MINIREVIEW

Lager-brewing yeasts in the era of modern genetics

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One sentence summary: Saccharomyces pastorianus is a hybrid yeast that has been domesticated since the 16th century. The review presents the slough of lager yeast research under the influence of genome science.

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ABSTRACT

The yeast Saccharomyces pastorianus is responsible for the annual worldwide production of almost 200 billion liters of lager-type beer. S. pastorianus is a hybrid of Saccharomyces cerevisiae and Saccharomyces eubayanus that has been studied for well over a century. Scientific interest in S. pastorianus intensified upon the discovery, in 2011, of its S. eubayanus ancestor. Moreover, advances in whole-genome sequencing and genome editing now enable deeper exploration of the complex hybrid and aneuploid genome architectures of S. pastorianus strains. These developments not only provide novel insights into the emergence and domestication of S. pastorianus but also generate new opportunities for its industrial application. This review paper combines historical, technical and socioeconomic perspectives to analyze the evolutionary origin and genetics of S. pastorianus. In addition, it provides an overview of available methods for industrial strain improvement and an outlook on future industrial application of lager-brewing yeasts. Particular attention is given to the ongoing debate on whether current S. pastorianus originates from a single or multiple hybridization events and to the potential role of genome editing in developing industrial brewing yeast strains.

Keywords: Saccharomyces pastorianus; strain improvement; hybrid heterosis; whole genome sequencing; genome editing

EMERGENCE AND INDUSTRIALIZATION OF LAGER BREWING

Beer brewing is tightly intertwined with human culture. Archaeological remains from the 12th millennium BC indicate that microbial fermentation of cereals may predate the agricultural revolution (Liu et al. 2018). Chemical archaeology and pictographic evidence show that beer brewing was customary as early as in the 4th millennium BC (Michel, McGovern and Badler 1992; Sicard and Legras 2011). Lager-style beer emerged only in 16th century Bavaria under the influence of novel regulations to standardize the brewing process and to improve quality. For example, the well-known 'Reinheitsgebot' of 1516 restricted ingredients used for brewing to water, barley and hops (Hornsey 2003). When, in 1553, beer brewing was legally restricted to winter months, bottom-fermenting yeast emerged as a consequence of the lower fermentation temperatures (Unger 2004). In contrast to the top-fermenting yeasts used at higher temperatures for brewing ale-type beers, bottom-fermenting yeast form flocs that sediment at the end of the fermentation (Oliver and Colicchio 2011). Bottom-fermenting yeasts were initially used to brew a dark brown beer, which was stored to enable consumption
during the summer months. This beer was designated as lager, in reference to the German ‘lager’ which means ‘to store’ (Meussdoerffer 2009). In 1842, Bavarian brew master Josef Groll, working in the Bohemian city Pilsen, started brewing a pale style of lager beer with fruity Saaz-type hops, which became known as Pilsner beer (Meussdoerffer 2009). The advent of Pilsner coincided with rapid technological advances that enabled industrialization of beer brewing. The discovery that yeast is responsible for fermentation (Pasteur 1876) and the isolation of pure lager-brewing strains (Hanssen 1883; Moritz and Morris 1891) enabled inoculation of beer fermentation processes with pure cultures, resulting in more consistent quality. Moreover, the invention of the steam engine and ammonia refrigeration enabled industrial mass production (Appel 1990; Poelmans and Swinnen 2011a). Finally, the invention of bottle production using iron molds, of crown corks and of beer filtration improved product stability and enabled exportation (Painter 1892; Kunze 2004; Lockhart 2007). As a result of these innovations, global beer production soared to 17.7 billion liters in 1899 (Michel 1899), and further increased to 193 billion liters in 2015, of which 89% was lager-type beer (Brickwedde et al. 2017).

