Chapter 16

Selecting new distilling yeasts for improved fermentation and for sustainability

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Introduction

Yeast is essential for the production of all distilled spirits and as such has provided much interest at previous Worldwide Distilled Spirits Conferences (Walker 2010a). The strain of yeast employed in potable spirit fermentations is crucially important not only for yielding high levels of ethanol, but also for production of minor yeast metabolites which collectively contribute to the development of spirit flavour (Watson 1981). Distillers must therefore pay careful attention to the type of yeast used to ensure consistency of fermentation performance in terms of both of these parameters (Watson 1984). Walker (1998) has provided a fundamental and detailed review of the physiology and biochemistry of yeast and Campbell (2003) concisely summarised yeast and fermentation aspects with regard to Scotch whisky production.

Watson (1984) has highlighted a number of yeast characteristics that are important in determining the efficiency of fermentation. These include carbohydrate utilisation, rate of fermentation, alcohol tolerance and culture stability. Scotch whisky distillers are strongly interested in extending this list of desirable yeast attributes.

Over the last decade or so, there has been a high level of interest in new distilling yeasts, and there has been much research into new strains and different types of yeast by distillers, yeast scientists in academia and yeast manufacturers. This expansion of research has become more important now that distillers are increasingly seeking initiatives that fulfil the sustainability requirements of the industry.

With the emergence of a rising awareness of environmental issues, such as global warming and climate change, distillers now have to take a longer term perspective on issues that will impact on the sustainability of their respective industries. These present challenges for distillers to meet through initiatives to reduce demands for energy, use less water and improve the utilisation of co-products. This means that Scotch whisky distillers will have to think 'out of the box' (Ingledew 2009) when considering new yeasts. As a result distillers' expectations for their yeast supplies are changing, with a greater requirement for efficient fermentation performance under more highly stressful conditions. Hence, it is essential to stimulate research into developing new yeast strains that will be capable of fulfilling these objectives.

Yeast producers already have research programmes that have the potential to deliver new types of yeast, capable of efficiently fermenting a much wider range of substrates. While many of these developments are directed principally at the burgeoning biofuels (fuel alcohol, or bioethanol) sector, there are clearly lessons to be learned from these applications and from the technology that has been developed along with it, that would also be of benefit for potable spirits distillers. It is therefore essential that lines of communication between yeast producers and distillers are kept open so that there continues to be an on-going dialogue to ensure that the distilling industry is able to achieve not only its production objectives, but also to address sustainability issues.

This paper highlights the priorities of the Scotch whisky industry, while also comparing and contrasting their yeast requirements with those of other potable spirit manufacturers and biofuel producers.

There are a very large number of potential substrates and process for the production of spirit beverages and Watson (1984) summarised the main substrates used for a range of different spirits. Buglass et al (2011) provided a more recent, detailed review of a wide range of products. For the purposes of this paper we have just highlighted a small number of the important ones.

The present contribution aims to provide a stimulus for discussion, which will encourage yeast producers and researchers to look more closely at the desirable attributes of yeast directed at the market for distilled beverages, to ensure that specific requirements over the whole range of products are considered when developing new distilling yeast strains.

Scotch whisky distilling yeast

Although there is now, in theory, a large selection of potential yeast strains available to distillers, in reality the bulk of the standard distilling strains of *Saccharomyces cerevisiae* used by the Scotch whisky industry are supplied from two major sources: Kerry Ingredients and Flavours (Glenochil, Menstrie) who produce 'M' and 'MX' strains, and Mauri Products (Hull) who produce Pinnacle yeast. Some distillers, particularly those producing grain whisky and neutral spirit also use yeast supplied by Lallemand Ethanol Technology (LET) from the former British Fermentation Products (BFP) plant (Felixstowe), which is now part of the Lallemand Group who also own Anchor Yeast, a South Africa based company specialising in dried yeast. LET has a range of yeasts (under the DistillaMax® brand) for different types of distilled spirit production and is now a supplier of cream yeast to grain distilleries in Scotland. LET also supplies both malt and a grain distillers yeast under the Anchor brand, both in dried form, which and are being used by some distillers to deliver natural flavour complexity, as well as high alcohol yield. All of these yeast manufacturers are developing new distilling yeasts, so there will always be potential for novel strains that will suit distillers.

