Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Food Science

Spoilage yeasts in beer and beer products

Inge M Suiker and Han AB Wösten

Microbial spoilage of beer and related products results in high economic loss. Undesired microbes can impact the quality of the end product at any stage of the production process. *Brettanomyces* and *Saccharomyces* wild strains, including *B. bruxellensis* and *S. cerevisae diastaticus* (*S. diastaticus*), are commonly isolated as spoilage yeast. Knowledge of the taxonomy, ecology, and mechanisms of resistance against antimicrobial activity of beer (products) and preservation methods is now emerging, which can be used to develop spoilage prevention strategies.

Address
Microbiology, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Corresponding author: Wösten, Han AB (h.a.b.wosten@uu.nl)

Current Opinion in Food Science 2022, **44**:xx–yy

This review comes from a themed issue on **Food mycology**

Edited by **Anderson Souza Sant'Ana**

https://doi.org/10.1016/j.cofs.2022.100815

Spoilage of beer and beer related products

The global revenue in the beer segment will amount more than 600 billion euro in 2022 and the market is expected to grow annually by 6.8% [1]. This is explained, at least in part, by the increasing popularity of alcohol free beer and radlers. Beer has a high microbiological stability because of its low pH and the presence of ethanol, hop bitter compounds, high levels of carbon dioxide, low levels of oxygen and low amounts of easily digestable carbohydrates such as glucose and maltose [2]. Moreover, the heating step at 100°C during wort production effectively inactivates spoiling microbes that have accumulated up to this stage. Still, preventive measures like sanitation protocols in the brewery and pasteurization or sterile filtration during packaging are needed to reduce the chance of spoilage. Despite these measures, beer can still be spoiled by microbes. Spoilage risk is higher in non-alcoholic beer and in radlers because of the absence of alcohol and the presence of higher levels of easily digestible sugars in these products.

The economic loss of microbial spoilage of beer (products) is not exactly known as not all cases are reported but costs are high at a global scale. The introduction of undesired microbes at any stage of the malting and brewing process can have major negative effects on the quality of the end product [3]. Table 1 shows the major stages of beer production and microbial species that have been found during these stages. It should be noted that little is known about the spectrum of spoilage microbes in non-alcohol beers and radlers, especially because the commercial tools to identify spoilage microbes have been developed for regular beer. The spectrum of spoiling microbes thus may be more diverse in radlers and non-alcoholic beer [4]. Bacteria, especially some lactic acid bacteria, are considered the most hazardous beer spoilage microorganism in breweries [2]. Yet, also yeasts and filamentous fungi spoil beer.

This review focusses on the fungal beer spoilers with emphasis on *Saccharomyces diastaticus* and *Brettanomyces bruxellensis* and discusses how spoilage incidence can be reduced. To this end, spoilers have to be correctly identified by revealing their taxanomy, entry of spoilers in the brewery should be decreased by understanding their habitat, and hurdles that inhibit growth of the spoiler should be introduced targeting molecular mechanisms that underly spoilage.

Fungal spoilers

The degree to which barley is contaminated with filamentous fungi and yeasts depends on the field and storage conditions. The many fungal genera on the field include *Fusarium* and *Cladosporium* species, while typical storage fungi comprise *Aspergillus* and *Penicillium* species [5]. Fungi such as *Fusarium*, *Aspergillus* and *Penicillium* are capable of producing mycotoxins that survive the brewing process. Therefore, strict quality standards for mycotoxin levels in malt are defined [3]. An additional problem of fungal contamination of barley is gushing. This vigorous overfoaming of carbonated beverages occurs when the container is opened and is caused by hydrophobins [6]. Growth of filamentous fungi and wild yeasts can also be deleterious for malt quality by competing with the malt embryo for oxygen, thus inhibiting its germination [7].

Wild yeasts, but not filamentous fungi, are known to spoil later stages of beer production (i.e. during wort production, inoculation, fermentation and conditioning and packaging; Table 1). Notably, *Saccharomyces* and *Brettanomyces* that are used in beer production [5,8] are also the main yeasts that spoil beer. The latter yeasts, in particular *B. bruxellensis* (see below), spoil beer through the production of acetic acid and highly volatile phenolic compounds, such as 4-ethylguaiacol and 4-ethylphenol, giving

Table 1

Microbes that have been found during the different stages of beer production. Adapted from Ref. [16••]

