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REVIEW ARTICLE

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Applications of diastatic *Saccharomyces cerevisiae* in brewing, distilling and biofuel production

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Abstract

Why was the work done: Diastatic variants of *Saccharomyces cerevisiae* are unusual in producing an extracellular glucoamylase which enables the breakdown of starch to fermentable sugars. Diastatic *S. cerevisiae* has long been viewed negatively as a contaminant of especially beer packaged in cans or bottles. However, this view is being reconsidered due to the opportunities that diastatic strains present for niche fermented products and distillation applications.
What are the main findings: This review highlights the utilisation of diastatic *S. cerevisiae* for its flavour potential, and processing applications in the brewing, distilling, and biofuel industries. Further, genetic differences are compared with non-diastatic strains of *S. cerevisiae*, together with commonly employed and emerging methods of detection.
Why is the work important: Diastatic yeast strains can be used to create flavour profiles that resemble traditional beverages and can be used to achieve fermentation with higher attenuation. This offers greater fermentation efficiency in, for example, the development of low-calorie beers. Additionally, the ability of diastatic strains of *S. cerevisiae* to convert non-fermentable oligosaccharides to fermentable sugars enables applications that range from novel beverages using unusual raw materials to more efficient distillation and biofuel production. The negative attributes that are associated with diastatic *S. cerevisiae* yeasts can be managed through co-inoculation or hybridisation with standard strains.

Keywords:

brewing, diastatic yeast, beer, genome, distilling, biofuel

Introduction

A diastatic variant of *Saccharomyces cerevisiae* was first characterised by Andrews and Gilliland (1952), who reported the ability of the yeast to secrete glucoamylase that could break down dextrin oligosaccharides from starch resulting in the 'super attenuation' of fermentation. This property lead to the conclusion that this diastatic yeast – *S. diastaticus* - was a strain that was independent from *S. cerevisiae* (Riu-Aumatell et al. 2011). Later, Tamaki (1978) reclassified the yeast as *Saccharomyces cerevisiae* var. *diastaticus* because of its ability to inbreed with *Saccharomyces cerevisiae*. This relationship was confirmed through whole genome sequencing (Liti et al. 2009; Pontes et al. 2020; Paraíso et al. 2023). However, the use of '*Saccharomyces cerevisiae* var. *diastaticus*' continues despite the genomic work to rectify this taxonomic issue (Hittinger 2013; Peter et al. 2018). Accordingly, diastatic strains of *S. cerevisiae* are considered subpopulations of *S. cerevisiae*, reflecting the functionally distinct STA genes, which allow the secretion of extracellular glucoamylase (EC 3.2.1.3) (Krogerus et al. 2019; Krogerus and Gibson 2020). Although the nomenclature suggests a single strain, it is a group of yeast strains, all of which have at least one STA gene (Krogerus et al. 2019; Krogerus and Gibson 2020). Here, those strains of *S. cerevisiae* able to secrete glucoamylase are referred to as diastatic strains of *S. cerevisiae.*

Diastatic variants of *S. cerevisiae* are considered a super attenuating contaminate of beer by metabolising the residual carbohydrates (dextrins and soluble starches) remaining from primary fermentation (Meier-Dörnberg et al. 2017). Dextrins contribute 10-20% of the total sugar content in brewing wort (Štulíková et al. 2021) and are considered to contribute to body and mouthfeel of beer, while also contributing to the calorie content. Fermentation of these residual carbohydrates by diastatic yeast can be problematic in packaged beers resulting in additional carbon dioxide production, causing the beer to gush or, in bottles or cans, burst (Boulton and Quain 2001). Diastatic contamination of beer occurs most commonly during the packaging of beer in bottles (Meier-Dörnberg et al. 2018), often due to poor cleaning practices (Meier-Dörnberg et al. 2017) resulting in biofilm production (Storgårds et al. 2006).