THE LAGER-BREWING YEAST

Saccharomyces pastorianus

Lager beers are fermented with S. pastorianus strains. These hybrids of Saccharomyces cerevisiae and Saccharomyces eubayanus have only been encountered in brewing-related contexts (Libkind et al. 2011). S. cerevisiae has a long history of use in bakery, wine fermentation and brewing of ale-type beers and has been intensively studied for well over a century (Gallone et al. 2016). In contrast, S. eubayanus was discovered only in 2011 (Libkind et al. 2011). First isolated in South America, S. eubayanus was subsequently isolated from oaks and other deciduous trees in North America, Asia and Oceania (Bing et al. 2014; Peris et al. 2014; Gayevskiy and Goddard 2016). Despite efforts of many European research groups, isolation of wild S. eubayanus strains has remained unsuccessful in Europe so far. While DNA from S. eubayanus was detected in samples from oak and spruce trees in Europe by ITS (Internal Transcribed Spacer) sequencing (Alsammar et al. 2018), this DNA does not prove the presence of wild S. eubayanus strains, as the DNA may also originate from hybrids such as S. pastorianus or Saccharomyces bayanus. Currently, Tibetan isolates of S. eubayanus have the highest degree of genetic identity to the S. eubayanus-derived genome sequences of S. pastorianus strains (Bing et al. 2014; Salazar et al. 2019). Based on this observation, trade along the Silk Road has been hypothesized to have enabled migration of S. eubayanus from Asia to the European birthplace of lager brewing (Bing et al. 2014). Alternatively, a now extinct or as yet undiscovered European S. eubayanus wild stock may be the ancestor of current S. pastorianus strains. In recent studies, hybrids between S. cerevisiae and S. eubayanus that were created in the laboratory were shown to outcompete their parental strains in lager-brewing related environments by combing the fermentative vigor of S. cerevisiae with the low temperature optimum of S. eubayanus (Hebly et al. 2015; Krogerus et al. 2015; Mertens et al. 2015). These observations are consistent with the emergence of S. pastorianus by spontaneous hybridization between an ale-brewing S. cerevisiae strain and a wild S. eubayanus contaminant, as well as with its subsequent dominance in lager-beer production. In this review, we will refer to lager yeast derived from spontaneous hybridization as S. pastorianus and to laboratory-made lager hybrids as S. cerevisiae × S. eubayanus.

In contrast to the genomes of laboratory-made hybrids, S. pastorianus genomes are extensively aneuploid, with 45 to 79 chromosomes instead of the allodiploid complement of 32 chromosomes (Fig. 1) (Dunn and Sherlock 2008; Nakao et al. 2009; Walther, Hesselbart and Wendland 2014; Van den Broek et al. 2015; Okuno et al. 2016). Based on genetic differences, two S. pastorianus subgroups were identified, Group 1 strains (‘Saaz’) and Group 2 strains (‘Frohberg’), which show marked differences in chromosome copy numbers (Liti et al. 2005; Dunn and Sherlock 2008). While both groups have an approximately diploid S. eubayanus chromosome complement, the S. cerevisiae chromosome complement is incomplete in Group 1 strains and diploid or higher in Group 2 strains (Fig. 1) (Dunn and Sherlock 2008; Van den Broek et al. 2015; Okuno et al. 2016). Genome-sequence comparison revealed group-specific genes, substantial differences in subtelomeric regions and different frequencies of synonymous nucleotide variations between both groups (Liti et al. 2005; Baker et al. 2015; Monerawela et al. 2015). While Group 1 strains display superior growth kinetics at low temperatures, they generally show limited maltotriose utilization, resulting in an overall inferior brewing performance relative to Group 2 strains (Gibson et al. 2013b).

EVOLUTIONARY HISTORY OF S. PASTORIANUS:

MULTIPLE HYBRIDIZATION EVENTS OR MAN-MADE POPULATION BOTTLENECKS?

Based on their phenotypic and genotypic differences, Group 1 and 2 strains were initially hypothesized to have emerged from two independent hybridization events (Fig. 2A) (Rainieri et al. 2006; Dunn and Sherlock 2008). Indeed, distinct haploid and diploid S. cerevisiae ancestors could explain the ploidy of Group 1 and 2 strains, respectively (Krogerus et al. 2016). However, identical recombinations between S. cerevisiae and S. eubayanus chromosomes were found at the ZU01, MAT, HSP82 and XRN1/KEM1 loci in all Group 1 and 2 strains (Hewitt et al. 2014; Walther, Hesselbart and Wendland 2014; Okuno et al. 2016). When evolved under lager-brewing conditions, laboratory-made S. cerevisiae × S. eubayanus hybrids acquired a diverse range of interchromosomal recombinations, but these did not include those present in S. pastorianus strains. While differences between the parental genomes of S. cerevisiae × S. eubayanus hybrids and S. pastorianus may affect likeness of individual recombinations, the diversity of recombinations obtained in individual S. cerevisiae × S. eubayanus isolates and the complete lack of recombinations shared with S. pastorianus indicate that recombination patterns emerge mostly serendipitously and point toward a common hybrid ancestry of all current S. pastorianus strains (Gorter de Vries et al. 2019b).

