

Coming 'ome

The return of brewing yeast genetics

I could be wrong but I suspect that 'yeast genetics' would be a poor bet to feature in the top ten of *B&D* reader's 'most want to read' articles. However, it was not always like this. For a decade or so from the early 1980s yeast genetics was big news in brewing and distilling research. Predating consolidation and globalisation, brewing companies around the world were supporting vibrant R&D laboratories. Most of whom were excitedly exploring new genetic techniques and the possibility of 'transformation' or 'modification' through the introduction of new non-brewing yeast characteristics into production yeasts. The myriad of targets included improved ethanol yield, no need for exogenous enzymes, adding value to spent yeast etc.

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Of course, as is often the way, the bubble burst and in recent years yeast genetics in the world of brewing has taken something of a back seat. Looking back, many of the somewhat bullish targets of genetic modification were achieved but never saw the light of day in a commercial fermenter. 'Why' is straight forward, as the application of genetic modification in 'foods' has been roundly rebuffed by consumers and generally given a bad press ('Frankenfood') for more than a decade. With hindsight, the targets for modification were inward and focussed on the process with no clear-cut marketable consumer benefit. As we'll see later in this article, genetic modification may well be more acceptable if it is consumer friendly.

Yeast genetics – back on the agenda?

Contrary to the decline in brewing and distilling but perhaps not in wine; fundamental studies on yeast genetics have continued to grow exponentially. Undoubtedly the trigger for this was the publication of the sequence of the yeast



genome in April 1996 (see Goffeau *et al.*, *Science*, 1996, 274, 546-567). This was something of a milestone as it used the internet and computer-based 'bioinformatics', as well as being the first eukaryote (organisms whose cells are organised into complex structures enclosed within membranes) to be sequenced. Since yeast (the third sequence), over 700 genomes have been sequenced including those of *Gallus galus* (chicken), *Canis familiaris* (dog) and with much hullabaloo in 2001, *Homo sapiens*. Important stuff, as cracking the DNA sequence of an organism is a door opener to understanding how it works and enables sophisticated comparisons with related and unrelated life forms.

One of the consequences of genome projects was that researchers started thinking 'globally' in terms of cell physiology and genetics. Also spinning out of this, has been a host of amazingly insightful techniques that over the years have become quicker, more easily interpreted and importantly more available/cheaper. It is perhaps this that has renewed enthusiasm for applied research in yeast genetics. Certainly something has changed (see Figure 1), as the number of papers on yeast genetics

presented at the last eight congresses of the European Brewery Convention has slowly but surely grown since 1997. Admittedly not a perfect measure but certainly directional.

A new 'ome'

In the mid 1990s, bioinformaticists and molecular biologists playfully applied the suffix 'ome' to effectively rebrand cellular processes and populations (see Table 1 presents a glossary of definitions of the different 'omes). First up the coded cell proteins became the proteome (first citation in 1995), gene expression was badged the transcriptome (1997) and cellular metabolites became metabolome (1998). Inevitably the analysis or study was tagged as an 'omic' giving proteomics, transcriptomics and so on. Before you ask, the term genome was first coined in 1932! Should you want to know more (and who could blame you!) see Greenbaum *et al.*, *Genome Research*, 2001, 11, 1463-1468.

The saga of lager yeast taxonomy

Taxonomy is a little dry and is defined (by Wikipedia) as 'the practice and science of classification'. Perhaps more appropriately for lager strains, 'taxonomy is described sometimes as a science and sometimes as an art, but really it's a battleground' (Bill Bryson, 'A Short History of Nearly Everything').

It's OK for ale yeast, always unapologetically *Saccharomyces cerevisiae*. Lager yeasts though I feel a little sorry for although I suspect they really don't care. Since the 1960s lager yeasts have been through four name changes (see Table 2). These days, bottom fermenting lager strains are taxonomically *Saccharomyces pastorianus*. Although not especially important in itself, this flags an important point of difference between lager and ale yeasts as *S. pastorianus* is a natural hybrid of two *Saccharomyces* species, *S. cerevisiae* and *S. 'another'*. To make this real, the genome of lager yeast is about twice the size of that ale yeast/*S. cerevisiae*. In the sequenced lager strain (Weihenstephan 3470), two or three copies are found of each of the 16 specific chromosomes. In this strain, of the total compliment of 35 chromosomes, 15 were derived from *S. cerevisiae*, 12 from *S. 'another'* and eight chromosomes were a hybrid ('chimera') between *S. cerevisiae* and *S. 'another'*. To find out more on the sequenced lager strain see Nakao *et al.*, *Proceedings of the EBC Congress, 2003, Dublin, 524-530*.