Contaminant microorganisms in biofilms are more resistant to stress, and are not easily removed; with recolonisation within 2-12 hours (Storgårds et al. 2006). Diastatic strains of *S. cerevisiae* can be aerosolised during filling and/or cleaning resulting in scatter contamination, often only affecting a few bottles or cans which is almost impossible to detect (Meier-Dörnberg et al. 2017). In some countries, breweries and food manufacturers are required by law to report if a product is contaminated with diastatic strains of *S. cerevisiae*, typically resulting in a public recall (Rees 2014; Post 2016). Reported contamination incidents caused by diastatic yeast have increased over the years, along with associated costs (Begrow 2017; Meier-Dörnberg et al. 2018; Suiker et al. 2021). Indeed, a product recall in the United States linked to diastatic yeast by Left Hand Brewing (Colarado), result in over 20,000 cases of beer (worth $2 million) distributed to over thirty-seven states being recalled and destroyed. This was linked to contamination of the primary brewing yeast, resulting in the brewery filing an, as yet unresolved, civil suit against the yeast supplier (Post, 2016).

Genetics

As described above, research and advancements in genomics have found that the amylolytic yeast contaminants were variants of *S. cerevisiae* (Liti et al. 2009). More specifically, the amylolytic *STA1* gene (formally *DEX2*) is uniquely associated with diastatic yeast, enabling the production and secretion of glucoamylase (Tamaki 1978; Yamashita et al. 1986). The enzyme removes glucose from non-reducing ends of starch and dextrins by hydrolysis of the α-1-4-glucosidic bond (Sauer et al. 2000). The *STA1* gene is most strongly associated with diastatic activity and the ability to produce/utilise glucoamylase. Genomic tools have been used to analyse the unlinked homologous *STA1–STA3* genes (Pretorius and Marmu 1988). Although the DNA sequences of *STA1-STA3* genes are almost identical, the genes have different names as they are located on different chromosomes and linkage groups (Tamaki 1978; Krogerus and Gibson 2020). A study by Burns et al (2021) analysed 15 *STA1+* yeast strains to assess different functional tests and the potential for refermentation. This work showed yeast with medium to high diastatic activity was associated with super attenuation. However, one strain

(OYL-501) with the *STA* gene did not result in refermentation perhaps reflecting a deletion in the sequence of the *STA1* promoter (Krogerus et al. 2019). Diastatic strains of *S. cerevisiae* are not common, with 54 of the genomes of 1169 sequenced *S. cerevisiae* strains (Gallone et al. 2016; Peter et al. 2018), having a 100% match with the sequence of the *STA1* gene.

The phenolic off flavour (POF) is a characteristic medicinal aroma linked to 4-vinyl guaiacol from the decarboxylation of ferulic acid by 'wild' strains of *Saccharomyces* (Stewart et al. 1983). Although now termed *PAD* (phenylacrylic acid decarboxylase), POF is used throughout this review. Although diastatic strains of *S. cerevisiae* are typically POF+ (Stewart et al. 1983; Meier-Dörnberg et al. 2018) resulting in super attenuation and phenolic off flavour, there are reports of *STA+* strains which are POF- (Gallone et al. 2016; Krogerus et al. 2019).

Yeast Family Tree

The isolation and use of pure yeast cultures in brewing dates back to work of Hansen in 1883 (Boulton and Quain 2001). Before this, brewers would inoculate wort with a volume of fermenting beer, a process known as 'backslopping'. This would result in a faster and more predictable fermentation and such controlled environments were ideal settings for domestication of indigenous microorganisms (Gallone et al. 2016; Garshol 2020). Accordingly, desirable traits were selected, whilst allowing the yeast to outcompete any microbial competition. Most domesticated ale and lager yeasts do not have functional *STA* genes but have been selected for their ability to use maltotriose via transmembrane transporters such as *AGT1*. Diastatic strains have mutations in the Agt1p permease and rely the extracellular glucoamylase to utilise maltotriose (Mukai et al. 2014; Pauley and Maskell 2017; Krogerus and Gibson 2020).

