Chapter 17

Current challenges and future opportunities for distillers yeast: discussion forum

Reporter: G M Walker

Forum panel members: Prof Mike Ingledew (University of Saskatchewan)
Prof Graham Stewart (GGStewart Associates)
Dr Mike Walsh (AB Mauri Global Technology Group)
Mr Jonathan Miles (Anchor Yeast/Lallemand)

Chair: Graeme Walker (University of Abertay Dundee, UK

Introduction

The Chair introduced the panel of experts who represented expertise in yeast science, fermentation technology, and the yeast supply sector of industry. This Distiller's Yeast Discussion Forum followed similar events held previously at the Scotch Whisky Research Institute (SWRI) in 2003 and the previous Worldwide Distilled Spirits Conference (WDSC) in 2008, to discuss key scientific and technological issues regarding yeast for the distilling industry. Table 1 summarises the main discussion points of these previous meetings

Some of the aspects outlined in Table 1 are still being considered by the distilled spirits industry at the present time. Therefore, current challenges and future opportunities for distillers yeast were firstly reviewed by the Chair who indicated there were different priorities for end-users (distillers), suppliers (yeast companies) and researchers (yeast academics). Key questions relevant to modern distilling, and which impact on the activities of the distiller, yeast supplier and researcher were considered to include the following:

Is it possible to obtain > 20% v/v ethanol by fermentation? Are there very stress-tolerant yeasts available for industrial fermentations operating at higher temperatures, elevated wort gravities etc? Can yeasts be provided that consistently yield flavoursome fermentations? Is there a simple, and rapid, vitality test available for distiller's yeast? How can genomics and proteomics be of practical value for the distiller, and will the sector ever embrace GM yeasts?

The concept of the *ideal* distillers yeast (Fig 1) was then raised in which multiple attributes

Table 1. Distiller's Yeast Discussion Forums 2003 & 2008

2003 Yeast Workshop at SWRI – Distiller's yeast attributes	*2008 Yeast Forum at WDSC – Key yeast questions raised*
1. consistent flavour congener production	7. what can GM yeasts do?
2. temperature tolerance	8. sources of innovative Scotch whisky yeasts?
3. faster fermentation	9. thermotolerant yeasts for tropical distilleries?
4. increased alcohol tolerance	10. is there a good vitality test for yeast?
5. increased substrate tolerance	11. matching yeasts strains with barley varieties?
6. increased substrate utilisation	12. liquid, cake or dried yeast formats?

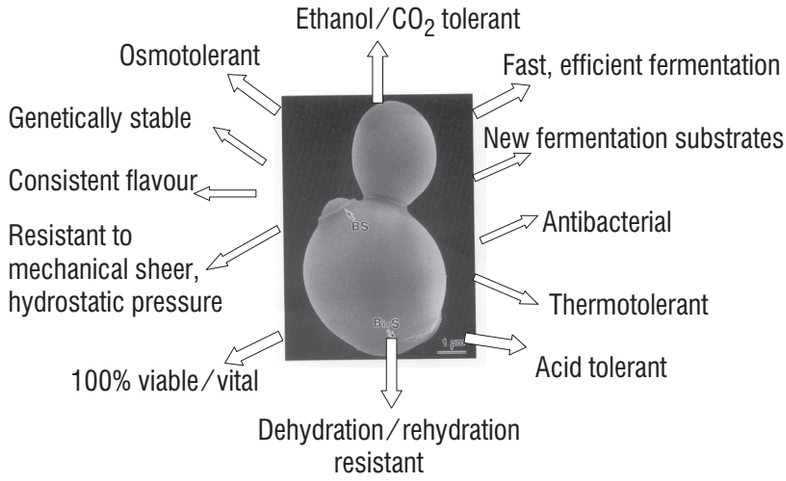

Figure 1. Conceptual aspects of the *ideal* distiller's yeast.

would be combined, but the Chair concluded such "super-yeasts" were not yet available for practical exploitation in all types of industrial fermentation processes.

The Chair then thanked conference delegates for their following questions to the panel.

Questions to the panel

Q1 What is the key desired attribute for a distilling yeast strain? (Tom Bringhurst – SWRI)

The panel commented that flavoursome fermentations as well as ethanol production were key attributes for distiller's yeast strains. Dr Jamieson (Heriot-Watt University) had already considered some of these attributes in his presentation to this conference (see Chapter 14). A multi-purpose distiller's yeast would be one that was equally effective in fermenting both malt-based sugars (primarily maltose) and sucrose (from sugar cane and beet). Maintenance of high viabilities and vitalities was also deemed to be a valuable attribute, especially in the fuel alcohol sector where some biofuel producers (especially in Brazil) conduct yeast recycling (a process uncommon in the distilled beverage sector). The attribute of high vitality (as a measure of yeast cells' physiological fitness or fermentation vigour) was deemed to be more important than viability (as a measure of yeast cells' reproductive capabilities). However, it should not be forgotten that yeast growth and fermentation are closely integrated activities. For some processes, it would be very desirable to achieve fast and predictable (i.e. minimal batch-to-batch variation) fermentations that reached over 20% v/v ethanol – these levels can be obtained by paying close attention to aspects of yeast nutrition. Professor Ingledew indicated that levels of 23.8% ABV have previously been achieved using very high gravity fermentation (VHG) technology. A small Scottish brewing company (Brewdog, Aberdeenshire) had recently claimed to reach 28% ABV by fermentation but unlike most breweries the yeast is not recycled.