Two theories have been forwarded to reconcile the evidence for a common ancestry of Group 1 and Group 2 strains with their genetic differences (Fig. 2): (2B) Group 1 and 2 strains shared an initial hybridization event, with Group 2 strains resulting from a subsequent hybridization between the initial hybrid and a distinct S. cerevisiae strain, or (2C) Group 1 and 2 strains resulted from the same hybridization event involving a heterozygous S. cerevisiae ancestor, after which different paths of loss of heterozygosity and loss of genetic material caused the two Groups to diverge (Okuno et al. 2016). Long-read nanopore sequencing and comparative genome analysis indicated that the S. cerevisiae genetic material is highly similar in both groups, thereby
Figure 1. Estimated chromosome copy numbers in *S. pastorianus* strains as determined by whole-genome sequencing. Chromosome copy number estimates of various Group 1 (red) and Group 2 (blue) strains were estimated from short-read sequencing data published by Van den Broek et al. 2015 (circles) and Okuno et al. 2016 (squares) (Van den Broek et al. 2015; Okuno et al. 2016). For each strain, the estimated total number of chromosomes derived from *S. eubayanus* is plotted against the estimated total number of chromosomes derived from *S. cerevisiae*. Due to copy number differences within individual chromosomes, copy number estimates should be interpreted as indicative.

Figure 2. Theories formulated about the emergence of Group 1 and 2 *S. pastorianus* strains. (A) Emergence by two independent hybridizations (Dunn and Sherlock 2008). While both groups shared a similar *S. eubayanus* ancestor, Group 1 emerged from hybridization with a haploid *S. cerevisiae* while Group 2 emerged from a diploid *S. cerevisiae*. (B) Emergence by two successive hybridizations (Okuno et al. 2016). *S. pastorianus* emerged from an initial hybridization between a haploid *S. cerevisiae* and a diploid *S. eubayanus*. Group 1 strains evolved directly from this ancestor, while Group 2 strains emerged from a subsequent hybridization between the *S. pastorianus* ancestor and a haploid *S. cerevisiae* strain of different origin. (C) Emergence by a single hybridization followed by different evolutionary trajectories (Okuno et al. 2016; Salazar et al. 2019). *S. pastorianus* emerged from the hybridization of a heterozygous diploid *S. cerevisiae* strain and a mostly homozygous diploid *S. eubayanus* strain. Group 1 and 2 strains both evolved from this ancestor. However, Group 1 and Group 2 strains were affected differently by loss of heterozygosity and by loss of *S. cerevisiae* genome content. As a result, Group 2 strains are more heterozygous than Group 1 strains and their *S. cerevisiae* subgenomes differ despite common ancestry.
reducing the likelihood of multiple hybridization events (Salazar et al. 2019).

Domestication has been shown to stimulate rapid genetic adaptation and diversification in widely different genetic contexts (Arnold 2004; Bachmann et al. 2012; Gibbons et al. 2012; Gibbons and Rinker 2015; Gallone et al. 2016; Peter et al. 2018). In hybrids such as S. pastorianus, genetic plasticity is exacerbated by an increased incidence of (segmental) aneuploidy and loss of heterozygosity (Delneri et al. 2003; Pérez Trévés et al. 2014; Peris et al. 2017; Gorter de Vries et al. 2019b). Therefore, genetically divergent S. pastorianus populations likely emerged during the centuries of extensive subsequent batch cultivations across Europe. Due to the sterility of S. pastorianus, the absence of genetic admixture through sexual reproduction enabled genetic diversification even within yeast populations of individual breweries. However, the industry practice of replacing locally evolved brewing strains by strains from successful breweries, as illustrated by the Bavarian origin of the Carlsberg strain isolated by Hansen (Meusdoerffer 2009), is likely to have expanded successful populations at the expense of genetic diversity. Even narrower bottlenecks may have occurred when Hansen isolated the first Group 1 strain at Carlsberg in 1883 and Elion isolated the first pure Group 2 strain at Heineken in 1886 (Hansen 1883; Struyk 1928). These isolates likely spread as other European breweries increasingly implemented pure-culture brewing, thereby replacing previously used mixed starter cultures. Furthermore, in the 19th and early 20th centuries, small breweries commonly used yeast starter cultures sold by large breweries such as Carlsberg and Heineken, thereby further reducing the diversity of industrial strains (Mendlik 1937). Rather than reflecting different origins, the differences between Group 1 and 2 strains may therefore reflect genetic divergence during domestication, followed by severe population bottlenecks caused by anthropological selection (Fig. 2C).