Gallone and colleagues (2016) sequenced the genomes of 157 *Saccharomyces* strains used to produce bread, beer (ale and lager), sake, spirits, and wine, as well as wild yeast and other strains of *S. cerevisiae*. Most of the brewing yeasts were in two families – 'Beer 1' and Beer 2' – together with three other clades representing wine, Asian (sake) and mixed (bread) strains. The 54 Beer 1 yeasts were from Belgium/Germany, the UK and the USA whereas the Beer 2 lineage was geographically diverse. Of the Beer 1 yeasts, nine were POF+ whereas of the 21 yeasts in Beer 2, 16 were POF+ (Gallone et al. 2016). In another study focussing on *STA1+* strains of *S. cerevisiae* (Krogerus et al. 2019), a search of 1169 publicly sequenced strains showed 54 contained the sequence for STA1. Of these, 51 were in the 'Beer 2' clade.

As diastatic strains of *S. cerevisiae* are defined by properties (linked to containing specific genes), diastatic strains are not a single species or subspecies of *Saccharomyces* yeasts but rather a variant (Liti et al. 2009; Meier-Dörnberg et al. 2018; Peter et al. 2018; Jespersen et al. 2000; Suiker et al. 2021; Suiker and Wösten 2022).

Detection Methods

Diastatic strains share genetic and physiological traits with other *Saccharomyces* resulting in practical difficulties in their differentiation and identification from production yeasts (Krogerus and Gibson 2020). For example, Figure 1 shows two ale strains viewed under light microscopy where one is a diastatic variant. There is far more variability within *S. cerevisiae* than is observable between these strains. Therefore, methods to detect diastatic variants must rely on traits other than physical appearance.

Methods for the detection of diastatic strains range from 'traditional' plating methods through to contemporary, more sophisticated approaches (Table 1). Plate tests include media containing copper or more specifically dextrin or starch. The use of copper is non-selective enabling the growth of diastatic yeasts together with 'wild' yeasts (Krogerus and Gibson 2020). Molecular methods tend be specific and quicker, and include the polymerase chain reaction (PCR) in the detection of *STA* (or DEX) genes (Yamashita et al. 1984). In a detailed study, Suiker et al (2021) looked for diastatic strains of *Saccharomyces* in nature (tree bark and soil) and in brewery biofilms. With enrichment and PCR of the samples, one *STA+* yeast was recovered from bark and 21 from biofilms in brewery packaging halls. Using the same technology, diastatic *Saccharomyces* have been reported in four samples of draught beer from public houses in the UK (Jevons and Quain 2022). Other methods include third-generation

Figure 1.

Light microscopy images of *Saccharomyces cerevisiae* var *diastaticus* (left) and *Saccharomyces cerevisiae* strain (right) taken using a Nikon Eclipse Ci-L microscope with an oil immersion objective lens.

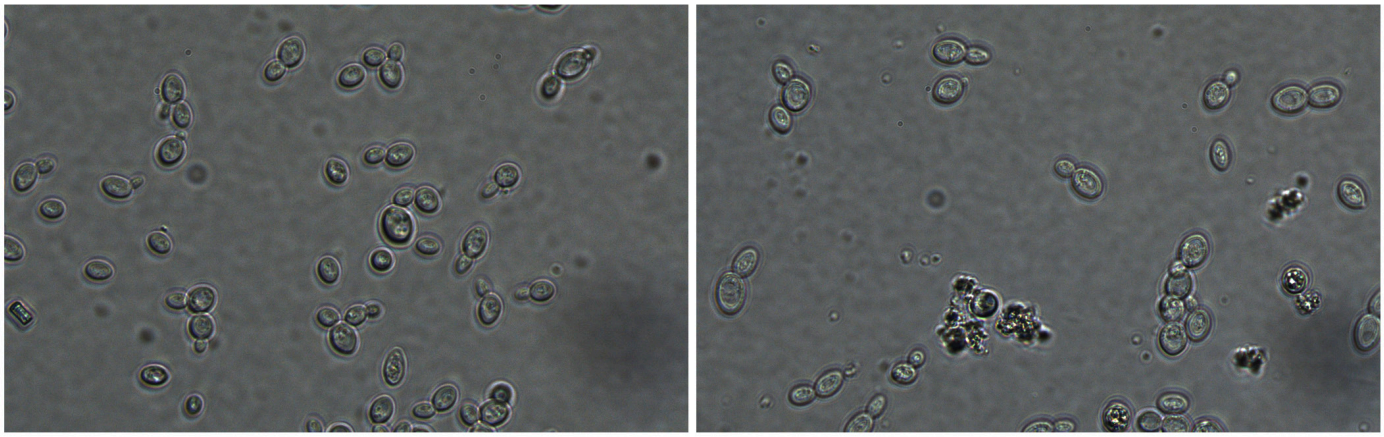

Table 1.