	Filamentous Fungi	Yeast	Bacteria
From Barley to Malt	*Absidia*	*Candida*	*Acetobacteriaceae*
	Aspergillus	*Debaromyces*	*Alcaligenes*
	Alternaria	*Hansenula*	*Bacillus*
	Aureobasidium	*Hanseniaspora*	*Enterobacteriaceae*
	Fusarium	*Rhodotorula*	*Flavobacterium*
	Botrytis	*Sporobolomyces*	*Lactobacillus*
	Cladosporium	*Trichosporon*	*Pseudomonas*
	Epicoccum		
	Penicillium		
Wort		*Saccharomyces*	*Enterobacteriaceae*
Inoculation		*Saccharomyces*	*Obesumbacterium*
			Rhanella aquatilis
Fermentation		*Saccharomyces*	*Lactobacillus*
			Pediococcus
Conditioning and Packaging		*Saccharomyces*	*Acetobacter*
		Bretanomyces	*Gluconobacter*
		Candida	*Lactobacillus*
		Hansenula	*Megaspaera*
		Hanseniaspora	*Micrococcus*
		Pichia	*Pectinatus*
		Schizosaccharomyces	*Pediococcus*
		Torulopsis	*Selenomonas*
			Zymomonas
			Zymophilus

beer an undesired aroma of bandages, sweat, and smoke. *S. diastaticus* is likely the main *Saccharomyces* spoiler. For instance, about two third of the *S. cerevisae* related spoiling events in craft beer in Andean Patagonia are related to *S. diastaticus* [9]. Spoilage of beer by this yeast manifests itself by fermentation of residual carbohydrates, including dextrins and starch, by producing phenolic off-flavors and by haze formation and superattenuation. This leads to increased alcohol percentage, overcarbonation and a weakened body [5,10,11]. The incidence of spoilage with *S. diastaticus* has increased between 2008 and 2017 [12]. About two twird of the cases are related to contamination that occurs post-fermentation, most likely during filling [12]. Biofilms are most probably the main source of these contaminations [13••]. Microbes in biofilms are highly resistant to antibiotics, disinfectants, UV light and dessication [14]. Moreover, biofilms are not easily removed; some locations were recolonized in 2–12 hours [15]. Thus, daily cleaning of colonized areas only temporarily reduces the number of microorganisms on surfaces. PCR and selective enrichment identified *S. diastaticus* in 46% and 39% of biofilms from breweries, respectively [16••]. *B. bruxellensis* was identified in 32% and 3% of the PCR-positive and PCR-negative *S. diastaticus* samples, respectively. Notably, both yeasts were only identified in samples that were positive for *Candida* and/or *Pichia* as well. Together, data suggest that *S. diastaticus* and *B. bruxellensis* only exist in mixed biofilms in the presence of *Candida* and/or *Pichia*. Primary colonisers of surfaces in brewery bottling plants are gram-negative bacteria such as *Pseudomonas* spp. In the next step, yeasts (particularly *Pichia anomala*, *Candida sake* and *Debaryomyces hansenii*) will adhere [13••]. Subsequent adherence of *S. diastaticus* and/or *B. bruxellensis* will result in a biofilm with spoilage potential. Yet, these yeasts can be relatively lowly abundant in these biofilms. Relative abundance of *S. cerevisiae* (including *S. diastaticus*) was only <0.05% of the total fungal population in 85% of the biofilms [16••].

Phylogeny and identification of *S. diastaticus* and *B. bruxellensis*

Brettanomyces was for the first time described in 1904 as the causative agent of secondary fermentation and characteristic flavours of English stock ales [17]. Nowadays, *Brettanomyces bruxellensis*, *Brettanomyces anomalus*, *Brettanomyces custersianus*, *Brettanomyces naardenensis*, and *Brettanomyces nanus* are accepted species within this genus, while *Brettanomyces acidodurans* has also been assigned to this genus despite its high genetic divergence relative to the other *Brettanomyces* species [18,19]. The former three species can grow in beer, while the former two are able to spoil beer [20]. Most reported cases of spoilage with *Brettanomyces* concern *B. bruxellensis* [21]. Sequencing of 53 strains of this yeast revealed that intra-strain genetic diversity is larger than in *S. cerevisae* and related to ploidy level with about 75% and 25% of the strains being diploid and triploid, respectively. The diploid and triploid strains form three and two strain clusters, respectively [22•].

Yeast strains capable of fermenting starch and dextrins were for the first time isolated by Andrews and Gilliland [11], who classified this yeast as a separate *Saccharomyces*

species called *S. diastaticus*. Genomics changed this perspective by showing that the amylolytic strains are varieties of *S. cerevisiae* [23•]. The amylolytic *STA1* gene is considered unique for *S. diastaticus*. Out of 1169 genomes [24,25], 54 were positive for *STA1* [26••]. These 54 strains and an additional 43 *S. diastaticus* strains cluster in 4 out of >30 *S. cerevisae* clades [27••]. The Beer/Mosaic clade, related to beer and breweries, comprises 74 *S. diastaticus* strains and represent 86% of the strains in this clade. The French Guiana Human clade, related to human feaces, consists of 20 *S. diastaticus* strains that represent 63% of the strains in this clade. Three *S. diastaticus* strains cluster in a clade with 65 *S. cerevisiae* strains, and one in a clade with three *S. cerevisiae* strains [16••]. Thus, *S. diastaticus* is not a monophyletic variant of *S. cerevisiae* and can not be considered a subspecies [23•,27••,28,29]. However, for historical reasons we will continue to label them as *S. diastaticus*. Presence of *STA1* is also not monophyletic since about 10% of the strains identified as *S. diastaticus* by a commercial PCR-based method do not have this gene and are positioned in different clades [27••]. Together, a commercial PCR-based method and the presence of *STA1* are not sufficient to assign a strain as *S. diastaticus*. Reversely, presence of *STA1* does not imply that the strain is a spoiler. Several strains contained a promoter deletion in this gene and thereby do not have amylase activity [26••].