The yeast strains used are based on a small number of stable commercial *S.cerevisiae* strains, primarily deriving from a baking/brewing (ale) yeast heritage. Scotch whisky distillers currently use yeast in three main formats; cream yeast, pressed (or cake) and dried yeast. Distilling yeast differs from brewing yeast in that it is expected to ferment the wort more completely, fully utilising the fermentable sugars deriving from starch (mainly maltose, glucose and maltotriose). The production of Scotch whisky differs from brewing in that the wort is not boiled, so sufficient enzyme activity will survive into fermentation, to provide secondary conversion of oligosaccharides to fermentable sugars.

The ability to efficiently ferment maltotriose in cereal based worts is considered to be an important characteristic of yeasts suitable for Scotch whisky production (Korhola et al 1989; de Amorim Neto et al 2009), and the presence of unfermented maltotriose in the co-products can cause serious problems (Stewart 2010). This contrasts with the yeasts for sugar and fruit based fermentations which are developed to ferment sucrose, fructose and glucose. Jones (1999) has described the former yeasts as "maltose + oligosaccharide type" and the latter yeasts as "sucrose + glucose" type.

Producers of similar products to Scotch whisky, such as bourbon, often propagate their own proprietary yeast strains, something that Scotch whisky distillers do not commonly do. Although there is no technical reason for them not to propagate their own yeast, the relatively small size of a typical Scotch malt whisky distillery makes this uneconomic. In addition, most Scotch malt whisky distilleries are in close proximity to yeast suppliers, so obtaining fresh yeast direct from them makes more sense in terms of both quality assurance and cost effectiveness.

Scotch malt whisky production

Scotch malt distilleries, which traditionally used pressed yeast, are increasingly moving over to cream yeast, which is, at least in theory, easier to handle and store. While there is a capital cost involved in the changeover, since it requires the installation of custom made handling facilities, this is more than offset by improved yeast storage conditions, and more precise control over subsequent inoculation (pitching) rates and temperature.

Malt whisky distilleries do not normally propagate yeast but add it to the wort, by injecting it into the wort line, putting it directly into the washback (fermenter), or adding it via a slurry tank. Pitching rates can vary but are normally in region of 0.4 percent (w/v). There is normally no temperature control. Control of fermentation is achieved by adjusting the wort to a suitable pitching temperature (typically 16 - 19°C). This is sufficient to achieve a maximum temperature (32 - 33°C) which is necessary to ensure efficient fermentation. Fermentation normally takes 2 - 3 days, depending on distillery capacity. A small number of malt distilleries, have a pre-multiplication conditioning step to help maximise the vigour of the yeast to encourage it to begin fermenting more rapidly, particularly if a high original gravity (>1070) is used. Scotch whisky distilleries do not recycle their yeast, in the way that brewers do. Instead the full contents of the fermenter are transferred to the wash still for distillation. This practice is considered to be fundamentally important from a flavour point of view.

The use of dried yeast is *essential* for a small number of remote malt distilleries, such as those in the Highlands and Islands of Scotland, where delivery of regular supplies of fresh yeast would be difficult. Although pressed and cream yeasts are most prevalent, dried yeast is also useful as a backup in case of disruption of other supplies. Use of certain dried yeasts can give distinctive sensory characteristics, hence providing an opportunity to diversify spirit flavour.