Pros and cons of commercial methods for diastatic yeasts.

Method	Description	Pros	Cons	References
Selective growth media	Used for the detection and quantification of specific microorganisms	Straight forward Inexpensive	Slow False Positives (MYGP+Cu)	Burns et al. 2021; Krogerus and Gibson 2020
Polymerase Chain Reaction (PCR) for diastatic genes	A common laboratory technique used to detect the presence or absence of specific DNA fragment.	Sensitive	Expensive reagents Initial equipment cost	Yamauchi et al.1998; Michel et al. 2016; Meier-Dörnberg et al. 2018; Schönling et al. 2019
Culture dependent + PCR	Identification using selective media and genomic methods.	High accuracy	Time consuming Expensive reagents Initial equipment cost	Jevons and Quain 2022
Third-Generation Sequencing (TGS)	State-of-the-art platform enabling long-read sequencing of DNA or RNA for constructing full genome or transcriptome or typing via multi-locus sequences.	High accuracy Fast	Unproven single nucleotide level resolution Initial equipment cost	Kurniawan et al. 2022; Shinohara et al. 2021
CRISPR-Cas12a	Using CRISPR-Cas nucleases to remove specific nucleic acid sequences on ssDNA or ssRNA.	High accuracy Fast, cheap Sensitive	Expensive reagents	Meng et al. 2021; Uotila and Krogerus 2022
Matrix Assisted Laser Desorption/Ionization – Time-of-Flight Mass Spectrometry (MALDI-TOF MS)	Analytical tool that separates particles according to their mass-to-charge ratio and are measured by the amount of time to travel through the detector.	Identification of diastatic strains Rapid and cost effective after initial set-up	High equipment cost High equipment cost	Condina et al. 2019; Lauterbach et al. 2017; Wieme et al. 2014

sequencing (TGS) and, notably, CRISPR-Cas12a which is both rapid and cheap (Uotila and Krogerus 2023). Instrumental methods include MALDI-TOF MS (matrix assisted laser desorption/ionization - Time-of-Flight Mass Spectrometry) for comparative analysis of ribosomal proteins (Mellmann et al. 2008).

Industrial Applications

While diastatic yeast have long been considered a contaminate, there are several applications which are changing this perception. These include the use of diastatic variants to replicate brewing styles, create novel beer styles, develop low calorie beers (glucoamylase enables greater attenuation (Stewart and Russell 1987)), increase efficiency of distillation, with application in biofuel production, and to explore high dextrin adjuncts. Some examples of studies in these areas are listed in Table 2.

Brewing Applications

Diastatic Yeast in Brewing

Diastatic yeasts have long been used to make farmhouse/Belgian style beers (such as Saison, Belgian Golden Strong, and Biere de Garde) (Krogerus and Gibson 2020). Farmhouse ales are characterised as having a drier mouthfeel coupled with a spicy/phenolic note (Krogerus and Gibson 2020). Despite diastatic yeasts being linked to poor sanitation, the acceptability and use of diastatic yeasts under controlled brewing conditions can be used to replicate farmhouse/Belgian styles, or lead to the development of new and unique flavours (Boulton and Quain 2001; Krogerus and Gibson 2020). A flavour study by Meier-Dörnberg et al (2018) brewed a lager style beer with seven different yeast strains (6 x POF+ diastatic *Saccharomyces* strains and 1 x *S. pastorianus*). It was demonstrated that the use of diastatic strains produced beers with phenolic compounds that were 'acceptable' in sensory trials with a fruity/tropical fruity flavour profile. Further beneficial traits included high flocculation and the utilisation of non-fermentable sugars (*aka* dextrins) (Meier-Dörnberg et al. 2018). However, Gallone et al (2016) found that the *Saccharomyces* yeasts in the Beer 2 clade (rich in STA+ and POF+ yeasts produced fewer aromatic and fruity ester compounds than the Beer 1 clade.

