220

WORLD BREWING CONGRESS 2016

World Brewing Congress
August 13-17, 2016
Sheraton Downtown Denver
Denver, CO 80202, U.S.A.

Phenotypic microarray: A high-throughput screening tool for evaluation of desirable brewing traits in novel yeast strains

(Stuart Wilkinson[1], Ed Louis[2], Daniela Delneri[3], Sue James[4], Katherine Smart[4], Chris Powell[1])

[1]ICBS University of Nottingham, UK, [2]University of Leicester, UK, [3]University of Manchester, UK, [4]SABMiller plc, UK

Introduction

Ale (*Saccharomyces cerevisiae*) and lager (*S. pastorianus*) brewing yeasts have different origins and genealogies. Ale-type strains can be traced back to Roman and Egyptian civilizations, while lager yeasts are believed to represent a more recent hybridization event (occurring ~2-300 years ago) between an *S. cerevisiae* strain and an '*S. bayanus*-like' species, most likely *S. eubayanus*. Irrespective of their origins, current production yeast strains have evolved over the interim period, largely through artificial-selection by the brewer for batches of beer with desirable traits. Yeast used to conduct these fermentations were traditionally selected and transferred to a fresh batch of unfermented beer with the knowledge that this was more likely to lead to a 'good' final product. This process has been relatively successful, giving rise to many of the strains used today. However, within the previous 20-30 years there have been major innovations within the industry which have led to increased demands on the current brewing yeasts employed. These center on requirements for increased process efficiency through faster fermentation times (quicker attenuation, shorter VDK rest), the ability to ferment higher gravity wort efficiently, and obtaining yeast populations with improved physiological condition both during and after fermentation (characterized by stress resistance). As such there is growing potential for the use of novel yeast strains which can meet these criteria.

Advances in molecular and cell biology, through both genetic modification and classical breeding approaches, as well as for selection of 'natural' strains from novel environments have created the possibility to generate libraries of new hybrid yeasts with potential for use in beer fermentations. However, screening of large numbers of yeast strains for brewing-specific phenotypes can present a technical problem, as performing traditional assays and small scale fermentations are simply not viable. As such, rapid screening assays are required in order to short-list potential candidate strains for more in-depth analysis. Here we present the use of a phenotypic microarray (PM) technique as a high-throughput screening tool for evaluation of novel yeast strains. The PM effectively conducts 'micro-fermentations' (ca. 100 µL) in 96-well plates and the system can simultaneously run up to 50 plates (4800 fermentations) at any one time.

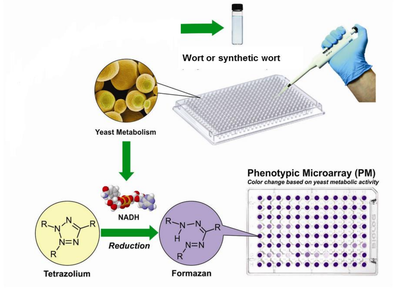


Figure 1: Phenotypic Microarray (PM) system
(adapted from Wilkinson et al., 2016*)

12 different yeast strains isolated from around the world were evaluated (Table 1) with regards to their metabolic output, and compared against a commercial lager brewing strain (*S. pastorianus* W34/74). The PM measures metabolic activity of yeast through the relative degree of colour change of a tetrazolium dye in response to the presence of NADH (Figure 1) using a specialised camera that is able to detect subtle colour changes and translate this into numerical data.