**COMPLEXITY OF S. PASTORIANUS GENOMES**

S. pastorianus genomes are alloaneuploid, with varying, strain-dependent copy numbers of homologous and homeologous chromosomes. This chromosome copy number variation affects the phenotype due to two general mechanisms: (i) a general aneuploidy-associated stress response, encompassing growth defects, genetic instability and low sporulation efficiency, and (ii) chromosome-specific copy-number effects, resulting from the cumulative impact of copy number differences of individual genes harbored by the affected chromosomes (Gorter de Vries, Pronk and Daran 2017b). In S. pastorianus, genetic differences between the S. cerevisiae and S. eubayanus subgenomes present an additional degree of complexity (Fig. 3). During genome evolution, recombinations between both subgenomes can create new genetic complexity, for example by creating novel, hybrid open-reading frames (Fig. 3A) (Dunn et al. 2013; Hewitt et al. 2014; Brouwers et al. 2019b). Since gene complements of the two subgenomes differ (Salazar et al. 2017; Brickwedde et al. 2018), genes and gene products that do not occur together in either of the parental genomes can interact in hybrids to generate novel, difficult to predict phenotypes (Fig. 3B). For example, protein subunits encoded by different subgenomes can assemble into novel, chimeric protein complexes (Fig. 3C) (Piatkowska et al. 2013), while non-specificity of regulatory elements can cause cross-talk of transcriptional regulation networks (Fig. 3D) and of protein modification (Fig. 3E) (Tirosch et al. 2009; Vidgren and Gibson 2018). Moreover, functional differences between homeologous genes (Fig. 3F) (Yamagishi et al. 2010; Bolat et al. 2013), as well as gene dosage-effects (Fig. 3G) (Ogata, Kobayashi and Gibson 2013; Yao et al. 2013), can result in complex interactions. Expression levels of homologous genes generally differ, resulting in stronger expression of one of the two versions (Fig. 3H) (Gibson et al. 2013a; He et al. 2014). Overall, understanding the complex interactions between subgenomes is critical, as they underlie the synergistic phenomenon of heterosis (Lippman and Zamir 2007; Chen 2013; Shapira et al. 2014), which enables hybrids such as S. pastorianus to outperform their parental species (Belloch et al. 2008; Heiby et al. 2015; Krogerus et al. 2016). For example, in S. pastorianus, interaction between maltotriose transporter genes from the S. eubayanus subgenome and the MAL regulator genes from the S. cerevisiae subgenome was shown to enable the trait of maltotriose utilization, which is critical to brewing performance (Brouwers et al. 2019a). The importance of subgenome interactions is consistent with the frequent loss of heterozygosity during evolution of Saccharomyces hybrids, since it facilitates elimination of non-beneficial genome content from the least adapted parental species (Smukowski Heil et al. 2017; Lancaster et al. 2019; Gorter de Vries et al. 2019b; Heil et al. 2019). The presence of mitochondrial DNA descending from S. eubayanus and the loss of mitochondrial DNA from S. cerevisiae in S. pastorianus strains may also have been beneficial for S. pastorianus strains (Rainieri et al. 2008; Baker et al. 2015; Okuno et al. 2016). Indeed, the loss of S. cerevisiae mtDNA was likely instrumental in the lager-brewing domestication process, as its replacement by S. eubayanus mtDNA enables improved growth at low temperatures (Baker et al. 2019).