To obtain the novel characteristics of diastatic strains of *Saccharomyces*, while mitigating the negative attributes, they can be co-inoculated alongside 'standard' brewing strains. This can result in the complex phenolic aroma and glucoamylase production with the ethanol tolerance of primary yeasts to complete the fermentation.

Low-calorie beers

Beer typically contains non-fermentable dextrins which are not compatible with popular low or no-carb diets (Bamforth 2005; Haimoto et al. 2008). The calories in beer come from alcohol (60%), residual carbohydrates (39.3%) and protein (0.7%) (Helbert 1978). The low-calorie beer category are important to consumers who desire 'healthier' drinking (Capece et al. 2018). Low-calorie (light/lite) beer account for about 40% of beer consumed in the United States, with anticipated growth over the next five years. Current production techniques for these beers focus on carbohydrate reduction in the final product (Capece et al. 2018) and are classified by the United States Tax and Trade Bureau as a low-calorie or 'low-carb' beer if they contain less than 20 g/L of carbohydrates (Bamforth 2005). Methods to produce low-calorie beers include the 'dilution' method where water is added, enzymatic treatment of wort, or the use of non-conventional yeasts (Ogata et al. 2017). The use of extracellular glucoamylase producing *Saccharomyces* yeasts enables the hydrolysis and subsequent fermentation of glucose oligosaccharides significantly reducing the residual sugar content (Capece et al. 2018; Erratt and Stewart 1978). However, this results in an increased ABV (alcohol by volume) and low-calorie beers often require dilution to the desired ABV prior to packaging (Capece et al. 2018; Markowski 2004).

Distillation

Spirits are produced from the distillation of low ABV beverages from the fermentation of sugars by yeasts, typically *Saccharomyces cerevisiae*. 'Sugars' are sourced from the hydrolysis of starch in cereals or more directly from fruit, sugar cane or molasses. Spirits from cereal starch include malt whisky, grain whisky and grain neutral spirit for vodka and gin. The mashing process resembles that of brewing, but the hop-free wort is not boiled and the amylolytic enzymes remain active during fermentation such

Table 2.

Emerging applications for diastatic strains of S. cerevisiae.

Application	Comment	Reference
Low calorie	'… the use of new brewing yeasts, in addition to alternative raw materials, represents an innovative approach for producing specialty and original beers'	Erratt and Stewart 1978; Capece et al. 2018
	'*Saccharomyces cerevisiae* var. *diastaticus* yeast strains show high potential in brewing in batches and can be also used in secondary or mixed fermentations to produce beers with special flavours and/or a low carbohydrate content.'	Meier-Dörnberg et al. 2018
Distillation	'Developments in yeast isolation, propagation techniques, and format have greatly expanded the diversity of the yeast strains available to Scotch whisky distillers. It is also no secret in the distilling industry that using alternative yeast strains, compared to the 'industry standards', is a perfectly viable production strategy.'	Walker and Hill 2016
Biofuel	'The metabolic engineering strategies reviewed here prove that yeasts are powerful microbial platforms for the production of fatty acid-derived hydrocarbon fuels.'	Lu et al. 2022
	'… STA1 also plays a central role in enabling maltotriose use during wort fermentations. This allows for the improved reliability of molecular detection methods for diastatic contaminants in beer and can be exploited for strain development where maltotriose use is desired.'	Krogerus and Gibson 2020

that dextrins are broken down to fermentable sugars. Unlike brewery fermentations, only a few yeast strains of *S. cerevisiae* are used commercially by distillers (Walker and Hill, 2016) and some are considered to have some diastatic activity (Watson, 1993).

Although the addition of exogeneous enzymes is not permitted for Scotch whisky (https://www.legislation.gov.uk/uksi/2009/2890/pdfs/uksi_20092890_en.pdf) they can be used elsewhere in the production of whisky or for other distilled spirits. Accordingly, it may be advantageous to use yeast that produces glucoamyloytic enzymes to ferment to a lower attenuation or to use raw materials that have a high dextrin content or lower enzymatic power. As diastatic yeast may have a lower ethanol tolerance than standard distillers' yeast, this can be mitigated though co-inoculation.