Q2 Are heat-tolerant yeasts as efficient in alcohol production at higher temperatures (40°C) compared with lower temperatures (25-30°C)? (Dennis Watson, Pernod Ricard)

The panel indicated that *efficiency* in this context required definition. Fermentation rates will be higher at 40°C than 30°C, but although yeast will

ferment at the higher temperature, yeast growth would be compromised. In addition, at this high temperature there would be volatilisation and loss of product. Dr Walsh commented that in work with bioethanol yeast strains showed that higher ethanol levels can be obtained at temperatures $<27^{o}$C. However, yeast will experience thermal stress, but with simultaneous saccharification and fermentation (SSF) systems, osmotic stress problems would be minimised. The added expense on effective cooling systems also requires careful consideration. Professor Stewart indicated that for some industrial fermentations, combined stresses on yeast will prove lethal, but this may not be problematic if yeast were not to be recycled following fermentation. The practice of over-pitching may compensate for yeast viability loss during stressful fermentations.

Q3 – What is the best yeast format for whisky fermentations – cake, cream or dried? (Jason Bennett, Abertay University)

Mr Miles commented that the choice of yeast format depended quite a lot on the location of the distillery. For example, creamed is ideal if the yeast supply company is in relative close proximity to the end-user. For the use of dried yeast preparations, it is very important to conduct rehydration protocols carefully to maintain cells' physiological state and high viability. In terms of fermentation performance, dried, pressed or cream yeast all compare favourably.

Q4 Is there a "reference method" for sampling of yeast from grain fermentations? (John Carvell, Aber Instruments)

The panel commented that such sampling is very difficult and lacks reproducibility. The presence of suspended solids in fermentation broths is particularly problematic for yeast enumeration. Dr Walsh indicated that when taking samples from 8 points in a 3000 hL fermentation vessel, a distinct lack of homogeneity can be observed. Professor Stewart mentioned, in relation to brewing practice, that wort clarity distinctly influences final beer flavour due to lipid and carbon dioxide effects, with some companies showing striations in multi-brew fermentations.

Q5 Is there more interest in yeast strain diversity for distilling in USA compared with Europe? (Tim Dolan)

Mr Miles commented that there is much diversity when considering yeast strain usage in the US. For example, some Kentucky distilleries have their own specific yeasts, and North American craft distillers are prepared to use any yeast to get diverse and interesting products. Some distillers are currently experimenting to get different flavours but many of the world's distillers are reticent to change from their currently employed yeast strain(s). Professor Smart (from the audience) commented that Dr Ed Louis at the University of Nottingham had a very large yeast culture collection tracing stains back to continent of origin. In Brazilian fuel alcohol plants, there was evidence of yeast strain diversity in fermentation processes based on sugar cane molasses. Dr Watson (from the audience) commented that for Scotch whisky processes there were strict definitions to abide by and the use of complex substrates without addition of enzymes. There was, nevertheless, potential to consider yeast strain "blends" in distillery fermentations.

Q6 Can we use nitrogen-assimilation characteristics as a distilling yeast attribute? (Luc Lurton, BNIC)

Professor Ingledew commented that attention to nitrogen nutrition for distiller's yeast was very important. Urea supplementations are beneficial for fuel alcohol fermentations, but such practices are not tolerated for potable spirits due to production of potentially carcinogenic

ethyl carbamate. Of course, for Scotch whisky, no nutritional supplements are permitted in the fermenters. With regard to amino acid utlisation by yeast, Professor Smart (from the audience) commented that ale and lager brewing yeast strains differed in their amino acid uptake profiles. For example, it was now known that lager yeast strains have the ability to utilise the amino acid proline, previously considered to be poorly assimilated by brewing strains. There is a need for further research into nitrogen nutrition in yeast strains specifically for distilled spirits. Other nutritional factors for distiller's yeast were also considered important, notably phosphorous, sulphur, magnesium and zinc. The latter was emphasised by Professor Stewart who indicated that zinc plays an important role in maintenance of yeast health, and some brewing companies add this nutrient to maintain the health of its 'old' yeast. Dr Walsh commented that a procedure called "accelerated evolution" may be employed to alter the physiological characteristics of yeast, including nutrient uptake capabilities (as has been reported for brewer's yeast).

Q7 Are "floc" yeasts more stress-tolerant than "non-floc" yeasts? (Dennis Watson, Pernod Ricard)

Professor Stewart commented that this question was somewhat provocative, but it was unclear if this was the case. Perhaps (but not proven) yeasts flocculate as a response to environmental stress? Dr Walsh indicated that nutrient limitation plays a role and there is a difference in cells nutrient status from the outside to the centre of the yeast floc. Epigenetic factors may also be involved. For example, the presence of repetitive sequences may relate to loss of flocculation characteristics with too many generations. There are 11 FLO genes identified, but why so many when only three seem to be active?

Q8 Are yeast suppliers reaching the end of the line in terms of yeast strain development, and will we now seriously need to consider the use of GM strains? (Tom Bringhurst, SWRI)

Mr Miles commented that there were numerous GM yeast strains sitting in laboratory culture collections that are not being used in industry due to current adverse consumer perceptions. Nevertheless, research by yeast suppliers continues to seek new yeast varieties of potential value to the distilled spirits and other sectors. Dr Walsh indicated that GM technology had the capability to solve many problems, but inventors require protection for their invention by patent. The appeal is that you can "paste" whatever gene you want, but there are different rules and conventions in different countries. Dr Walsh noted a new phrase – gene repair – relating to the addition of a particular gene to a yeast that has not got one. This is different from cloning. However, do not be fooled as it is GMO and such yeasts are widely used in the enzyme industry. Professor Ingledew mentioned that GM technology was widespread for research currently, but practical advances will soon accelerate in the bioethanol industry, especially for lignocellulose-derived fuel alcohol and biobutanol. In the US, GM corn is used in the biofuel industry which shows distinct benefits. Professor Stewart concluded the discussion on GM yeasts for distillers by commenting: never say never!