful for delineating responses to uncharacterised transcription factors. The utility of this method is demonstrated by the example of microarray analysis of one-carbon metabolism.

Tetrahydrofolate (THF) mediated one-carbon metabolism provides single carbon units at various oxidation states for purine, methionine, dTMP and serine biosynthesis. Exogenous glycine has been shown to inhibit one-carbon unit generation and induce genes encoding sub-units of the glycine decarboxylase complex *via* an unknown transcription factor responsive to cytosolic 5,10-methylene-THF levels. In order to determine whether this response was part of a more general one-carbon regulon, a time-course analysis of the glycine response was performed.

Cluster analysis demonstrated that several other one-carbon metabolism genes, as well as genes encoding members of the purine biosynthesis pathway, were rapidly induced by glycine. This induction occurred with very similar timing to that of the glycine decarboxylase genes, which indicates that these genes are co-regulated by cytosolic one-carbon unit levels. A number of uncharacterised genes were co-expressed with the one-carbon metabolism genes, and may have functions connected with this pathway.

The use of a detailed time course facilitated not only the identification of the one-carbon unit responsive genes, but revealed a temporal sequence of transcriptional responses to glycine. Genes encoding respiratory proteins were induced after the primary induction of one-carbon metabolism genes, and remained induced over four hours, from which it can be inferred that respiratory metabolism increases under conditions of increased glycine catabolism. Amino acid biosynthetic genes were induced in a transient manner approximately an hour after glycine addition, suggesting flux through amino acid metabolism pathways was disturbed by the depletion of one-carbon units and subsequent increase in mitochondrial one-carbon unit generation.

Analysis at the transcriptional level was complemented by genome-wide screening of viable deletion mutants for defects in glycine catabolism. This study identified genes with functions that are important for glycine utilisation, including those involved in one-carbon metabolism, mitochondrial gene expression, terminal respiration, nitrogen and carbon source regulation and several general transcription factors.

# RAPID OPTICAL ESSAY FOR IDENTIFICATION OF DIFFERENT STRAINS OF BAKER'S YEAST *SACCHAROMYCES CEREVISIAE*.

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Fluorescence and fluorescence excitation spectroscopy are optical techniques of significant utility in many industrial problems that require rapid diagnostics and remote and contact-less sensing. We report here the application of these techniques to differentiate between industrially relevant strains of baker's yeast, *Saccharomyces cerevisiae*. We identified spectroscopic fingerprint regions of the excitation curves between 200 nm and 240 nm, with the detection set at 340 nm. In these regions where differentiation between strains number 255 and 167 could be done by inspection of the data. Sixteen other strains were also optically studied. Their behaviour belonged to one of the three classes, it was either similar to 167, 255 or to the third "intermediate" class. A statistical method based on Fischer distance was used to fully confirm differentiation.

This work can be used as a basis for a rapid optical test for distinction between selected strains of *Saccharomyces cerevisiae*.

# INVESTIGATION OF THE FORMATON OF VOLATILE PHENOLS AND FATTY ACDS BY *Dekkera* AND *Brettanomyces* YEAST

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The yeast *Dekkera,* the perfect form of *Brettanomyces,* when allowed to grow in wine can cause a range of flavour modifications. These changes are generally considered to be undesirable. Some of the sensory characters that these yeasts can produce in wine are attributed to volatile phenols and fatty acids. The volatile phenols, 4-ethylphenol and 4-ethylguaiacol have been described as; stable, farmyard, horsy and 'band-aid', while the fatty acid, iso-valeric acid has a rancid cheese or vomit-like aroma.

A range of species and strains of *Dekkera* and *Brettanomyces* have been investigated for the production of these aroma compounds. The yeast were grown in chemically defined media and analysed by sensory evaluation and gas chromatography/mass spectrometry. The production of the volatile phenols appears to be a unique characteristic of *Dekkera*, with only trace amounts or none produced by the *Brettanomyces* species. The results confirmed that the precursor of 4-ethylphenol is *p*-coumaric acid and that ferulic acid is the precursor of 4-ethylguaiacol. No volatile phenols were produced in the absence of the phenolic acid compounds. All strains of *Dekkera* investigated could produce iso-valeric acid. In the presence of exogenous L-leucine a stimulation of iso-valeric acid was observed. A better understanding of the conditions that lead to the formation of these flavour compounds will lead to the development of strategies to limit their concentration in wine.