Elucidation of the genetic complexity of S. pastorianus strains was initially limited by the accuracy of available genome assemblies (Brickwedde et al. 2017). The first S. pastorianus genome was published in 2009 and consisted of 25 Mbp divided over 3184 contigs (Nakao et al. 2009). While many more strains were sequenced since, short-read sequencing invariably yielded incomplete and fragmented genome assemblies with, at best, hundreds of contigs (Walther, Hesselbart and Wendland 2014; Van den Broek et al. 2015; Okuno et al. 2016). Short-read sequencing cannot resolve repetitive sequences, such as TY-transposons and paralogous genes within each subgenome, or homeologous gene pairs (Kim et al. 1998; Mattheson, Parsons and Gammie 2017). As a result, subtelomeric regions, which are known hotspots of genetic plasticity and inter-strain diversity (Pryde, Huckle and Louis 1995; Liti et al. 2005; Brown, Murray and Verstrepen 2010; Bergström et al. 2014; Monerawela et al. 2015) and harbor many industrially-relevant genes (Teunissen and Steenmans 1995; Denayrolles et al. 1997; Teste, François and Parrou 2010; Jordan et al. 2016), were poorly assembled.

Recent developments in long-read sequencing enabled the generation of chromosome-level S. pastorianus genome assemblies that include most telomeres (Salazar et al. 2019). Saccharomyces genome assemblies based on long-read sequencing typically capture up to 5% more genes than high-quality short-read assemblies (Goodwin et al. 2015; Giordano et al. 2017; Istance et al. 2017; Salazar et al. 2017; Brickwedde et al. 2018; Salazar et al. 2019). Such added genes were of particular interest due to their role in brewing-relevant traits; such as FLO genes involved in the calcium-dependent flocculation process that causes bottom fermentation of S. pastorianus, MAL genes encoding maltose and maltotriose transporters and hydrolases, and HXT genes encoding the uptake of glucose and other hexose sugars (Salazar et al. 2019). Despite the near-complete assembly of all its chromosomes, the first long-read S. pastorianus genome assembly captured only 23 Mbp of the 46 Mbp genome of strain CBS 1483 because assembled chromosomes were consensus
sequences of all chromosomal copies, and intra-chromosomal variation of multi-copy chromosomes was not captured (Salazar et al. 2019). Nevertheless, alignment of short-read and long-read sequences allowed retrieval of sequence and structural heterozygosity (Okuno et al. 2016; Salazar et al. 2017; Salazar et al. 2019).

**IMPROVEMENT STRATEGIES FOR LAGER-BREWING STRAINS**

Industrial strain improvement typically relies on five pillars: exploration of existing diversity, mating, laboratory evolution, mutagenesis and selection and genome editing (Pattnaik 2008; Steensels et al. 2014b). The complex genetics of *S. pastorianus* and, in particular, the lack of customer acceptance of genetic modification have restricted genetic modification for strain improvement of brewing yeasts (Gibson et al. 2017); therefore, development and potential of genetic modification are discussed in a separate section.

Compared to ale brewing *S. cerevisiae* strains, the genetic and phenotypic diversity of *S. pastorianus* is limited (Dunn and Sherlock 2008; Gibson et al. 2013b; Steensels et al. 2014a; Gallone et al. 2016; Okuno et al. 2016; Salazar et al. 2019). While diversity has been successfully expanded by crossing spores of an *S. pastorianus* strain with *S. cerevisiae* (Bilinski and Casey 1989; Sanchez, Solodovnikova and Wendland 2012), mating strategies are constrained by the low sporulation efficiency of alloaneuploid *S. pastorianus* strains (Gjermansen and Sigsgaard 1981; Liti, Barton and Louis 2006; Ogata et al. 2011; Santaguida and Amon 2015). As illustrated by the mating of a non-sporulating allopolyploid *S. bayanus* strain with beer-brewing *S. cerevisiae* strains (Sato et al. 2002), low sporulation efficiencies could be circumvented by using rare mating based on spontaneous or induced mating-type switching (Gunge and Nakatomi 1972; Alexander et al. 2016). Although labor- and time-intensive, non-sexual crossing methods such as spheroplast fusion can also be applied (Barney, Jansen and Helbert 1980).

