Alcohol, stress and working with yeast

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Ethanologenic fermentation is traditionally associated with beverage production. However, more recently it has become increasingly important in the biofuel sector. Although the harnessing of yeast for societal outcomes has resulted in many improvements to the fermentation process, there are still considerable gains to be made in this area. In this regard, microbial sensitivity to ethanol toxicity is a major factor affecting fermentation productivity, its impact causing lower fermentation rates, reduced ethanol yields and decreased microbial lifespan. One approach to developing more ethanol stress tolerant yeast is to improve the protective response elicited by cells during ethanol stress.

Ethanol stress causes many changes in yeast including an increased frequency of petite mutations, the inhibition of metabolism, reductions in growth and nutrient intake [1-4], and the disruption of membrane structure leading to increased membrane permeability and subsequent loss of electrochemical gradients and membrane-associated transport activity [5,6].

Although limited, yeast have evolved protective adaptive responses to the effects caused by ethanol exposure. One of the earliest recognised ethanol stress adaptive responses is an alteration in the fatty acid profile of cellular membranes leading to increased fatty acid length and increased proportions of unsaturated fatty acids and sterols in yeast cell membranes [7]. Yeast also accumulate intracellular trehalose leading to suggestions that trehalose functions as a cellular protectant during stress tolerance, potentially by stabilising proteins and preserving the integrity of cellular membranes [8-11]. Moreover, increased expression of many heat shock protein (Hsp) genes has been observed when yeast is exposed to ethanol stress; although of these only Hsp104p [12-14] and Hsp12p [15] have been shown to influence yeast tolerance to ethanol. A number of signalling pathways appear to be activated in the *S. cerevisiae* response to ethanol. One protein that has recently been suggested to have a signalling role specific to the ethanol stress response is Asr1p (alcohol sensitive ring/PHD). It has been shown to constitutively shuttle between the nucleus and cytoplasm but accumulates in the nucleus upon exposure to alcohol [16,17].

Gene array studies on *S. cerevisiae* confirm earlier findings on the ethanol stress response, with all reports observing the up-regulation of genes associated with the trehalose pathway *(TPS1, TPS2* and *TLS1),* and genes encoding HSPs, with *HSP12, HSP26, HSP78* and *HSP104* being amongst the most highly up-regulated [18-20]. These studies also report the ethanol-induced up-regulation of members of the *HSP70* family *(SSA1, SSA2, SSA3, SSA4, SSE1, SSE2).* However, in interpreting these results it must be noted that the transport of specific mRNAs out of the nucleus is modulated during ethanol stress in yeast [21].

It is becoming clear that cellular energetics are particularly affected by ethanol exposure, with gene array-based studies reporting the up-regulation of glycolysis-associated genes *GLK1, HXK1, TDH1, ALD4* and *PGM2,* and high affinity hexose transporter genes, HXT6 and HXT7, in yeast during ethanol stress [18,19]. Other energy production-associated genes, *GPD1, HOR2, GRE3, HOR7* and *DAK1,* were also found to be up-regulated during ethanol stress [18]. These results led Chandler *et al* [19] to propose that yeast subjected to ethanol stress enter a pseudo-starvation state, even though ample glucose may be present in the medium; the impact of ethanol on cell function either impedes glucose transport, or the cells lack sufficient glycolytic activity. Another interesting result from array studies is the down-regulation of genes associated with protein biosynthesis, cell growth and RNA metabolism, possibly reflecting growth arrest during stress to facilitate energy conservation and cellular adaptation [19,22].

As well as global expression studies, several genome-wide screens have been performed with *S. cerevisiae* to identify genes required for ethanol tolerance [17,23,24]. Results identified cell membrane and cell wall architecture genes that were predicted based on previous physiological and genetic studies [17,25]. Many ethanol tolerance genes are required for vacuolar and mitochondrial function. Mitochondria are most likely required for the removal of reactive oxygen species generated from ethanol exposure [26] whereas vacuolar function is anticipated to be necessary for pH homeostasis [23]. Genes that impact on the cytoskeleton, morphogenesis and the cell cycle were also identified as being

required for ethanol tolerance in genome-wide screens. Ethanol has been shown to disrupt the yeast cytoskeleton, which in turn has been proposed to activate the morphogenesis checkpoint and delay the cell cycle [24].

A more recent study has used mapping of quantitative trait loci (QTL) to identify regions in the *S. cerevisiae* genome that explain the difference in ethanol sensitivity between two strains [27]. Two vacuolar protein sorting genes *VPS16* and *VPS28,* also identified in the genetic screens and expression studies, locate within two of the five identified QTL. These results strongly support the significance of the vacuole for ethanol tolerance. The identification of QTLs in the region of *HXK1* and *PFK26* reinforce the proposal that ethanol causes a pseudostarvation state.