Traditionally, culturing is used to detect spoilage yeast [30,31]. Use of enrichment media (containing for instance ethanol, cupper, or starch) increases sensitivity of this detection method [30–33]. However, culturing is accompanied with long incubation periods (up to a few weeks), may give false-positives, and *B. bruxellensis* may not grow despite being viable [30,31]. Direct measurements using PCR-based methods (such as amplifying (different parts of) *STA1* of *S. diasaticus*) may lack sensitity and thus needs a pre-culturing step [27••,34]. This also applies to detection techniques monitoring spoilage yeast metabolites [30]. Moreover, primers used in PCR-based methods should be selective and cover all spoiler strains. As mentioned above this is still an issue in the case of *S. diastaticus*.

Habitat

Brettanomyces can be isolated from different substrates such as fruit peels, kombucha, kefir, tea, olives, sodas and wooden barrels but also from ecosystems with limited nutrient conditions not often inhabited by other yeasts [35•]. Various studies have shown a high phenotypic diversity of *B. bruxellensis* with respect to sugar metabolism, nitrogen source utilisation and to abiotic factors like temperature, pH, oxygen availability, and sulfur dioxide (see Ref. [36]). This phenotypic diversity may contribute to the variety of ecosystems from which *B. bruxellensis* can be isolated.

It is easy to isolate *S. cerevisiae* from places associated with fermentation and making alcohol, such as vineyards, wineries, breweries, bakeries and distilleries. Yet, a study indicates that it is not adapted to a specific niche [37••] and it can also be isolated from oak trees, soil, plants, insects and fruit, as well as from humans as a commensal or even as a pathogen [38–42]. These environments have high or low carbon and nitrogen concentrations and range from pH 3 to pH 8, from water to 1.3 M NaCl, and from 0°C to 45°C [38–47]. However, it is unclear whether the cells are actively growing, are metabolically active or are dormant in these niches [40]. In contrast to *S. cerevisiae, S. diastaticus* is not easily isolated from nature. Out of 97 strains, only six originate from nature; two from Ecuador (from water on a leaf and an insect), three from French Guiana (from agouti paca and fruit), and one from the Netherlands (from bark) [16••,26••,27••]. The other 91 strains have been isolated from beer, wine, spirits and human feces. Results thus indicate that *S. diastaticus* is a lowly abundant variety of *S. cerevisiae* in nature but it can be easily isolated from biofilms in breweries.

Molecular mechanisms involved in spoilage

The physiological and developmental state of cells

S. cerevisiae cultures enter the quiescent state when carbon source in the medium has been depleted [48,49]. Alternatively, cells can form ascospores when a non-fermentable carbon source such as acetate is present [50,51]. These spores represent the most resilient phase of *S. cerevisiae* [52–54]. For instance, they are more resistant to temperature treatment, mild alkali and acid conditions and to digestive enzymes when compared to vegetative cells during logaritimic or stationary growth or to quiescent cells [40,52,54]. Yet, vegetative cells in the stationary growth phase are similarly resistant to freeze-thaw and desiccation.

Ascospores of *S. diastaticus* were shown to grow in all tested alcoholic and non-alcoholic beers and radlers but vegetative cells did not grow in lemon or lime-based radlers (LL radler) [16••]. The latter may be explained by the low pH of this beer product and the presence of D-limonene. The ability of ascospores to grow under these conditions may be explained by their dormancy, which would enable them to adapt to the new medium before germination. Adaptation would also explain why vegetative cells that are pre-cultured in alcohol free beer or in mixtures of alcohol-free beer and LL radler do colonize this type of radler [16••]. Reversely, spoilage capacity is lost when vegetative cells grown in LL radler (resulting from germinated ascospores) are grown for 13 generations in YPD but not when grown for 4 generations. These findings suggest a cellular memory of acquired stress resistance. Gradual increase of LL radler in the growth medium or the low pH of non-alcoholic beer may be experienced as a mild stress by yeast, thereby acquiring

cross-protection against a range of stresses [55•]. This may enable the yeast to grow in LL radler.