The low mating efficiency of existing *S. pastorianus* strains was circumvented by mating different *Saccharomyces* species in
the laboratory to obtain novel S. pastorianus-like lager-brewing strains (Hebly et al. 2015; Krogerus et al. 2015). In addition to sharing the hybrid vigor of S. pastorianus, laboratory-made S. cerevisiae × S. eubayanus hybrids displayed phenotypic diversity depending on their ploidy and on the genetic background of parental strains (Mertens et al. 2015; Krogerus et al. 2016). Moreover, hybrids of S. cerevisiae with other cold-tolerant Saccharomyces species such as S. arboricola, S. mikatae and S. uvarum displayed similar fermentation performance at low temperature as S. pastorianus (Goncalves et al. 2011; Nikulin, Krogerus and Gibson 2018). Laboratory hybrids are typically made by crossing strains with complementary selectable phenotypes and selecting hybrid cells which combined both phenotypes. In some cases, natural traits of the parental strains, such as growth at low temperature or the ability to utilize melibiose, can be used as selectable phenotypes (Sato et al. 2002). In the absence of such pre-existing selectable phenotypes, selectable genotypes can be introduced prior to mating. For example, uracil auxotrophy can be selected by growth in the presence of 5-fluorooorotic acid, lysine auxotrophy can be selected by growth in the presence of α-aminoadipate and respiratory-deficient strains can be obtained by growth in the presence of ethidium analogues (Chattoo et al. 1979; Fukunaga et al. 1980; Boeke et al. 1987). After crossing strains with different auxotrophies or deficiencies, hybrids can be isolated by selection on appropriate media (Krogerus et al. 2016; Magalhaes et al. 2017; Krogerus, Holmström and Gibson 2018). Alternatively, selectable phenotypes may be introduced using genome editing, for example by introducing genes conferring antibiotic resistance (Jimenez and Davies 1980; Gritz and Davies 1983; Goldstein and McCusker 1999). By combining an uncommon auxotrophy and an introduced antibiotic resistance gene in one parental strain, it can be crossed with a large array of other strains without requiring any additional pre-existing or introduced selectable phenotypes (Hebly et al. 2015), however GM status of such strains complicates industrial application.

The requirement for phenotypic and genetic markers can be completely circumvented by staining parental strains with fluorescent dyes prior to mating and, subsequently, sorting double-stained cells using fluorescence-activated cell sorting. Indeed, a recent study shows how hybrids could be obtained with this method without the use of any selectable phenotype (Gorter de Vries et al. 2019a). Such laboratory hybrids generally display increased evolvability, which can be beneficial for strain improvement, as illustrated by faster and superior evolution of ethanol tolerance in hybrids during laboratory evolution under high-ethanol conditions (Krogerus, Holmström and Gibson 2018). Despite their increased plasticity, cultivation of laboratory-made S. cerevisiae × eubayanus hybrids under lager-brewing conditions during >100 repeated batches demonstrated that genetic instability was far more limited than it is in S. pastorianus and that phenotypic deterioration only occurred after far more brewing cycles than are customary in the lager-brewing industry (Gorter de Vries et al. 2019b). Overall, laboratory-made hybrids show high potential for brewing applications (Krogerus et al. 2017).

Both S. pastorianus strains and laboratory-made lager-brewing hybrids can be further improved by laboratory evolution and/or mutagenesis and selection (Table 1). Generation of novel phenotypes can occur by spontaneous acquisition of mutations during growth. Alternatively, the mutation frequency can be increased by mutagenesis using irradiation (such as ultraviolet light) or by exposure to mutagenic compounds (such as ethyl methanesulfonate (EMS), methyl benzimidazole-2-ylcarbamate (MBC), N-methyl-N’-nitro-N-nitroso-guanidine (MNNG)). Mutants of interest can be isolated by screening for desirable phenotypes, or by growth under conditions that confer a selective benefit to mutants with a desirable phenotype. When growth under conditions favoring desired phenotypes is not only applied to select pre-existing mutants, but also to generate new mutants in the process, it is designated as laboratory evolution. This strategy has been successfully applied to select for lager-brewing-relevant phenotypes of Saccharomyces strains, including superior fermentation in ‘high gravity’ processes, increased ethanol tolerance, improved sugar utilization, increased performance under nutrient limitation, altered flocculation behavior and altered flavor profiles (Table 1). For an overview of relevant taste compounds in beer brewing and of relevant phenotypic properties of brewing yeast, we refer to recent reviews (Lodolo et al. 2008; Holt et al. 2019). Readers should keep in mind that strain improvement methods developed by commercial brewers are rarely published; therefore, the list in Table 1 is not exhaustive.