# COMPARATIVE PROTEOMICS: IDENTIFYING NOVEL PROTEINS IN THE WINEMAKING STRAIN *SACCHAROMYCES CEREVISIAE*.

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Extensive phenotypic variation can exist between individuals of a single species. Elucidating the genetic basis for this variation, especially in the post genome era, has received considerable attention in view of its importance in to a broad range of objectives including predicting human disease, defining biodiversity and improving agricultural yield. *Saccharomyces cerevisiae* wine yeast strains have been selected over thousands of years of winemaking for properties that include fast growth in high-sugar grape juices, high yield and tolerance to ethanol and, more recently, sulfite resistance and the biosynthesis of flavour and aroma compounds at concentrations beneficial for wine quality. However, the genes that contribute to these valuable properties have not yet been elucidated. As ethanol is the main stress encountered during grape juice fermentation, we have chosen to study how wine strains respond to an ethanol stress and compare this to the response of less ethanol tolerant laboratory strains. By using a comparative approach, we hope to establish which genetic factors play a role in the greater ethanol tolerance of wine strains. A proteomic approach is being taken so that differences in protein modification as well as expression can be monitored. The results have shown that many proteins are differentially expressed in wine strains compared to laboratory strains. Proteins identified using mass fingerprinting (MALDI-TOF) include glycolytic enzymes, proteins involved in amino acid metabolism, and signaling proteins. However, limitations in the sensitivity of the current methods and apparatus used to date and problems in comparing gels between experiments have made it difficult to identify all proteins of interest. Our results achieved thus far and the difficulties encountered with a comparative proteomics approach will be presented.

# PHYSIOLOGICAL REGULATION OF THE UNFOLDED PROTEIN RESPONSE IN YEAST

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The Unfolded Protein Response (UPR) is an intracellular signalling pathway between the endoplasmic reticulum (ER) and the nucleus. The accumulation of unfolded proteins in the ER results in the transcriptional upregulation of genes encoding ER resident chaperones and folding catalysts. In *S. cerevisiae*, the ER to nucleus (ERN) signal transduction pathway is mediated by a sensor molecule Ern1p which spans the ER membrane. UPR stress results in the oligomerisation and autophosphorylation of Ern1p where upon it functions as an endoribonuclease splicing *HAC1* mRNA encoding a b-ZIP transcription factor. Hac1p recognises the unfolded protein response element (UPRE) found in the promoters of genes transcriptionally upregulated in response to UPR stress.

We have shown that splicing of *HAC1* mRNA is modulated by osmotic stress. Under conditions of low osmolarity the cell integrity (CI) MAP Kinase module is activated in a Rho1p and protein kinase C dependent manner. Deletion analysis of CI pathway components indicates that modulation of *HAC1* mRNA splicing is primarily regulated through the action of *PKC1* directly while the CI MAP Kinase pathway is responsible for the level of accumulation of Hac1p. Furthermore we have demonstrated that a pathway from the plasma-membrane receptors Wsc1p and Mid2p through the RhoGEF (GTP/GDP Exchange Factor) Rom2p to Rho1p modulates the UPR.

The CI MAP Kinase pathway primarily mediates the cell integrity/cell proliferation response in yeast and is activated in the cell-cycle at the stage of bud emergence. We propose that under conditions of cellular stress during cell-wall remodelling the synthesis of cell wall components and plasma membrane is co-ordinated by the interaction between the CI pathway and the UPR.

# TALKING TO YEAST: APPLICATION OF GENOME-WIDE EXPRESSION ANALYSIS TO COMMUNICATE CONDITIONS IMPORTANT FOR SUCCESSFUL INDUSTRIAL FERMENTATIONS

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Industrial fermentation processes are dynamic systems that subject yeast to an ever-changing range of stresses and differing metabolic requirements. However, the majority of research into these processes is carried out using defined biological systems that do not always reflect the true nature of the industrial process. To determine whether microarray technology could produce a better understanding of environmental conditions important to industrial fermentations, the genome-wide expression response of an industrial yeast strain during the early stages of large-scale beer fermentation was analysed. During the first hour of fermentation genes involved in ergosterol biosynthesis were induced. Cellular ergosterol levels were measured and shown to correlate with the increased expression of the ergosterol biosynthetic genes. Metabolic activity analysis of yeast mutants deficient in ergosterol biosynthesis showed that its presence is essential for efficient adaptation to conditions of fermentation. Genes involved in maintaining cell redox balance and protection against oxidative stress were also induced during the initial stages of the fermentation. Many of these were part of the thioredoxin and glutathione systems. This was a surprising result since the only aeration that occurred was that caused by the agitation of the wort during the filling of the fermentation vessel. This amount of aeration would not normally be regarded as a condition that would cause an oxidative stress response in yeast cells. Genome-wide expression analysis was found to be an effective communicator, through yeast gene expression profiles, of environmental conditions that are important in industrial fermentations.