Biofuel

Bioethanol is a primary source of renewable biofuel with the United States and Brazil the largest producers (Balat and Balat 2009). First generation ethanol refers to biofuel produced by *Saccharomyces* yeasts from crops and plant material and accounts for up 96% of the global biofuel production. A downside of this is the use of crops used in food such as sugar cane and wheat (Sánchez and Cardona 2008; Nigam and Singh 2011; Mohd Azhar et al. 2017; da Silva Fernandes et al. 2022), however there are examples of biofuel being derived from processing waste streams. For example, sweet potato and cassava are rich in starch and bioethanol can be produced from all parts of both plants (Sivamani et al. 2018). However, cassava has a short shelf life - due to its high moisture content - and must be processed within a month of harvest (Sivamani et al. 2018).

Second generation biofuels utilise non-food lignocellulosic biomass including crop residues, woody crops, and waste products such as rice bran (Almeida et al. 2022) and brewery spent grain (Nigam 2017). Second generation biofuels are attractive as they reduce the demand on food crops (Fung Min et al. 2013) while also valorising waste streams. Other raw materials (rice straw, corn cob, wheat straw, sugarcane bagasse) are abundant,

renewable, and relatively inexpensive (Zaldivar et al. 2001; Hahn-Hägerdal et al. 2006; Cardona and Sánchez 2007). In addition, second generation biofuels are evolving to include higher alcohols and fatty acids to expand the use into heavier vehicles (Lu et al. 2022). However, these processes are hampered by the lack of readily fermentable sugars in the substrate, and high levels of cellulose which require extensive hydrolysis to be fermentable by conventional yeasts.

The production of first generation bioethanol involves gelatinisation of starch, liquefaction, saccharification, fermentation, and distillation/ separation (da Silva Fernandes et al. 2022). The saccharification step is expensive and requires the addition of amylolytic enzymes (alpha-amylase and glucoamylase) to break down the starch to fermentable sugars (da Silva Fernandes et al. 2022). Accordingly, any reduction in enzyme addition would improve the profitability of the process. Wang et al (2021) have reported the integration of glucoamylase into *S. cerevisiae* and a corresponding reduction of 40% in the enzyme addition required for fermentation. Work has also been conducted in modifying *S. cerevisiae* to improve enzymatic production to increase biofuel production efficiency and functionality (Lu et al. 2022).

The application of diastatic yeasts to produce amylolytic enzymes is useful for biofuel production (Verma et al. 2000: Krogerus and Gibson 2020). Diastatic yeasts are resistant to some environmental stressors (Krogerus and Gibson 2020), however to be economically acceptable, the inoculum for biofuel production must grow quickly and efficiently metabolise fermentable sugars (da Silva Fernandes et al. 2022). Therefore, co-inoculation and hybridisation are methods to resolve some of the limitations of diastatic yeasts. For example, a lack of a starch-binding domain in diastatic yeasts (Latorre-García et al. 2005) has been addressed through co-culture of *Aspergillus niger* and expression of glucoamylase-encoding *STA1* gene in *Saccharomyces cerevisiae* (Latorre-García et al. 2008).

Conclusions

Whilst diastatic strains of *S. cerevisiae* are regarded as microbiological contaminates, the yeast has growing application in brewing and distilling. These yeasts have *STA* genes that produce glucoamylase that hydrolyse glucose oligosaccharide (dextrins) enabling more extensive fermentation. Further these yeasts produce phenolic aroma compounds that are associated with specific beer styles. There are a number of industries (brewing, distilling and biofuel) where indigenous glucoamylase offers significant economic potential by reducing the requirement for exogenous enzymes. However, there are also drawbacks and open questions that remain to be resolved or managed. Modern methods of detection allow for the rapid identification of diastatic strains; however, methods can be slow and/or expensive. The ability of diastatic strains of *S. cerevisiae* to break down dextrins make these yeasts of interest for biofuel and spirit production.

Author contributions

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Boce Zhang: writing (original draft, review and editing).
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Conflict of Interest

The authors declare there are no conflicts of interest.

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