Until fairly recently, distillers used a proportion of brewers' yeast (typically 30 - 50 percent of the pitching rate) obtained from the recycling of aged yeast from brewery fermentations. This was acid washed, repackaged and supplied to distilleries, and was considered to provide a useful supplement to normal distillers' yeast, in terms of supporting fermentation performance and providing additional flavour complexity (Watson, 1981). More recent work reported at the 2005 Worldwide Distilled Spirits Conference (Yomo et al 2008; Noguchi et al 2008) confirmed the synergistic effects of using a mixture of distilling and brewers' yeast and highlights the flavour benefits of using brewer's yeast in providing 'richer' 'full bodied' and 'more complex' characters. However, the use of brewer's yeast was largely phased out by Scotch whisky distillers (Munro 2008) during the last decade, when it was realised that the quality, consistency and keeping properties of commercially available material from breweries, had declined to such an extent that it no longer provided significant benefits. Nonetheless, a small number of distilleries do still use it for 'traditional' reasons.

Scotch grain whisky production

In some ways, the yeast and fermentation requirements of Scotch whisky grain distilleries are similar to those of malt whisky distillers. The main difference is one of scale, since the aim of these production units is to produce the maximum quantity of alcohol from the raw materials, within the confines of the legal definition of Scotch whisky production. One of the ways of doing this is to ferment cereal derived worts in larger batches, but with less yeast. In grain distilleries pitching rates (eg. 0.1% v/v using cream yeast) are generally about 25 percent of that used in a malt distillery. Although the flavour attributes of grain spirits are important, there is greater separation of flavour congeners from alcohol in a grain still than in a malt whisky still, making flavour control in many ways easier to achieve. Consequently this puts fewer constraints on the available options for increasing production efficiency, provided that they are consistent with the legal definition of Scotch whisky. This means that grain distillers can operate closer to the process limits, particularly in terms of wort specific gravity, where wort concentrations approaching 1080°IBD (19 - 20°Plato) can be used.

Currently, nearly all Scotch whisky grain distilleries are using cream yeast, although they could use other formats (pressed or dried) if necessary. Because of the scale of production, there is often some form of yeast propagation, multiplication or conditioning. Grain distillery fermentations may operate under more stressful conditions, such as high original gravity (> 1080°IBD), higher alcohol strength (>10 percent v/v) and at elevated temperatures, and it is essential for yeasts to have the ability to perform efficiently under these conditions. Some, but not all grain distilleries, have at least some capacity for temperature control of washbacks. Due to the (semi) continuous nature of grain whisky production, it is desirable that fermentations are as short as practically possible. Hence, as well as using the standard malt whisky yeasts, grain distillers may use additional strains, that are better adapted to these conditions.

Neutral spirit (vodka and gin)

Scotch whisky grain distilleries may also produce neutral (or plain) spirit for vodka or gin production, using a fermentation process that is similar, if not identical, to that for grain whisky. The main difference is that the production of neutral spirit is not constrained by the Scotch whisky definition, so a wider range of processing options is available. The most important of these is that distillers producing neutral grain spirits are allowed to add food grade enzymes to degrade starch to ease the processing of cereals and produce as much ethanol as possible. Hence the composition of the initial substrate can be slightly different.

The process used in the production of neutral spirit will give even greater separation of flavour congeners from alcohol than in grain whisky production, the aim being to reduce the level of flavour compounds to below the limits of sensory detection. Hence, there is no requirement for the yeast to provide any specific flavour congeners, although distillers have to ensure that the selected strains do not produce any unusual or undesirable flavours that could potentially pass through the process.

Other sugar/fruit based spirits (rum, tequila, and cognac/brandy)

Sugar and fruit based spirits use a wide range of yeasts for their fermentation processes. Some more specialised products (eg. tequila/mescal, cachaça etc) may use spontaneously fermenting indigenous microflora, or local yeast cultures isolated from previous fermentations. Producers of the major spirit types (Cognac, brandy, rum) prefer to use specific, pure or cultivated yeast strains. In all cases, as with Scotch malt whisky, flavour is an important consideration and the selection of suitable strains is vital in determining the flavour profile of these spirits.

Other fruit and pomace spirits are prepared by fermenting the fruit pulp and/or juice, and are distilled from the fermented fruit wine (or cider, perry). These are mostly fermented by *S. cerevisiae* cultures, but some products use the natural microflora (ie. wild, or non-*Saccharomyces* yeasts), associated with the fruit skins, or present in the must. In some cases the naturally occurring microflora has to be supplemented with cultured yeast to ensure complete fermentation.