# YEAST STRAIN DYNAMICS DURING MIXED CULTURE WINE FERMENTATION AND EFFECT ON WINE COMPOSITION

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In most spontaneous or uninoculated wine fermentations, a number of *Saccharomyces spp.* and strains are present. Although individual strains of *S. cerevisiae* and *S. bayanus* can produce a characteristic flavour profile, the effect of conducting multiple strain fermentation with *Saccharomyces spp.* has not been studied under controlled conditions. Using Chardonnay juice, mixed yeast cultures were inoculated and allowed to ferment at 15ºC. The relative population of each of the inoculated yeast strains was determined at different stages of the fermentation by microsatellite-PCR banding profiles from colonies plated at each time point. Relative proportions of each of the strains changed throughout fermentation and were dependent on nutrient status of the grape juice. Differences in the wines were tested by examining the chemical and sensory profiles of the mixed culture wines, and comparing them to a blended wine using monocultures wines of the yeasts. Assay of yeast metabolites indicates that some mixed culture wines are different to the blended wines. Interactions between yeast strains by sharing metabolites may account for these differences, which affect the composition of flavour volatiles and sensory qualities of the wines.

**MODELLING *Pneumocystis carinii* DRUG RESISTANCE IN YEAST.**

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DHPS (dihydropteroate synthase) is an important target for antifolates in both prokaryotic and eukaryotic pathogens. The understanding of DHPS and antifolates in eukaryotes has been limited due to technical limitations. Antifolate assays are inadequate and the culturing of eukaryotic pathogens such as *Plasmodium falciparum*, *Pneumocystis carinii* and *Mycobacterium leprae* is extremely difficult or impossible. For this reason we developed model systems to examine the role of DHPS in drug resistance and to enable efficient screening of potential antifolates. Lethal DHPS knockout strains of yeast (*S. cerevisiae*) and *E. coli* were made that could be rescued by DHPS complementation in trans using expression vectors. Optimisation of DHPS expression led to the finding that DHPS expression levels were critical to growth, viability and sulfa drug sensitivity. These data suggest that regulation of DHPS expression *in vivo* is a potential drug resistance mechanism. Furthermore, mutant alleles of DHPS that map to the active site and have been implicated in drug resistance of organisms such as *P. carinii*, *M. leprae,* and *Plasmodium spp.* were cloned into the DHPS genes *of S. cerevisiae*, *M. leprae* and *P. falciparum*. These mutant alleles also led to altered growth, viability and drug resistance in both models. These data together with existing knowledge of folate biosynthesis will be collated to present a new model for sulfa drug resistance and the implications to the prevention and therapy of a broad range of human pathogens and parasites.

# UVB SENSITIVITY OF MESOPHILIC YEASTS AND PSYCHROPHILIC ANTARCTIC YEASTS AT DIFFERENT STAGES OF GROWTH

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Previous studies have shown that yeast cells are, in general, highly sensitive to ultraviolet B (UVB) irradiation. In the present studies, the mesophilic yeasts *Saccharomyces cerevisiae*, *Pichia membranefaciens*, *Zygosaccharomyces rouxii*, *Metschikowia biscupidata*, the Antarctic psychrophilic yeasts *Cryptococcus victoriae, Mrakia gelida* and three newly isolated Antarctic yeasts 41b, 1130a and 130b were studied for survival following UVB irradiation. Cells were exposed to UVB radiation at different stages of growth using a Vilber-Lourmat Bio-Sun apparatus to generate known amounts of UVB radiation from 50 to 500 mJ/cm$^2$ (calibrated at 312 nm).