Table 1: Yeast strain selection screened

Species	Geographical origin	Key
S. pastorianus	Bavaria	Strain B
S. eubayanus	Patagonia	Strain 1
S. eubayanus	West China	Strain 2
S. eubayanus	Tibet	Strain 3
S. cerevisiae	West Europe	Strain 4
S. cerevisiae	West Africa	Strain 5
S. cerevisiae	North Africa	Strain 6
S. cerevisiae	South Africa	Strain 7
S. cerevisiae	North America	Strain 8
S. uvarum	Argentina	Strain 9
S. uvarum	Canada	Strain 10
S. uvarum	New Zealand	Strain 11
S. uvarum	New Zealand	Strain 12

The PM system was used to screen the metabolic performance of novel yeast strains for a variety of key performance indicators such as nutritional requirements (such as carbohydrates), as well as tolerance to stress factors (ethanol toxicity and osmotic stress) associated with industrial fermentations. This approach was used to conclude if novel strains exhibited improved and desirable phenotypes over a current brewing yeast strain.
It is anticipated that the use of PM analysis will be used in the future to determine the suitability of novel strains to ferment a variety of wort types and to optimize fermentation efficiency.

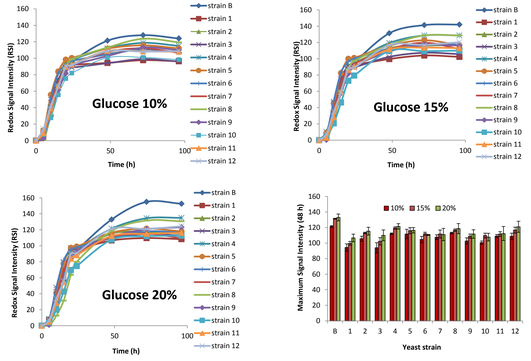
The performance metric of yeast metabolic output was the 'maximal signal height' after 48 h. Bespoke microarray plates were produced (Table 2) using YPD media (2% peptone and 1% yeast extract) as a base, with subsequent variations in carbon source. This included using glucose and maltose (at 10%, 15% and 20% w/v) to simulate HG and VHG brewing conditions. Glucose utilization was employed as a metric since this is key for HG brewing when using significant quantities of adjuncts (i.e. glucose based syrups). Conversely maltose utilization was evaluated in order to specifically evaluate the maltose metabolism of different yeast strains, crucial for when all-malt worts are utilised.

Table 2: Parameters used for phenotypic microarray-based screening

Parameter	Level
Glucose utilisation	10% w/v 15% w/v 20% w/v
Maltose utilisation	10% w/v 15% w/v 20% w/v
Ethanol tolerance	4% v/v 8% v/v 12% v/v
Osmotic tolerance (sorbitol)	10% w/v 30% w/v 50% w/v
Using lager wort (15°P)	50% wort

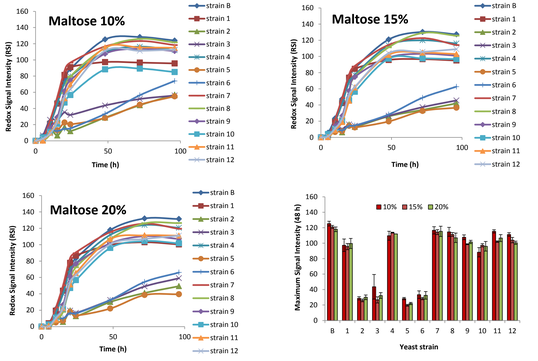
In addition, basic stress tolerance of the yeast strains was evaluated using base YPD media (prepared using 5% glucose [5% YPD]) spiked with external ethanol (at 4%, 8% and 12% ABV) and sorbitol (at 10%, 30% and 50%; to simulate the osmotic stress encountered by yeast during HG fermentations). In addition, the 12 yeast strains (and the reference lager yeast strain) were evaluated using 15°P all-malt lager wort.
All screening was conducted using yeast metabolic output measurements using the PM over the course of 96 h microscale fermentations at 20 ± 1°C.