The ability to convert starch and dextrins

As mentioned above, spoilage of *S. diastaticus* is related with the ability to convert starch and dextrins, which is due to the secretion of extracelular glucoamlyase (1,4-α-D-glucan glucohydrolases; EC 3.2.1.3) [11,56–58]. This enzyme is encoded by three highly homologous *STA(1–3)* genes, which are chimers from *FLO11* and *SGA1* [59,60]. The 3′ end of *STA1* is homologous to *SGA1*, present in all *S. cerevisiae* strains, that encodes an intracellular glycogen degrading glucoamylase that is specifically expressed during sporulation. The 5′ part of *STA1* is homologous to *FLO11* that encodes a membrane-bound flocculin that promotes flocculation of *S. cerevisiae* [60], and which makes that the STA1 is secreted.

Heat resistance

Heat resistance of ascospores of *S. diastaticus* strains within the Beer/Mosaic clade varies considerably with a 4.41 $\log_{10}$ decimal reduction between the most sensitive and the most resistant strain. In fact, the latter strain is among the most heat resistant yeast strains [27••,53,61]. Notably, ascospores of *S. diastaticus* strains of the Beer/Mosaic clade are on average more heat resistant than spores from *S. diastaticus* strains of other clades [27••]. Therefore, it is tempting to speculate that breweries provide a selective pressure that makes *S. diastaticus* more heat resistant. This selective pressure may be exposure to heat. *S. diastaticus* rapidly acquires heat resistance during eight cycles of heat exposure of ascospores with a D_{60} value increase from 6.5 to 9 min [27••]. Notably, the D_{52} value of vegetative cells also increases from 9.2 min to 16.2 min during the eight cycles. Apparently, heat treatment of ascospores selects for a mechanism that also functions in vegetative cells. Apart from the genetic background of the strain, also environmental growth conditions and the menstruum impact heat resistance. Vegetative cells grown in YPD are more heat resistant when treated in physiological saline when compared to treatment in non-alcoholic beer with D_{52}-values of 10.3 and 4.0 min, respectively [16••]. This may be explained by the pH of 4.3 of the latter menstruum when compared to physiological saline (~6.5). Also, vegetative cells grown in non-alcoholic beer are 10 times more heat resistant than cells grown in YPD [16••]. This could be due to induced cross-protection against environmental stresses for instance by strengthening the cell wall [62,63]. Finally, the developmental state of the cells impact heat resistance. Vegetative cells in the post diauxic shift phase are more heat resistant than vegetative cells in the logarithmic growth phase. Furthermore, young ascospores are more susceptible to heat inactivation than mature spores, even though the spore wall already seems fully formed in these young spores [27••].

Genes *SRD1*, *OSW1*, *CWP1*, *CWP2* are higher expressed in young ascospores of *S. diastaticus* when compared to their equivalents in *S. cerevisiae* [16••]. Sporulation is not initiated in the *S. diastaticus* ΔΔ*srd1* strain, while spores walls do not mature in the ΔΔ*osw1* strain. Consequently, cells of these deletion strains do not survive heat treatment at 60°C. The transcriptional regulator *SRD1* is thus involved in spore formation and its increased expression in *S. diastaticus* may result in spores that are more heat resistant. Sporulation in the *S. diastaticus* ΔΔ*cwp1,2* strain is similar when compared to the wild type strain and malachite green staining indicates that mature ascospores are formed. Yet, ΔΔ*cwp1,2* cells are more sensitive to a heat treatment [16••]. Cell wall proteins Cwp1p and Cwp2p are mannoproteins that are covalently linked to the cell wall and that lower permeability of the cell. Especially when cells are under stress, Cwp complexes are formed [64–66]. Such complexes may play a role in the protection of ascospores by stabilizing the ascus wall. The higher expression of these genes in spores of *S. diastaticus* may contribute to the increased heat resistance of these strains compared to other *S. cerevisiae* strains. The difference in heat resistance in the vegetative cells of the *SRD1*, *OSW1*, *CWP1*, *CWP2* deletion strains needs to be confirmed. Preliminary results indicate that the vegetative cells of ΔΔ*osw1* and ΔΔ*srd1* have a similar heat resistance when compared to the wildtype strain, which is expected since these genes are both sporulation specific. The vegetative cells of ΔΔ*cwp1,2*, however, seem to be less heat resistant, possibly due to weakened cell walls.

Conclusion and future perspectives

Brettanomyces and *Saccharomyces* are considered the most common yeasts that spoil beer (products), of which *S. diastaticus* and *B. bruxellensis* are notorious examples. These spoiling yeasts can be found within biofilms in breweries but their source from the environment is not clear. This knowledge may provide leads to prevent entry of the spoilage yeasts at beer production sites.