The results showed that, in the case of stationary phase cells, all the mesophilic yeasts were highly sensitive to UVB-radiation with 60% and 30% survival at 150 and 250 mJ/cm$^2$ respectively and essentially no survivors at 500 mJ/cm$^2$. By contrast, the Antarctic yeasts and, in particular, the pigmented species were less sensitive to even high levels of radiation with 50-60% survival at 500 mJ/cm$^2$. However, all strains, including the pigmented Antarctic yeasts, were sensitive to irradiation during early exponential phase of growth. Depletion of glutathione from mesophilic yeasts by treatment with iodoacetamide (IAA) resulted in higher sensitivity to UVB, which suggested a key role for glutathione in photo-oxidative stress. On the other hand, the Antarctic yeast 1130a maintained high resistance to UVB even in the absence of glutathione, although a reduction in pigmentation was observed in IAA-treated 1130a cells. It was concluded that resistance to UVB-radiation might be related to the presence of carotenoid pigments in Antarctic yeasts. Work is currently underway on the effects of selected antioxidants on UVB protection in mesophilic and psychrophilic Antarctic yeasts.

# RECENT ADVANCES IN LASER SCANNING MICROSCOPY.

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Many techniques have been developed to monitor “average” status and performance of yeasts throughout biotechnological processes. However within a population cells may vary in age, physiological or functional state. To be able to fully interpret averaged data, it is important to understand the potentially different contributions of different subpopulations.

Analysis of subpopulations may be enabled by flow cytometry or microscopy. Confocal Laser Scanning Microscopy (CLSM) has been applied to studies of green fluorescent protein (GFP) expression in yeasts. Multi-Photon Laser Scanning Microscopy (MPLSM) offers advantages over confocal microscopy, and has been applied to studies of animal and plant tissues, model membranes and mammalian cells.

We have applied MPLSM to study membrane fluidity modulation in yeasts in relation to physiological state (growth phase and availability of glucose), heat and ethanol stress [1]. These studies have provided important information on differential responses of individual cells.

[1] Learmonth, R.P. and Gratton, E. (2002) In Fluorescence Spectroscopy, Imaging and Probes - New Tools in Chemical, Physical and Life Sciences (R Kraayenhof, AJWG Visser and HC Gerritsen, Eds.), Springer Series on Fluorescence: Methods and Applications, Vol. 2, Springer, Heidelberg, pp241-252.

# FOREIGN GENE EXPRESSION IN *Saccharomyces cerevisae*

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This presentation is a reflection on a diversity of projects that developed from yeast expression technology in CSIRO. Hopefully it will provide some useful lessons and insights.

The capacity to transform the budding yeast *Saccharomyces cerevisiae* with recombinant plasmids was first demonstrated in 1978. Since that time there have been many advances in molecular biology approaches to produce valuable foreign products when and where required. Such products are of considerable importance for commercial production of heterologous proteins used in the medical, pharmaceutical, veterinary and food industries. Yeast-derived products such as Hepatitis B vaccine and human insulin are the most notable examples, having saved millions of lives. Some of the early products produced by CSIRO included the second viral subunit vaccine (for IBDV) for use in chickens, Blue tongue virus antigen and house dust mite allergen as diagnostic agents. Later work focused on yeast to elucidate the function of HIV-1 proteins and to use yeast to screen for inhibitors of those functions. Likewise some current work involves inhibitor screening of well-known targets. The developments in expression systems have occurred in every dimension including transformation approaches, plasmid development, integration strategies, expression cassettes, secretion modules and host modifications. Pathway engineering is frequently possible and is exploited for bioengineering. Despite the many successes many challenges still remain in making yeast suitable for all of the occasions demanded by industry or researchers. The progress to date and some of the future obstacles will be reviewed.

# PHYSIOLOGICAL AND GENETIC CHARACTERISATION OF BREWING STRAINS OF *Saccharomyces cerevisiae* DISPLAYING CONSTITUTIVE UTILISATION OF MALTOSE AND SUCROSE.