As ever, the identity of the 'other yeast' in lager strains continues to be debated.

Yeast genetics papers at the EBC 1991 – 2007

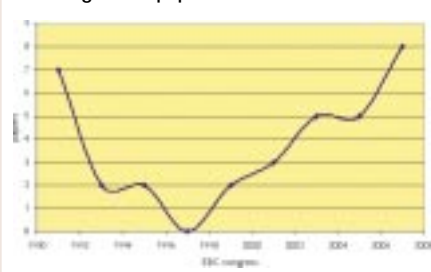


Figure 1: The number of papers on yeast genetics presented at the last eight congresses of the European Brewery Convention has slowly but surely grown since 1997.

Table 1: A glossary of definitions of the different 'omes

Genome	The DNA sequence in its entirety
Transcriptome	All the RNA molecules, including messenger RNA for the synthesis of proteins
Proteome	All the proteins in the cell
Metabolome	All the small chemicals and metabolites in the cell

Table 2: The taxonomy of brewing yeasts over recent years.

	Ale yeast	Lager yeast
Pre 1970	<i>Saccharomyces cerevisiae</i>	<i>Saccharomyces carlsbergensis</i>
1970	<i>S. cerevisiae</i>	<i>Saccharomyces uvarum</i>
1990	<i>S. cerevisiae</i>	<i>Saccharomyces cerevisiae</i>
Mid 1990s	<i>S. cerevisiae</i>	<i>Saccharomyces pastorianus</i>

However, much circumstantial and increasingly direct evidence indicates that *S. another* is a related *Saccharomyces* species, *S. bayanus*. This yeast is found in winemaking and, being cryophilic, probably contributes the lager yeast markers of fermenting at comparatively low temperatures and active fructose transport (wine musts are rich in this sugar). Interestingly, although it could be derived from either parent, the mitochondrial DNA of lager strains originates from *S. bayanus*.

S. cerevisiae appears to be particularly amenable to the formation of natural hybrids. Although beyond the scope of this article three-way hybrids of *S. cerevisiae*, *S. bayanus* and another closely related species have been reported. Further, the creation of hybrids of *S. cerevisiae* and *S. bayanus* in the laboratory has been achieved (Rainieri et al., Proceedings of the EBC Congress, 2007, Venice, 420-424). A development which opens the door to model and systematically study the structure/function relationship together with fermentation performance of hybrid yeasts. Finally, hybrid strains appear not to be unique to lager yeasts. A recent paper (Gonzalez et al., Applied and Environmental Microbiology, 2008, 74, 2314-2320) describes a new hybrid between *S. cerevisiae* and *Saccharomyces kudriavzevii* which was first found in Japan in 2000. Whilst comparatively early days, it interesting that of the six brewing strains reclassified as this hybrid, three were isolated from Trappist beer in Belgium.

Compared to other *Saccharomyces* species, *S. bayanus* and *S. cerevisiae* are not closely related with 19 (potential) proteins unique to the former and 18 unique to the latter. Memorably, Kellis and colleagues (Nature, 2003, 423, 241-254) noted that the 'sequence

divergence between *S. cerevisiae* and *S. bayanus* is similar to that between human and mouse! For the record this sequence divergence represents only 0.5-1% of the genome.

Genome

The yeast genome sequencing project has been subject to continual refinement and development since its publication in 1996. The immensity of knowledge acquisition and growth is managed through the 'Saccharomyces Genome Database' (SGD) found at www.yeastgenome.org. It is very much 'everything you ever wanted to ask' about the yeast genome and provides a mindboggling array of resources. As a measure of value the SGD site has been accessed over 87 million times since 1994!

As of April 2008, the genome of 16 chromosomes consists of 6608 potential proteins of which 71% have been characterised, with the remainder being 'uncharacterised' (17%) or 'dubious' (12%). Of course the genome has a big say in what the yeast cell does and how it does it. At a high level the genome can be cut by the distribution of gene products involved in (i) molecular functions, (ii) biological process and (iii) cellular components. It is a veritable treasure-trove full of tools and analysis. My favourite resource is the gene summaries which, for ca. 2200 genes, provide a synopsis of the gene in question together with appropriate publications. Irrespective of your interest in yeast genetics, as an example of knowledge management, the site is well worth a look!