Fuel alcohol (bioethanol)

Walker (2010b; 2011) has reviewed bioethanol fermentation processes and compared fuel and potable ethanol production.

Global bioethanol production in 2008 was 65.7 billion litres and this will soon exceed 100 billion litres, with the largest increases in the US (using maize) and Brazil (using sugarcane). In Europe, bioethanol production is increasing sharply and the main producers are France, Germany and Spain using primarily feedstocks of cereals (mainly wheat) and sugarbeet. *First-generation* bioethanol feedstocks refer to plant biomass sources that are also sources of human and animal nutrition (namely: cereal starches and sugar crops), whilst *second generation* raw materials for bioethanol production typically refer to non-food biomass sources, mainly lignocellulosic biomass (eg. biowastes, woody residues, energy crops etc). The predominant microorganism responsible for first generation bioethanol fermentations is the yeast species, *Saccharomyces cerevisiae*, but other yeasts and certain bacteria have future potential for cellulosic second generation fermentations (see Walker, 2010b). Microbes for lignocellulosic hydrolysate fermentations are subject to intense research activity (Mousdale, 2008), mostly involving genetically engineered yeasts and bacteria. When cellulose and hemicellulose polymers are hydrolysed, the resultant monomeric sugars represent a mixture of C5 (pentose) and C6 (hexose) sugars. Conventional distilling strains of yeast are unable to metabolise pentose sugars such as xylose and arabinose. However, GM strains of yeast and bacteria are being developed to ferment both C5 and C6 sugars (see Walker, 2011). To achieve efficient fermentation of lignocellulose hydrolysates, it is necessary to further develop stress-resistance in industrial yeast strains, particularly with regard to the ability to withstand chemical inhibitors such as acids, phenols and furans.

The undesirability of production of secondary fermentation metabolites (notably glycerol) in bioethanol plants leads to potential loss of ethanol and efforts are made to dissipate this, including simultaneous saccharification and fermentation (SSF) processes to prevent osmotic pressure stress on yeast cells. Construction of yeast strains with reduced glycerol production is also possible (eg. Guo et al, 2009).

For first generation bioethanol processes (using starch and sugar based feedstocks), commercially available strains of *S. cerevisiae* have now been developed that can produce >10%v/v ethanol and can ferment effectively in high (>20%) solids. For example, through careful attention to yeast nutritional physiology, it is now possible to produce over 20%v/v ethanol in high gravity wheat fermentations (Thomas and Ingledew, 1992).

Some bioethanol distilleries operate yeast recycling and this circumvents the need to regularly purchase new batches of yeast from commercial suppliers. Other plants conduct their own aerobic yeast propagation in order to boost biomass required as starter cultures for fermentation. To maximise bioethanol production, it is important to ensure yeast is highly viabile and vital and also to minimise levels of contaminant bacteria – especially *Lactobacillus* spp. that can subtract significantly from bioethanol yield.

Aspirational targets for distilling yeasts

In the past Scotch whisky distillers have consulted with yeast experts, both within academia and individual yeast companies, to develop a 'wish list' for new distilling yeast strains (Walker et al 2008). In 2003 the Scotch Whisky

Research Institute hosted a Yeast Workshop, which included a wide range of stakeholders representing each of the above groups. The aim of this workshop was to identify and prioritise the main attributes of yeast that would be of most benefit to Scotch whisky distillers, so that they could be targeted by manufacturers and researchers when looking at new developments in distilling yeast.

The 2003 Yeast Workshop identified 6 key attributes (Table 1) for new distilling yeast strains that could be prioritised to deliver strong potential benefits for distillers, namely (1) consistency of flavour, (2) temperature tolerance, (3) increased fermentation rate, (4) increased alcohol tolerance, (5) increased substrate tolerance and (6) increased substrate utilisation. While the main focus of this workshop was on yeasts for Scotch whisky production, it was clear that many of these attributes are also highly relevant both for other potable distilled spirits, as well as potentially for bioethanol production.