**Florante Jon Meneses**[1,2] And Vladimir Jiranek[1]

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Efficient utilisation of maltose and sucrose is of special importance to brewing fermentation since these are the predominant sugars in primary and secondary fermentation of beer, respectively. Physiological and genetic studies were performed to define the ability of some brewing strains to utilise maltose and sucrose in the presence of glucose and fructose. Three patterns of maltose and sucrose utilisation was observed: repressible, constitutive and non-utilisers. Constitutive maltose utilisation was linked to strong expression of the maltose permease gene at the *MAL1* locus (*AGT1*) compared to non-constitutive maltose utilisers. Additionally, the strong levels of expression were likely to be due to the absence of nucleotide sequence from the promoter of *AGT1*, which correspond to the recognition site of the repressor protein encoded by *MIG1*. On the other hand, sucrose utilisation by brewing strains was seen in two forms: cell-associated and non-cell-associated. The pattern of sucrose utilisation was however, not strongly linked to the kinetics of *SUC2* expression. This study revealed a number attributes of brewing yeasts that could be optimised as a means of producing strains with rapid and efficient utilisation of maltose and sucrose.

# MOLECULAR EPIDEMIOLOGY OF *C. neoformans* REVEALING A POSSIBLE LINK BETWEEN THE OLD AND THE NEW WORLD

**Wieland Meyer** & the IberoAmerican Cryptococcal Study Group. Molecular Mycology Laboratory, The University of Sydney at Westmead Hospital, Sydney, Australia

*Cryptococcus neoformans* has been subdivided into 3 varieties: *C. neoformans* var. *grubii* (serotype A), var. *neoformans*, (serotypes D), hybrid (serotype A/D) and var. *gattii* (serotypes B, C). 340 clinical, environmental and veterinary isolates from Argentina, Brazil, Chile, Colombia, Mexico, Peru, Venezuela, Guatemala and Spain were divided by PCR-fingerprinting with a minisatellite specific primer (M13) and RFLP analysis of the orotidine monophosphate pyrophosphorylase (*URA5*) and phospholipase (*PLB1*) genes into 8 molecular types. The majority of the isolates 68.2% (n=232) were VNI (var. *grubii*, serotype A). This is in accordance with the fact that this variety causes the majority of all human cryptococcal infections worldwide. 5.6% (n=19) were VNII (var. *grubii*, serotype A); 4.0% (n=14) VNIII (AD hybrid); 2.0% (n=7) VNIV (var. *neoformans*, serotype D); 3.5% (n=12) and VGI; 6.2% (n=21) VGII; 9.2% (n=31) VGIII, 1.5% (n=5) VGIV (all var. *gattii*, serotypes B and C). VNIII, AD hybrid isolates, mainly from Chile and Spain, revealed two RFLP patterns one corresponding to VNI, VNII and VNIV and the other to VNII and VNIV suggesting different recombination events between var. *grubii* and var. *neoformans* leading to diploid or triploid strains. These findings suggest an epidemiological link between the old and the new world and support an ongoing speciation within the *C. neoformans* complex.

# THE TRANSCRIPTIONAL RESPONSE OF ETHANOL-STRESSED YEAST IN THE PRESENCE AND ABSENCE OF ACETALDEHYDE

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During fermentation, yeast is subjected to range of stressful conditions; the major stressors being ethanol, low pH, osmotic stress and nutrient limitation. Yeast stress during fermentation is strongly associated with reduced growth rates and diminishing viability, leading to poor ethanol yields and low productivity. An interesting development in studies on yeast stress is the discovery that the adaptation rate to ethanol stress is improved significantly by the addition of small quantities of acetaldehyde, however the biochemical and molecular processes underpinning this effect are unknown. Identifying the mechanisms associated with the acetaldehyde-mediated yeast response to ethanol stress may facilitate the production of yeast strains with improved ethanol tolerance or the development of strategies for improving the ethanol tolerance of yeast.

The presentation will focus on the level of gene expression in *Saccharomyces cerevisiae* during ethanol stress in the presence and absence of acetaldehyde. Yeast were inoculated into fresh, nutrient-rich medium alone, or medium with added acetaldehyde only, ethanol only or both acetaldehyde and ethanol. Samples for biomass and RNA extraction were taken at regular intervals during the initial growth lag period (*ie*. the period of adaptation to ethanol stress). Growth curves showed a 65% increase in the stress adaptation rate in cultures containing both ethanol and acetaldehyde compared to cultures containing ethanol only. Northern analysis was conducted using probes specific for known ethanol-stress response genes and a housekeeping gene.