Data suggest that *S. diastaticus* and *B. bruxellensis* exist in mixed biofilms in the presence of *Candida* and/or *Pichia*. In fact, relative abundance of *S. cerevisiae* (including *S. diastaticus*) in most biofilms is <0.05% of the total fungal population, making it very challenging to perform in depth analysis of the physiological state of these cells in such communities. This is of great importance to assess prevention strategies since the environmental growth conditions and the developmental stage of the cells have a great impact on stress resistance. Labelling with, for example, antibodies followed by cell sorting could reveal whether the spoilage yeasts occur as vegetative cells or ascospores and whether these cells are more stress resistant than cells that have been cultured in the lab. Possibly, resistance of cells in biofilms is higher and, as a consequence, temperature and time needed for pasteurization should be increased or other hurdles should be

introduced to avoid spoilage in breweries. It is clear that the menstruum impacts stress resistance of *S. diastaticus*. Therefore, challenge tests should be directly done in commercial products.

S. diastaticus is not monophyletic and is found in four S. cerevisiae clades mixed with non-*S. diastaticus* strains. A commercial PCR method does not strictly correlate with the presence of *STA1* in the genome of a strain. Thus, it is still difficult to assign strains as *S. diastaticus*. Future studies should expand the phylogeny of spoilage related *S. cerevisiae* to address which loci make them unique compared to non-spoilage yeasts and whether these loci can be used to improve identification and risk analysis with respect to spoilage incidents. The first steps have been taken showing that part of the *S. diastaticus* strains have an inactive copy of the *STA1* gene, making them non-spoilers.

Understanding molecular mechanisms underlying spoilage potential of *S. diastaticus* and *B. bruxellensis* may provide leads to control these yeasts. For instance, plant extracts may contain molecules that interfere with these mechanisms. So far, little is known about such mechanisms in *B. bruxellensis* but the first steps have been taken in *S. diastaticus*.

Role of funding source

The funding organization had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement

Nothing declared.

CRediT authorship contribution statement

Inge M Suiker: Writing – original draft, Writing – review & editing. **Han AB Wösten:** Writing – original draft, Writing – review & editing.

Acknowledgement

This work was supported by TiFN, a public–private partnership on precompetitive research in food and nutrition.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Statista.com: Statista, Hamburg, Germany. https://www.statista.com/outlook/10010000/100/beer/worldwide?currency=eur. 2020.

2. Sakamoto K, Konings WN: **Beer spoilage bacteria and hop resistance**. *Int J Food Microbiol* 2003, **89**:105-124.

3. Bokulich NA, Bamforth CW: **The microbiology of malting and brewing**. *Microbiol Mol Biol Rev* 2013, **77**:157-172.

4. Suzuki K: **Emergence of new spoilage microorganisms in the brewing industry and development of microbiological quality control methods to cope with this phenomenon–a review**. *J Am Soc Brew Chem* 2020, **78**:245-259.

5. Priest FG, Campbell I (Eds): *Brewing Microbiology*. Springer; 2003.

6. Sarlin T, Nakari-Setälä T, Linder M, Penttilä M, Haikara A: **Fungal hydrophobins as predictors of the gushing activity of malt**. *J Inst Brew* 2005, **111**:105-111.

7. O'Sullivan TF, Walsh Y, O'Mahony A, Fitzgerald GF, van Sinderen D: **A comparative study of malthouse and brewhouse microflora**. *J Inst Brew* 1999, **105**:55-61.

8. Colomer MS, Funch B, Forster J: **The raise of Brettanomyces yeast species for beer production**. *Curr Opin Biotechnol* 2019, **56**:30-35.

9. Latorre M, Hutzler M, Michel M, Zarnkow M, Jacob F, Libkind D: **Genotypic diversity of *Saccharomyces cerevisiae* spoilers in a community of craft microbreweries**. *Monatsschrift Brauwiss* 2020, **73**:51-57.

10. Hutzler M, Riedl R, Koob J, Jacob F: **Fermentation and spoilage yeasts and their relevance for the beverage industry - a review**. *BrSc* 2012, **65**:33-52.

11. Andrews BJ, Gilliland RB: **Super-attenuation of beer: a study of three organisms capable of causing abnormal attenuations**. *J Inst Brew* 1952, **58**:189-196.

12. Meier-Dörnberg T, Jacob F, Michel M, Hutzler M: **Incidence of *Saccharomyces cerevisiae* var. diastaticus in the beverage industry: cases of contamination, 2008-2017**. *Tech Q MBAA* 2017, **54**:140-148.

13. •• Timke M, Wang-Lieu NQ, Altendorf K, Lipski A: **Identity, beer spoiling and biofilm forming potential of yeasts from beer bottling plant associated biofilms**. *Antonie Leeuwenhoek* 2008, **93**:151-161

This article shows that *S. cerevisiae* comprises 46 % of all isolates of wild yeasts in biofilms in two breweries, while Candida pelliculosa strains make up 24 % of the isolates. Notably, the latter but not the former yeast forms biofilms in a microplate, suggesting that C. pelliculosa is involved in primary biofilm formation, while *S. cerevisiae* is a late colonizer of a preformed biofilm.