Table 1 Aspirational targets for new distilling yeasts (from the 2003 Yeast Workshop at SWRI)

Consistency of flavour:- Consistently having the sensory characters associated with Scotch whisky
Temperature tolerance:- Have the ability to withstand and operate efficiently at temperatures up to 37 -38°C (aspiring to 40°C)
Increased fermentation rate:- The ability to reduce fermentation times substantially, perhaps to less than 30h
Increased alcohol tolerance:- Display increased alcohol tolerance and be able to produce up to 15 percent (v/v) alcohol (or higher) (12 percent (v/v) for malt distilleries)
Increased substrate tolerance:- Capable of operating efficiently at higher original gravities up to 1080 or even 1100° IOB
Increased substrate utilisation:- Reduce biomass and waste (co-) product production and form more alcohol

Since this list was compiled, time and technology have moved on, primarily as a result of the research initiatives that have been stimulated, individually and collectively by distillers, yeast suppliers, and importantly in the academic sector. Partly this has been driven by the emergence of the bioethanol sector. While the 2003 targets are still considered to be highly relevant, recent years have seen the increasing importance of other sustainability issues, as described earlier. This has resulted in a growing awareness that distillers should revisit the criteria for selecting new distilling yeasts, so they can take into account the increasing priority of these aspects.

Options open to Scotch whisky distillers to address sustainability issues, include operating their distilleries to give increased wort concentration (specific gravity), higher alcohol strength, as well as aiming to ferment at higher temperatures to reduce the need for cooling. All of these place additional stresses on the yeast, compromising their fermentation performance. Addressing these issues requires a focus on specific yeast attributes, such as increased stress tolerance (thermotolerance, osmotolerance, alcohol tolerance). However, any developments need to take into account the impact on spirit flavour, which remains of primary importance.

In revisiting the targets for new yeasts, the need to widen our horizons to address not only the requirements of Scotch whisky distillers, but also for those of other distillers, became apparent. The continuing emergence of the fuel alcohol sector will drive the development of new yeasts for this market, so it is essential that distillers of potable spirits continue to communicate with those involved to ensure that their own specific requirements are not overlooked.

Priorities for distilling yeasts

Although the previous sections of this paper have shown that there are many similarities in yeast requirements between different distilling sectors, there are also some important differences.

Discussions between the University of Abertay Dundee and the Scotch Whisky Research Institute have resulted in the development of a convenient check-list (Table 2) which summarises these requirements. This highlights what we consider to be the key desirable properties of yeast strains employed for various types of alcohol fermentations: whisky and neutral spirit fermentations based on starchy/cereal substrates; rum and cognac fermentations based on sugar and fruit; and provides a comparison with fuel alcohol fermentations based on starch, sugar and cellulose substrates.

The aim of the check-list is to stimulate discussion between distillers (in all sectors), yeast manufacturers and academic yeast experts on the options that are available. This should help to ensure that future research resources effectively address the needs of all end users, to provide the distilling industry with a variety of new yeast strains that are tailored to specific individual needs.

Defining yeast fermentation performance

As with the development of our understanding of yeast and fermentation, recent years have seen an expansion in the analytical techniques that are available to researchers and distillers to assess