# CELLULAR FUNCTIONS IN GLUTATHIONE HOMEOSTASIS IDENTIFIED BY GENOME-WIDE SCREENING / ENGINEERING YEAST FOR GLUTATHIONE PRODUCTION

**Gabriel G. Perrone**[1], Chris M. Grant[2] and Ian W. Dawes[1]

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Glutathione is an essential metabolite protecting cells against oxidative stress and aging. Genome-wide screening has identified 270 yeast deletion mutants that secrete significant amounts of glutathione. These have identified a surprising set of functions that are important for glutathione homeostasis. The highest secretors were affected in the late endosomal sorting pathway which appears to play a crucial role in the balance between secretion and storage of glutathione. Other functions included nitrogen/carbon source signalling, mitochondrial electron transport, ion transport and those maintaining cellular integrity. These results have significant implications for understanding mechanisms affecting glutathione depletion in degenerative diseases and cell aging, and for engineering yeast cells to increase glutathione production.

# STRATEGIES FOR AVOIDING PROBLEMS ASSOCIATED WITH NITROGEN LIMITATION DURING WINE FERMENTATION

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A key research interest of this group is the identification of solutions to problem wine fermentations that arise out of a deficiency of assimilable nitrogen. Two strategies that we are pursuing seek to achieve a more efficient utilization of existing assimilable nitrogen in grape juice. Such strategies will yield outcomes that include a reduction in the frequency with which problem fermentations occur and/or a reduction in the requirement of nitrogen supplements to allow completion of fermentation.

One line of investigation arises out of a recognition that proline is a predominant amino acid in grape juice but one that is largely unutilized during fermentation. We constructed strains capable of constitutive proline uptake. Such strains take up more proline than the wild type under oenological conditions and can be induced to take up increased amounts by oxygenation. Even under the near anaerobic conditions found in wine fermentations there are still advantages for the proline-transport-capable strain in terms of culture viability at the crucial latter stages of the fermentation.

A second investigation seeks to determine the genetic basis for strain differences in relative efficiency of assimilable nitrogen utilization. Using transposon mutagenesis, a number of mutants that catabolise more sugar than the wild type when presented with the same, limited amount of assimilable nitrogen (75mg FAN/L) have been identified. Work is now underway to determine the nature and function of the disrupted genes.

Both projects are progressing towards the introduction of their various modifications/mutations into a wine yeast background so as to allow a determination of the broader oenological consequences of these changes.

# Beer Brewers or Yeast Farmers? - Managing Yeast in the Modern Brewery.

Jeff Potter

Lion Nathan Australia, 20 Hunter St., Sydney 2000

Among the commercial scale users of yeast the brewing industry is one of the few that rely on a constant supply of yeast that is produced during the process of manufacture of the main product – beer. As a result brewers must be masters of both beer production and management of the supply, health and wellbeing of their yeast.

In the modern brewery the demands of both consumer quality and economic productivity mean consistent fermentation performance is critical. The physiological state of brewing yeast slurry has a major effect on its fermentation performance and this state is a reflection of the management and handling practices used in the brewery. This paper reviews the process of yeast management in a modern ale and lager breweries from master strain culture management through to propagation, fermentation, storage and re-use. Recent work on techniques for measuring yeast physiological condition and their application in optimising the design and operation of large scale yeast handling equipment will be reviewed.

# R&D Opportunities in Fuel Ethanol Production

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The development of a fuel ethanol industry in Australia offers the potential for an enhanced R&D focus on both the related fermentation technology and on associated higher value inputs/products (e.g., enzymes, specific sugars).

Ethanol fuel blends (usually 10% v/v ethanol) are used now widely in both the US and Brazil, and have been mandated recently in India and Thailand. They have the advantages of reduced net greenhouse gas (GHG) production and decreased vehicle emissions (CO, $NO_x$, $SO_x$), as well as providing stimulus for the agricultural sector and associated regional development.

Fuel ethanol is currently produced from sugar and starch-based raw materials using yeasts and in many cases with continuous/cell recycle technology. Ethanol production costs from these raw materials are usually higher than those of petrol/diesel thereby requiring government support/subsidy to sustain the industry. However in the longer term the use of lignocellulosic materials (e.g., agricultural and forestry residues) are projected to reduce production costs to economically-viable values ($A 0.30-0.35/litre). As a result, R&D is now focussed on reducing enzyme (cellulase) costs as well as developing recombinant yeasts and bacteria capable of fermenting both hexose and pentose sugars.