Figure 2: Glucose utilisation



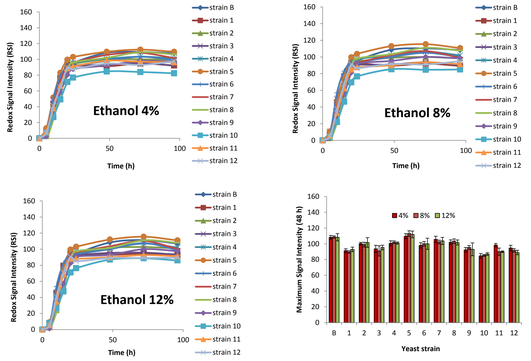
Glucose utilisation (as indicated by redox signal intensity; RSI) of each strain is shown over the course of a 96 h fermentation in Figure 2. In addition, each yeast strain is subsequently ranked in terms of maximum redox signal achieved after 48 h fermentation to provide an overall measure of comparison.

Figure 3: Maltose utilisation



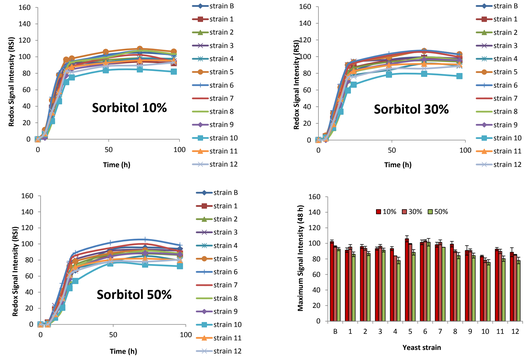
Maltose utilisation (as indicated by redox signal intensity) of each strain is shown over the course of a 96 h fermentation in Figure 3. In addition, each yeast strain is subsequently ranked in terms of maximum redox signal achieved after 48 h fermentation to provide an overall measure of comparison.

Figure 4: Ethanol tolerance



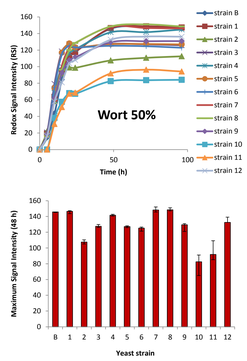
Ethanol tolerance (as indicated by redox signal intensity) of each strain is shown over the course of a 96 h fermentation (using 5% YPD) in Figure 4. In addition, each yeast strain is subsequently ranked in terms of maximum redox signal achieved after 48 h fermentation to provide an overall measure of comparison.

Figure 5: Osmotic tolerance



Osmotic tolerance (as indicated by redox signal intensity in the presence of sorbitol) of each strain is shown over the course of a 96 h fermentation (using 5% YPD) in Figure 5. In addition, each yeast strain is subsequently ranked in terms of maximum redox signal achieved after 48 h fermentation to provide an overall measure of comparison.

Figure 6: Fermentation performance with 15°P wort



Fermentation performance of each strain in 15°P wort (all-malt) diluted to 50% with Biolog IFY-0 buffer. In addition each yeast strain is subsequently ranked in terms of maximum redox signal achieved after 48 h fermentation to provide an overall measure of comparison.

Conclusions

Here we have demonstrated the effectiveness of the phenotypic microarray (PM) as a rapid, high-throughput tool for screening large collections of novel yeast strains for desirable brewing characteristics. Whilst PM technology is a powerful tool for the evaluation of certain phenotypic characteristics it is not inevitably only an indication of strain suitability. However, PM technology can enable the shortlisting of candidate yeast strains for further in-depth trials (larger scale fermentations) facilitating subsequent analysis looking at specific brewing phenotypes. These additional brewing traits may include; flocculation characteristics, variation in the production (profile and concentration) of flavour-active compounds, storage tolerance, thermo-tolerance (relative cell growth over a range of different temperatures), variation in the release and uptake of VDKs (i.e. diacetyl), pH tolerance, oxidative stress tolerance, and ability to tolerate nitrogen and micronutrient deficiencies. Further development of the PM technology is currently on-going in the pursuit of expanding the array of brewing specific phenotypes that can be evaluated.

*Wilkinson, S., Greetham, G., Tucker, G.A. (2016). Evaluation of different lignocellulosic biomass pretreatments by phenotypic microarray-based metabolic analysis of fermenting yeast. Biofuel Research Journal 9, 357-365.

ICBS International Centre for Brewing Science

SAB MILLER