To date, research on strain improvement has been mostly aimed at improving microbial ethanol tolerance, in the belief that such improvement will lead to higher ethanol productivity and yield [28]. There are few papers that report the creation and isolation of ethanol-tolerant mutants. Incomplete knowledge of the key cellular mechanisms underpinning ethanol tolerance means there are few successful targeted approaches to generating ethanol-tolerant variants. One successful non-targeted approach is adaptive evolution in continuously growing cultures [29] using ethanol as the selection pressure, otherwise known as evolutionary engineering. In such cases the authors reported an increase in ethanol tolerance of isolates according to their growth profile or CO_2 output, but a more thorough analysis of the phenotype was not conducted [30,31]. This is important to note as the relationship between selection conditions and isolate-phenotype can often be counter-intuitive [32]. Selecting mutants on the basis of their ethanol tolerance may not necessarily lead to increased ethanol productivity or yield. This was the case for a recent study where two ethanol-tolerant mutants of *S. cerevisiae* were created using EMS mutagenesis and an evolutionary engineering approach [32]. Both isolates were significantly more ethanol tolerant, but their ethanol productivity and yields were inferior to the parent strain.

Alper *et al* [33] have used 'global transcription machinery engineering' to isolate ethanol tolerant strains. In this method, the binding preferences of key global transcription factors are modified by a combination of mutagenesis and selection. The paper specifically describes the introduction of mutations in the TATA-binding protein gene *SPT15* using the polymerase chain reaction, followed by serial subculturing in a 6% ethanol, 120 g/l glucose medium, of cells expressing mutated transcription factors. The best performing isolate displayed a prolonged exponential growth phase, faster and more complete glucose utilisation and, most importantly, increased ethanol yield under a number of different conditions and glucose concentrations. The desired phenotype was shown to be due to three mutations in the *SPT15* gene that appear to alter the gene product's interaction with *Spt3p* – a subunit of the SAGA histone acetyltransferase that regulates a number of RNA polymerase II-dependent genes. Microarray analysis of the *spt15* mutant has demonstrated the overexpression of a number of *Spt3p*-dependent genes with broad function. While overexpression of these genes individually did not produce the desired effect, many of the most highly overexpressed genes were essential for the spt15-dependent tolerance.

Our knowledge of the molecular events that occur in yeast during ethanol stress has improved substantially in recent times. The creation and isolation of ethanol-tolerant mutants is evidence that genetic engineering approaches can potentially be used to improve yeast ethanol tolerance. However, the ability to design ethanol tolerant yeast will remain elusive until we better understand the key molecular mechanisms underpinning ethanol stress tolerance. This knowledge may not be far away with recent findings of possible signalling proteins in yeast specific to ethanol stress and the use of global transcription machinery engineering to create yeast with improved ethanol tolerance and productivity.

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EDSIG
Workshops at ASM 2007

Two workshops are planned for Monday 09 July, which means that you are free to immerse yourself in the conference program over the rest of the week. Furthermore, the workshops are open so that people can register for them without attending the conference. However, the final conference plenary session will consider the future of microbiology education.

Workshop: The value of educating university lecturers about teaching and learning.

Chaired by Cheryl Power

Should all university lecturers, demonstrators and tutors receive training in how to teach? If so, how is this best done? Is it a waste of time? Are teachers 'born' not created? Are basic communication skills all that are really needed? Alternatively, should they receive drilling in crowd control?

This workshop will discuss all of the above and more.

Dr Spencer Benson, Director, Center for Teaching Excellence
Associate Professor, Department of Cell Biology and Molecular Genetics,
University of Maryland College Park, College Park, USA will open the workshop describing his work over the years in teacher education.

Recent graduates of teaching courses from several Australian universities will then outline their course structures and objectives and discuss the value and benefits of their participation in the course. Speakers will include Dr James Botten (University of Adelaide), Dr Chris Burke (University of Tasmania) and Ms Liz Fagan (University of Melbourne). Audience involvement will be welcomed.

Workshop: Scholarly Evaluation of Teaching and Learning.

Chaired by Chris Burke

Speakers will include:

Dr Spencer Benson, Director Center for Teaching Excellence, University of Maryland.

Dr Chris Burke, University of Tasmania.

How do we know that the teaching and learning practices we use are enabling effective learning by our students? There is broad anecdotal literature on different techniques and their effectiveness or otherwise, but education research deserves the same level of evaluation that we make of our science research. This workshop will overview the basis of the scholarship of teaching and learning. Attendees will have the opportunity to apply and discuss the issues in the context of their own teaching.