The presentation will review global trends in the fuel ethanol industry and compare the characteristics of the various recombinant yeasts and bacteria being constructed for conversion of lignocellulosics to ethanol.

**Studying mitochondrial turnover by autophagy in *S. cerevisiae* using of a fluorescent protein biosensor**

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Mitochondria are important organelles that are sites for the production of cellular ATP. Autophagy is a ubiquitous cellular response to starvation that is responsible for the turnover of organelles during normal, as well as, stress, conditions, and is implicated in cellular homeostasis. Autophagy involves the sequestration of organelles or bulk proteins by vesicles targeted from the relatively alkaline cytosol to the acidic (pH ~5.0) vacuole for degradation. Although many proteins are turned over by the proteasome, only autophagy is implicated in organellar turnover.

Autophagy is poorly characterised at the molecular genetic level. No assay is currently available that allows monitoring of the process *in vivo*. However, much of the available data has been obtained from studies in yeast (*Saccharomyces cerevisiae)*.

We have developed a novel fluorescent protein biosensor that incorporates a pH sensitive green fluorescent protein variant, pHluorin, with the pH insensitive properties of red fluorescent protein. The biosensor acts as a ratiometric biosensor for pH, allowing us to monitor the autophagy process in living cells. In addition, this assay is compatible with a number of fluorescence detection systems, including fluorescence microscopy and spectroscopy.

We are using the biosensor to investigate, in yeast, the role of autophagy in mitochondrial turnover under different conditions. Information obtained from these yeast studies will be important in understanding the role of autophagy in cellular homeostasis, tumour progression, neurodegenerative disorders and programmed cell death in mammalian cells.

CYSTEINE SCANNING MUTAGENESIS OF YEAST MITOCHONDRIAL $F_1F_O$-ATP SYNTHASE SUBUNIT 8.

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The yeast mitochondrial $F_1F_O$-ATP synthase (mtATPase) is a large, multi-subunit enzyme complex embedded in the inner mitochondrial membrane. It functions as an energy-transducing machine, coupling ATP synthesis or hydrolysis on the $F_1$ sector to proton pumping across the membrane through the $F_O$ sector. Subunit 8 (Y8) is a small, highly hydrophobic intrinsic membrane protein essential for the formation of the proton pore and thus a functional mtATPase. The membrane topography, structural arrangement within the complex and function of Y8 remain unclear. In order to understand how ATP synthesis and hydrolysis are coupled to proton pumping, the structure and function of small protein subunits such as Y8 must be determined.

We have adopted a cysteine scanning mutagenesis approach to determine the structure and function of Y8 in yeast mitochondria. Each of the 48 amino acid residues of Y8 have been systematically replaced with unique cysteine residues and these variant proteins expressed in yeast cells lacking endogenous Y8. In each case *in vivo* respiratory function is restored to these cells, indicating that no amino acid residue of Y8 is directly required for proton translocation through the membrane. Using thiol-specific chemical compounds, we have endeavoured to determine the size and boundaries of the extra membranous regions of Y8. The first 14 amino acids of the N-terminus of Y8 are accessible from the intermembrane space of the mitochondrion, whilst the C-terminus of Y8 is exposed from residue 36 in the mitochondrial matrix. In addition, the pattern of labelling indicates that protein-protein interactions between Y8 and other proteins of the mtATPase occur in each of these regions. Using chemical cross-linking, the location and arrangement of Y8 to neighbouring proteins is also being examined. This strategy has already revealed interactions between Y8 and the protein subunits d and f, each a structural component of the stator stalk [**1**].

Our results indicate that Y8 is a structural, rather than functional, component of the mtATPase and is involved in protein-protein interactions in the assembled enzyme complex. The nature of these interactions is currently under investigation. Using an epitope-tagging approach, the stoichiometry of Y8 will also be determined.

[1]**.** Stephens *et al.* (2000). *Eur. J. Biochem.* **267** pp.6443 – 6451

# BIOGENIC AMINES IN WINES OBTAINED WITH DIFFERENT YEAST STRAINS: RELATIONSHIP WITH THEIR PRECURSOR AMINO ACIDS

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The presence of amines in wine, in high concentrations, can produce a negative impact on the aroma of the product and, in some cases, a pharmacological response. Several factors influence the content of amines in wine, among them the yeast strain and the amino acid composition of the must. The aim of the study was to examine the influence of different strains of *Saccharomyces cerevisiae* on the concentration of biogenic amines in rosé wines. The relation between the concentrations of these amines and the utilization of their precursor amino acids during fermentation was also studied. Depending on the yeast strain involved in the fermentation, there was a slight difference in the content of biogenic amines in the wines, although high concentrations were never reached. No relationship was found between the content of biogenic amines in the wine and the utilization of their precursor amino acids during fermentation for the three *S. cerevisiae* wine yeast studied.