14. Quain D, Storgards E: **The extraordinary world of biofilms**. *Brew Distill Int* 2009, **5**:31-33.

15. Storgards E, Tapani K, Hartwall P, Saleva R, Suikho M: **Microbial attachment and biofilm formation in brewery bottling plants**. *J Am Soc Brew Chem* 2006, **64**:8-15.

16. •• Suiker IM, Arkesteijn GJA, Zeegers PJ, Wösten HAB: **Presence of *Saccharomyces cerevisiae* subsp. diastaticus in industry and nature and spoilage capacity of its vegetative cells and ascospores**. *Int J Food Microbiol* 2021, **347**:109147

This article shows that *S. diastaticus* is only lowly abundant in nature but abundant in breweries where it is present in mixed biofilms with Candida and/or Pichia.

17. Claussen NH: **Eine Methode zur Anwendung von Hansens Reinzuchtsystem bei der Herstellung von englischen gelagerten Biersorten**. *Wochenschr Brau* 1904:370-383.

18. Steensels J, Daenen L, Malcorps P, Derdelinckx G, Verachtert H, Verstrepen KJ: **Brettanomyces yeasts-from spoilage organisms to valuable contributors to industrial fermentations**. *Int J Food Microbiol* 2015, **206**:24-38.

19. Péter G, Dlauchy D, Tóbiás A, Fülöp L, Podgoršek M, Čadež N: ***Brettanomyces acidodurans* sp. nov., a new acetic acid producing yeast species from olive oil**. *Antonie Van Leeuwenhoek* 2017, **110**:657-664.

20. Shimotsu S, Asano S, Iijima K, Suzuki K, Yamagishi H, Aizawa M: **Investigation of beer-spoilage ability of Dekkera/Brettanomyces yeasts and development of multiplex PCR method for beer-spoilage yeasts**. *J Inst Brew* 2015, **121**:177-180.

21. Crauwels S, Zhu B, Steensels J, Busschaert P, De Samblanx G, Marchal K, Willems KA, Verstrepen KJ, Lievens B: **Assessing genetic diversity among Brettanomyces yeasts by DNA**

fingerprinting and whole-genome sequencing. *Appl Environ Microbiol* 2014, **80**:4398-4413.

22. • Gounot JS, Neuvéglise C, Freel KC, Devillers H, Piškur J, Friedrich A, Schacherer J: **High complexity and degree of genetic variation in *Brettanomyces bruxellensis* population**. *Genome Biol Evol* 2020, **12**:795-807
This article describes the intra-strain diversity of *B. bruxellensis* which is higher than observed in *S. cerevisae* and is related to ploidy level.

23. • Liti G, Carter DM, Moses AM, Warringer J, Parts L, James SA, Davey RP, Roberts IN, Burt A, Koufopanou V *et al.*: **Population genomics of domestic and wild yeasts**. *Nature* 2009, **458**:337-341
This article describes the differences in genomes of domesticated and wild *S. cerevisae*. Inspection of their data shows that *S. diastaticus* is not a species or subspecies but simply varieties of *S. cerevisae*.

24. Peter J, De Chiara M, Friedrich A, Yue JX, Pflieger D, Bergström A, Sigwalt A, Barre B, Freel K, Llored A *et al.*: **Genome evolution across 1,011 *Saccharomyces cerevisiae* isolates**. *Nature* 2018, **556**:339-344.

25. Gallone B, Steensels J, Prahl T, Soriaga L, Saels V, Herrera-Malaver B, Merlevede A, Roncoroni M, Voordeckers K, Miraglia L *et al.*: **Domestication and divergence of *Saccharomyces cerevisiae* beer yeasts**. *Cell* 2016, **166**:1397-1410.

26. •• Krogerus K, Magalhães F, Kuivanen J, Gibson B: **A deletion in the STA1 promoter determines maltotriose and starch utilization in STA1+ *Saccharomyces cerevisiae* strains**. *Appl Microbiol Biotechnol* 2019, **103**:7597-7615
This article describes that the *S. diastaticus* strains are mainly found in the Mosaic/Beer and French Guiana human clades of *S. cerevisae* and that the presence of the STA1 gene does not per se leads to diastase activity.

27. •• Suiker IM: **Heterogeneity between and within strains of the beer spoilage yeast *Saccharomyces cerevisiae* subspecies diastaticus**. *PHD Thesis*. Utrecht University; 2021
This Thesis describes that *S. diastaticus* strains can be found in four clades of *S. cerevisae* and that this yeast can easily acquire heat resistance by inbreeding. Moreover, it describes the first genes involved in heat resistance of this spoilage yeast.

28. Jespersen L, van der Aa Kühle A, Petersen KM: **Phenotypic and genetic diversity of Saccharomyces contaminants isolated from lager breweries and their phylogenetic relationship with brewing yeasts**. *Int J Food Microbiol* 2000, **60**:43-53.