The commonly used laboratory yeast at the centre of the genome project – S288c – has an interesting provenance with little or no connection with beer fermentations. S288c was derived from EM93 which was isolated from a rotting fig in California in 1938. The current 'best bet' is that EM93 was a wine yeast which was transported to the figs by an

insect.

Inevitably, as the fine detail of the S288c sequence has been explored, this laboratory yeasts' comparative limitations and anomalies have been reported. This is no great surprise given S288c's history and the diversity of domesticated and wild populations of *S. cerevisiae*. A clearer focus for such comparison has resulted from the recent sequencing of a further strain of *S. cerevisiae*. In 2005, an isolate (RM-11) from a Californian vineyard, shares no recent history with S288c.

Comparative genomics

As is only too obvious, the growth of technology is increasingly rapid. In the case of the yeast sequencing project in 1996, involved 633 scientists from 96 laboratories worldwide and took four years. In 2008, sequencing technology has moved on sufficiently for the Genome Sequencing Center (Washington University Medical School, USA) to be sequencing 24 strains and in the UK, a collaboration between the Sanger Institute and the Institute of Genetics, University of Nottingham to be working on 35 strains in the 'resequencing project'. To emphasise the diversity of *S. cerevisiae*, isolates can be grouped into 'brewery', 'clinical',

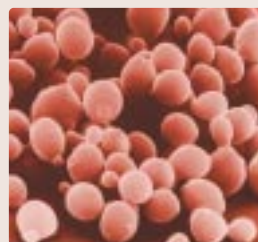
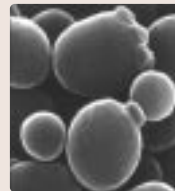
'fermentation', 'laboratory', 'nature', 'sake' and 'vineyard'. By comparing so many strains these groups aim to advance understanding of yeast genomic variation and evolution. Fantastic though this is, it is difficult not to be parochial and lament the lack of true brewing yeasts in these studies. For reasons beyond my ken the 'brewing' yeast in both projects is NCYC 361 or *S. diastaticus*, an amyolytic yeast used in the early transformation work in the 1980s.

Transcriptome

In terms of brewing yeast, the biggest impact of the 'genome project' has been manifest in a number of publications monitoring gene expression during fermentation. This has been made possible by the development and cost effective availability of DNA microarrays (or DNA/gene chip) containing 6400 'genes' (note 71% are characterised) from the

genome of *S. cerevisiae* S288c. Although a gross simplification, microarrays enable expression profiling (i.e. the transcription of DNA to RNA) of the entire genome (or thereabouts) to be monitored both quantitatively and qualitatively. For a readable overview of microarrays see Lucchini et al., (Microbiology, 2000, 147, 1403-1414) which is freely downloadable from the Society for General Microbiology website.

In addition to the obvious gene arrays (human, rat, mouse and yeast), a diverse catalogue of genomes can be mined including barley, wheat, *E.coli* and the tomato! Of



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course underpinning this technology - as with all of today's family of genetics techniques - is sophisticated and fast software to treat and analyse what would be manually, overwhelmingly complex information. As noted earlier, these technologies inevitably encourage a global perspective on the cell's behaviour.

Since 2003, there have been a number of publications describing gene expression in yeast during fermentation. Although 'thinking big' has its upsides, some authors clearly struggle to digest the 'vast amount of data' that expression profiling of fermentation generates. Having gone big, a number of authors take a reductionist approach and focus down on a specific area of metabolism e.g. sulphur (Kondo et al., Proceedings of the EBC Congress, 2003, Dublin, 531-543). The cell's response to the numerous sources of stress has also proved increasingly popular.

Comparisons though are, as ever, not straightforward. As noted elsewhere (Smart, Yeast, 2007, 24, 993-1013), these studies often only have yeast gene profiling in common as they involve (i) different yeast strains, (ii) different worts (and collection gravities, temperature), (iii) scale (tall tubes to cylindrical vessels) and (iv) different sampling times and frequencies which may miss 'events'. Further, gene arrays reflect the genome of *S. cerevisiae* S288c, which may preclude insight into the expression of the *S. bayanus* element of the lager yeast genome. Whether or not this is a major issue is not entirely clear. One piece of insight (using a different transcriptome technology) from VTT in Finland (Rautio et al., Yeast, 2007, 24, 741-760) reports that expression profiles for genes from *S. cerevisiae* or *S. bayanus* are either very different (alcohol dehydrogenase, a gene involved in sterol synthesis) or the same (genes involved in maltose utilisation, isoleucine synthesis and acetate ester synthesis).