# PRODUCTION OF VOLATILE METABOLITES DURING FERMENTATION OF GRAPE MUST INOCULATED WITH TWO DIFFERENT NITROGEN REQUIRING YEAST STRAINS

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*Saccharomyces cerevisiae* yeast contribute to the aroma and flavour of wine by forming esters and alcohols from sugar and amino acid metabolism during the alcoholic fermentation of grape must. Yeast strain and the concentration of amino nitrogen are known to affect the formation of volatile compounds. This study investigated the behaviour of two strains of *S. cerevisiae* with different nitrogen requirements in the production of esters and higher alcohols during alcoholic fermentation. To carry out the study a Chardonnay must with a high content of nitrogen compounds was used. The results showed that the strain with the highest nitrogen demand produced a higher concentration of esters, except for ethyl acetate, which was similar for both strains, during fermentation and gave rise to a wine with a slightly lower content of higher alcohols. The pattern of evolution of individual esters and alcohols, with the exception of ethyl acetate, depended on the yeast strain and could be expected to contribute a different aroma profile. These differences were probably related to the consumption of nitrogen by the strains as the nitrogen nutrients act as precursors in the synthesis of esters and alcohols and regulate their production.

# THE ACETALDEHYDE EFFECT: AN OVERVIEW OF RECENT RESEARCH IN THE BIOCHEMICAL ENGINEERING LABORATORY

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The addition of small quantities of acetaldehyde to ethanol-stressed cultures of *Saccharomyces cerevisiae* was previously shown to have a strong ameliorative effect on growth. To determine whether acetaldehyde is a global ameliorator of stress in *S. cerevisiae*, we have examined its effects on cultures exposed to various environmental stresses, including inhibitors found in lignocellulosic hydrolysates used for ethanol fermentations, as well as other toxic chemicals, high substrate loadings and changes in pH and temperature. Incremental addition of acetaldehyde significantly reduced fermentation time in high gravity fermentations. The extent to which the acetaldehyde effect is applicable to yeasts other than *S. cerevisiae* was also investigated. The effects of acetaldehyde were shown to be mimicked to varying degrees by the addition of alternative electron acceptors. Acetaldehyde addition significantly affected glycerol synthesis while stimulating the rate of glycolysis; these findings are consistent with suggestions that its stimulatory properties are due to its effects on the cellular redox balance.

# DEVELOPMENT OF HETEROTHALLIC WINE YEAST STRAINS FOR OENOLOGICAL RESEARCH

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The metabolic processes of wine yeasts that influence the fermentation and quality of wine are highly complex. A characterisation of such processes in wine strains, which are typically homothallic, would be greatly facilitated with the application of the standard classical and recombinant genetic techniques that have been successfully used to delineate the physiology of heterothallic laboratory yeasts

We report on the development of a number of heterothallic wine yeast strains of *Saccharomyces cerevisiae*, possessing minimal foreign DNA sequences. These strains were generated by gene disruption of the *HO* gene, which is responsible for switching of mating type and hence re-diploidisation of haploids.

The gene disruption is based on the introduction of the dominant selectable marker, *KanMX*, and acquisition of geneticin resistance (Wach *et al.*, 1994, *Yeast* **10:**1793-1808). The *KanMX* module was removed after gene disruption, in a homozygous *ho/ho* diploid strain using meiotic recombination. Haploid (△*ho*) strains of both mating types were isolated by classical genetics. Southern blot analysis and DNA sequencing confirmed the loop out of the selectable marker, *KanMX* within the *HO* gene. Further, the presence of non-*Saccharomyces* sequences at the target site was restricted to a single direct repeat sequence, derived from the disruption cassette. Small-scale fermentation trials of parental and modified strains were carried out to compare their fermentative performance and production of key metabolites.

A further benefit of the loop out of the *KanMX* marker is that further genes can be disrupted using similar constructs, without the need for other selectable markers and screening techniques.