29. Meier-Dörnberg T, Kory OI, Jacob F, Michel M, Hutzler M: ***Saccharomyces cerevisiae* variety diastaticus friend or foe? Spoilage potential and brewing ability of different *Saccharomyces cerevisiae* variety diastaticus yeast isolates by genetic, phenotypic and physiological characterization**. *FEMS Yeast Res* 2018, **18**:foy023.

30. Tubia I, Prasad K, Pérez-Lorenzo E, Abadín C, Zumárraga M, Oyanguren I, Barbero F, Paredes J, Arana S: **Beverage spoilage yeast detection methods and control technologies: a review of Brettanomyces**. *Int J Food Microbiol* 2018, **283**:65-76.

31. Krogerus K, Gibson B: **A re-evaluation of diastatic *Saccharomyces cerevisiae* strains and their role in brewing**. *Appl Microbiol Biotechnol* 2020, **104**:3745-3756.

32. Rodrigues N, Gonçalves G, Pereira-da-Silva S, Malfeito-Ferreira M, Loureiro V: **Development and use of a differential medium to detect yeasts of the genera Dekkera/Brettanomyces**. *J Appl Microbiol* 2001, **90**:588-599.

33. Burns LT, Sislak CD, Gibbon NL, Saylor NR, Seymour MR, Shaner LM, Gibney PA: **Improved functional assays and risk assessment for STA1+ strains of *Saccharomyces cerevisiae***. *J Am Soc Brew Chem* 2021, **79**:167-180.

34. Renouf V, Lonvaud-Funel A: **Development of an enrichment medium to detect Dekkera/*Brettanomyces bruxellensis*, a spoilage wine yeast, on the surface of grape berries**. *Microbiol Res* 2007, **162**:154-167.

35. • Smith BD, Divol B: ***Brettanomyces bruxellensis*, a survivalist prepared for the wine apocalypse and other beverages**. *Food Microbiol* 2016, **59**:161-175
This review describes the natural niches of *B. bruxellensis* showing it can survive harsh environments.

36. Avramova M, Cibrario A, Peltier E, Coton M, Coton E, Schacherer J, Spano G, Capozzi V, Blaiotta G, Salin F *et al.*: ***Brettanomyces bruxellensis* population survey reveals a diploid-triploid complex structured according to substrate of isolation and geographical distribution**. *Sc Rep* 2018, **8**:4136.

37. •• Goddard MR, Greig D: ***Saccharomyces cerevisiae*: a nomadic yeast with no niche?** *FEMS Yeast Res* 2015, **15**:fov009
This article describes that there is limited evidence that *S. cerevisiae* is adapted to fruit but rather has no niche being able to survive in a range of habitats.

38. Sampaio P, Gonçalves P: **Natural populations of *Saccharomyces kudriavzevii* in Portugal are associated with oak bark and are sympatric with *S. cerevisiae* and *S. paradoxus***. *Appl Environ Microbiol* 2008, **74**:2144-2152.

39. Angebault C, Djossou F, Abélanet S, Permal E, Ben Soltana M, Diancourt L, Bouchier C, Woerther PL, Catzeflis F, Andremont A *et al.*: **Candida albicans is not always the preferential yeast colonizing humans: a study in Wayampi Amerindians**. *J Infect Dis* 2013, **208**:1705-1716.

40. Knight SJ, Goddard MR: **Sporulation in soil as an overwinter survival strategy in *Saccharomyces cerevisiae***. *FEMS Yeast Res* 2015, **16**:fov102.

41. Wang QM, Liu WQ, Liti G, Wang SA, Bai FY: **Surprisingly diverged populations of *Saccharomyces cerevisiae* in natural environments remote from human activity**. *Mol Ecol* 2012, **21**:5404-5417.

42. Goddard MR, Anfang N, Tang R, Gardner RC, Jun C: **A distinct population of *Saccharomyces cerevisiae* in New Zealand: evidence for local dispersal by insects and human-aided global dispersal in oak barrels**. *Environ Microbiol* 2010, **12**:63-73.

43. Petrovska B, Winkelhausen E, Kuzmanova S: **Glycerol production by yeasts under osmotic and sulfite stress**. *Can J Microbiol* 1999, **45**:695-699.

44. Salvadó Z, Arroyo-López FN, Guillamón JM, Salazar G, Querol A, Barrio E: **Temperature adaptation markedly determines evolution within the genus Saccharomyces**. *Appl Environ Microbiol* 2011, **77**:2292-2302.

45. Serrano R, Martín H, Casamayor A, Ariño J: **Signaling alkaline pH stress in the yeast *Saccharomyces cerevisiae* through the Wsc1 cell surface sensor and the Slt2 MAPK pathway**. *J Biol Chem* 2006, **281**:39785-39795.