So what does the 'big science' of gene profiling tell us about the yeast transcriptome during brewery fermentation? Well, so far, as Katherine Smart noted in the above review there have been 'few surprises'. She notes that 'during the first 1-2 days of fermentation, genes involved include glycolysis, protein biosynthesis...were highly expressed, as would be expected of cells exhibiting growth and division'. It is ironic then that a very recent paper from the Nottingham team (Gibson et al., FEMS Yeast Research, 2008, 8, in press) should report the unexpected observation that anti-oxidant encoding genes (and intracellular antioxidants) increase as the cells enter

stationary phase. As conditions here are anaerobic this is somewhat surprising especially as this response would have been anticipated at the outset of fermentation when oxygen is present in the wort. The authors suggest that these events reflect a general stress response to growth limiting conditions which is not triggered by oxygen.

Proteome

Proteomics focuses on the identification of all the proteins in the (yeast) cell.

The proteome is considered to be more dynamic than the transcriptome but less so than the metabolome. As with the other 'omes', the proteome has been analysed for different species and strains of yeasts growing under a host of different conditions. The technology underpinning proteomics is relatively demanding involving separation (2D gel electrophoresis or increasingly liquid chromatography), identification (mass spectrometry) and quantification (image analysis or mass spectroscopy).

There have been relatively few reports of the proteomics of brewing yeasts. Typically the proteome of strains of lager yeasts (*S. pastorianus*) are similar to each other but different to that of ale strains (*S. cerevisiae*). Reassuringly, the proteins unique to the lager yeasts are most closely related to proteins found in *S. bayanus*.

Metabolome

Of all the 'omes', metabolome is by far my favourite - particularly as it can be extended by the prefix endo or exo. We digress, the metabolome is defined as the low molecular weight compounds (metabolites) found inside (endo-metabolome) and outside (exo-metabolome) the cell. Further these metabolic soups have the potential to differentiate through metabolic footprinting (the exo-metabolome) or metabolic fingerprinting (the endo-metabolome). Although an emerging technology, metabolomics is gaining ground as it is more dynamic and responsive than the transcriptome or proteome. The down-side is that the metabolome encompasses a large number of extremely diverse compounds (inorganics, alcohols, esters, ketones, amino acids, organic acids, lipids and so on) such that as yet there is no single analytical technique that can measure the complete metabolome. Most studies though use gas chromatography/mass spectroscopy coupled with other acronyms for other sophisticated analytical techniques. As with all the 'omes', software based data handling and mining is all important as, increasingly, is

automation/robotics.

Of course, looking at the compounds in the exo-metabolome is similar - but not the quite the same - as the analysis of beer. Accordingly, the technologies being used to probe the metabolome of yeast could clearly drift into tools that enable better insight into beer composition. Although there has been more take up in wine, metabolomics has found application in the differentiation of brewing yeasts via metabolic footprinting (Pope et al., Yeast, 2007, 24, 667-679) and during small scale fermentations at high and very high wort gravity. In the latter case (Pidcocke et al., Proceedings of the EBC Congress, 2007, Venice, 468-474), the sequenced lager strain (Weihenstephan 3470) was used and 37 metabolites monitored, mostly amino acids and organic acids.

And finally ... genetic modification

So as promised, an update on developments in the genetic modification of yeast, not brewing but wine strains. Two strains have been developed and both offer a consumer benefit. One (from First Venture Technologies) reduces the levels of the probable carcinogen ethyl carbamate (or urethane) in wine and the other ML01 - through integration of the malolactic fermentation - contains reduced levels of biogenic amines that are implicated in headaches. Both yeasts have been approved for use in wine making by the US Food and Drug Administration and by Health Canada.

To find out more and the current status of these developments please visit www.firstventuretech.com and for ML01 www.landfood.ubc.ca/wine/vanvuuren/vanvuuren_malolactic-yeast.

To wrap up

Yeast genetics, in all its 'omes' has been chugging away excitedly over the last decade and, from a brewing perspective, is starting once more to find application and to generate powerful insights into yeast behaviour. Inevitably this article is unashamedly 'scientific' and - through the occasional reference - will hopefully stimulate renewed interest in an old friend who has now returned to the fold! ■

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