Yeast attributes	Malt whisky fermentations	Grain whisky fermentations	Neutral spirit fermentations	Sugar-based fermentations	Fuel Alcohol fermentations		
			Grain/Molasses	Sugar crops /Fruits	Starch	Sugar	Cellulose
Rapid fermentation (eg. 1-2days)	**	****	****	****	*****	*****	*****
Continuous fermentation	*	**	***	**	***	*****	*****
Alcohol yield	**	****	****	****	*****	*****	*****
Acceptable spirit character	*****	****	**	***	*	*	*
Maltotriose utilisation	****	****	**	*	*****	*	*
Less yeast biomass	**	***	***	**	**	*****	**
Stress tolerance							
High gravity	**	****	****	**	*****	*****	*****
Temperature	***	****	****	****	*****	*****	*****
Ethanol	**	****	****	****	*****	*****	***
Competitive (v. bacteria)	**	***	****	***	*****	*****	***
Consistency of fermentation							
Yield	***	*****	*****	*****	*****	*****	*****
Flavour	*****	***	***	****	*	*	*
Yeast vitality	*****	*****	*****	*****	*****	*****	*****
Available Format							
Cream/liquid yeast	***	*****	*****	***	*****	**	***
Cake/pressed yeast	***	*	**	***	***	**	**
Dry yeast	****	*	**	**	***	**	**
Freshly propagated	*	****	****	**	***	**	*****
Recycled	*	*	***	*	**	*****	**

*****	= very high priority (or essential)
****	= high priority
***	= moderate priority
**	= low priority
*	= very low priority (or not applicable)

the performance of new yeast strains (Table 3). While some of these are well established, modern techniques, such as flow cytometry are coming forward, which are able to provide an unprecedented level of detail regarding yeast viability, vitality and performance during fermentation. Additionally there have been advances in our understanding of the genetic aspects of yeast, and this will ultimately allow yeast researchers to select for more specific quality and performance traits (Ingledew et al 2009). This will help to provide distillers with a palette of yeasts that can be selected to meet their specific requirements. In addition such advances will not only help to ensure consistent spirit quality for existing products, but should also result in a wider choice of strains that will give specific desired flavour attributes.

Conclusions

Research into new distilling yeast strains has progressed significantly over the last decade. Distillers now have a much clearer idea of the desirable attributes that they would like to see in new yeasts, while the direction of research and improved analytical techniques is in the fulfilment of these wishes. However, as seen previously with the emergence of sustainability issues, distillers' requirements can change. It is vital to communicate these changing needs to the other stakeholders in the supply chain, so that they can develop strains with the required characteristics.

Effective communication on distillers yeast matters has proved to be useful in the past. Some areas of interest originally identified by the 2003 Yeast Workshop are now being actively studied by multidisciplinary groups (e.g. the BBSRC Sustainable Bioenergy Centre (BSBEC), based at the University of Nottingham). Although some research is focussed primarily on bio-ethanol, this shares some of the key targets for improved distilling yeasts, such as maximising ethanol production and improved process tolerance (osmo-tolerance, ethanol tolerance, thermo-tolerance and pH tolerance). In addition, investigations of the genetic aspects controlling yeast attributes will ultimately result in a more fundamental understanding of yeast physiology during industrial fermentations (Ingledew et al 2009), and will help to identify potential new strains that are more suited to these environments.

Finally, and with specific regard to Scotch whisky production, this continues to be a 'traditional' industry with a strong cultural and regional identity, and is enshrined in its legal definition. Consequently, the options available to Scotch whisky distillers to address sustainability issues and deliver production improvements are constrained by the limits set out in the regulations, in ways that other distillers are not. Improving raw materials, such as developing new yeast strains, offers one of the few realistic options

Table 3. Distilling yeast fermentation performance: quantitative criteria

Criteria (fermentation performance)	*Analytical method (examples)*
Spirit yield	Calculations based on conversion of cereal starch to ethanol
Carbon dioxide evolution rate	Gas flow meter
High gravity fermentation	Original Gravity, yeast viability, pH decline
Sugar utilisation spectrum (eg. maltotriose)	HPLC
Spirit character (off-notes, fusel oils)	Sensory panel evaluation, GC
Yeast viability/vitality	Flow cytometry
Response (s) to stress	" "
Yeast cell cycle characteristics	" "
Distillery/Product/Application specific attributes*	Genetic Markers

(* Possibility of 'designing' yeast with attributes for specific applications/product profiles/processes) for delivering the necessary improvements in production performance, while at the same time addressing the industry's sustainability targets. It is hoped that the information will provide useful qualitative guidance for distillers and yeast suppliers in selecting new yeast strains that will enable them to fulfil their needs.

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