46. Sweeney JY, Kuehne HA, Sniegowski PD: **Sympatric natural *Saccharomyces cerevisiae* and *S. paradoxus* populations have different thermal growth profiles**. *FEMS Yeast Res* 2004, **4**:521-525.

47. Wenger JW, Piotrowski J, Nagarajan S, Chiotti K, Sherlock G, Rosenzweig F: **Hunger artists: yeast adapted to carbon limitation show trade-offs under carbon sufficiency**. *PLoS Genet* 2001:7-8:e1002202.

48. Gray J, Petsko GA, Johnston GC, Ringe D, Singer RA, Werner-Washburne M: **"Sleeping beauty": quiescence in *Saccharomyces cerevisiae***. *Microbiol Mol Biol Rev* 2004, **68**:187-206.

49. Werner-Washburne M, Roy S, Davidson GS: **Aging and the survival of quiescent and non-quiescent cells in yeast stationary-phase cultures**. In *Aging Research in Yeast.* Edited by Breitenbach M, Jazwinski S, Laun P. Springer; 2011:123-143.

50. Freese EB, Chu MI, Freese E: **Initiation of yeast sporulation by partial carbon, nitrogen, or phosphate deprivation**. *J Bacteriol* 1982, **149**:840-851.

51. Neiman AM: **Sporulation in the budding yeast *Saccharomyces cerevisiae***. *Genetics* 2011, **189**:737-765.

52. Coluccio AE, Rodriguez RK, Kernan MJ, Neiman AM: **The yeast spore wall enables spores to survive passage through the digestive tract of Drosophila**. *PLoS One* 2008, **3**:e2873.

53. Milani EA, Gardner RC, Silva FVM: **Thermal resistance of Saccharomyces yeast ascospores in beers**. *Int J Food Microbiol* 2015, **206**:75-80.

54. Reuter M, Bell G, Greig D: **Increased outbreeding in yeast in response to dispersal by an insect vector**. *Curr Biol* 2007, **17**:81-83.

55. • Guan Q, Haroon S, Bravo DG, Will JL, Gasch AP: **Cellular memory of acquired stress resistance in *Saccharomyces cerevisiae***. *Genetics* 2012, **192**:495-505
This article describes that *S. cerevisiae* cells have a memory of prior stress exposure. Stress resistance persists for four to five generations after stress exposure and was transmitted to daughter cells that did not experience this exposure.

56. Errat JA, Stewart GG: **Genetic and biochemical studies on glucoamylase from *Saccharomyces diastaticus***. In *Current Developments in Yeast Research.* Edited by Stewart GG, Pergamon Russel Ise .. 1981:177-183.

57. Kleinman MJ, Wilkinson AE, Wright IP, Evans IH, Bevanii EA: **Purification and properties of an extracellular glucoamylase from a diastatic strain of *Saccharomyces cerevisiae***. *Biochem J* 1988, **249**:163-170.

58. Yamashita I, Fukui S: **Isolation of glucoamylase-non-producing mutants in the yeast *Saccharomyces diastaticus***. *Agric Biol Chem* 1984, **48**:131-135.

59. Lo WS, Dranginis AM: **FLO11, a yeast gene related to the STA genes, encodes a novel cell surface flocculin**. *J Bacteriol* 1996, **178**:7144-7151.

60. Yamashita I, Nakamura M, Fukui S: **Gene fusion is a possible mechanisms underlying the evolution of STA1**. *J Bacteriol* 1987, **169**:2142-2149.

61. Shearer AEH, Mazzotta AS, Chuyate R, Gombas DE: **Heat resistance of juice spoilage microorganisms**. *J Food Prot* 2002, **65**:1271-1275.

62. Causton H, Ren B, Koh S, Harbison C, Kanin E: **Remodeling of yeast genome expression in response to environmental changes**. *Mol Biol Cell* 2001, **12**:323-337.

63. Gasch AP: **Comparative genomics of the environmental stress response in ascomycete fungi**. *Yeast* 2007, **24**:961-976.

64. Kapteyn JC, Montijn RC, Vink E, de La Cruz J, Llobell A, Douwes JE, Shimoi H, Lipke PN, Klis FM: **Retention of *Saccharomyces cerevisiae* cell wall proteins through a phosphodiester-linked β-1,3-/β-1,6-glucan heteropolymer**. *Glycobiology* 1996, **6**:337-345.

65. Klis FM, Boorsma A, de Groot PWJ: **Cell wall construction in *Saccharomyces cerevisiae***. *Yeast* 2006, **23**:185-202.

66. Shimoi H, Limura Y, Obata T: **Molecular cloning of CWP1: a gene encoding a *Saccharomyces cerevisiae* cell wall protein solubilized with *Rarobacter faecitabidus* protease I**. *J Biochem* 1995, **118**:302-311.