GENOME-EDITING TECHNIQUES IN S. PASTORIANUS AND THEIR POTENTIAL FOR INDUSTRIAL APPLICATION

Compared to the plethora of genome-editing techniques (also referred to as gene-, genetic- or genome engineering) in S. cerevisiae (DiCarlo et al. 2013; Nielsen et al. 2013; Jakočiūnas, Jensen and Keasling 2016; Nielsen and Keasling 2016), there are only very few accounts of targeted genome editing using cassette integration in S. pastorianus (Vidgren et al. 2009; Duong et al. 2011; Murakami et al. 2012; Bolat et al. 2013; Gorter de Vries et al. 2017a), supposedly due to limited homologous recombination efficiency (Gorter de Vries et al. 2017a). Even simple gene deletion studies were, until recently, complicated by the presence of several gene copies, which required repeated rounds of cassette insertion and marker removal. Instead, functional characterization often relied on expressing S. pastorianus genes in S. cerevisiae strains (Kobayashi et al. 1998; Yoshimoto et al. 1998; Kodama, Omura and Ashikari 2001; Salema-Oom et al. 2005; Bolat et al. 2013). While introduction of a double-strand break can drastically increase genome editing efficiency (Pâques and Haber 1999), Cas9 genome editing tools developed for S. cerevisiae were not immediately applicable in S. pastorianus strains (DiCarlo et al. 2013; Mans et al. 2015; Gorter de Vries et al. 2017a). However, polymerase-II-based expression of gRNAs flanked by self-cleaving ribozymes was successful in S. pastorianus, in laboratory-made S. cerevisiae × S. eubayanus hybrids and in both parental species (Gorter de Vries et al. 2017a; Brickwedde et al. 2018; Gorter de Vries et al. 2019b). While application of genetic modification (GM) to generate industrial strains is limited by customer acceptance issues (Akada 2002), non-GM strain improvement can also benefit from the prior application of efficient gene-editing techniques. The single-step deletion of all 9 copies of the ATF1 and ATF2 genes in S. pastorianus illustrated the potential of Cas9 to facilitate functional characterization by enabling fast and complete gene deletion (Gorter de Vries et al. 2017a). Furthermore, genome editing can be used to evaluate the desirability of mutations prior to the use of laborious non-GM techniques, as illustrated by the deletion of FDC1 and PADI1 genes in S. eubayanus prior to mutagenesis to obtain non-GM strains with low phenolic off-flavors (Didierich et al. 2018). In addition, when a phenotypic improvement is achieved through non-GM strain improvement methods such as laboratory evolution or mutagenesis, Cas9 can facilitate elucidation of the causal
Table 1. Non-GM mutagenesis, selection and/or laboratory evolution methods that resulted in lager-brewing-relevant phenotypic changes in Saccharomyces strains. For each method, the used Saccharomyces species, applied mutagenesis methods, applied selection and/or laboratory methods, and the selected phenotype are indicated. For mutagenesis methods, ultraviolet radiation (UV), ethyl methanesulfonate (EMS), methyl benzimidazole-2-ylcarbamate (MBC), N-methyl-N’-nitro-N-nitroso-guanidine (MNNG) are distinguished. RBS denotes the use of a repeated batch setup.

| Application | Strain | Mutagenesis | Selection and/or laboratory evolution | Selected phenotype | Reference |
|-------------|--------|-------------|---------------------------------------|--------------------|-----------|
| Substrate utilization | S. cerevisiae | MNNG mutagenesis | Differential staining with Triphenyltetrazolium chloride | Crabtree-negative mutants | (Böker-Schmitt, Francisci and Schweyen 1982) |
| | S. cerevisiae | – | Growth on solid medium with 2-deoxyglucose | Loss of glucose repression | (Jones, Russell and Stewart 1986) |
| | S. cerevisiae | – | Growth on solid medium with glucosamine | Loss of glucose repression | (Hockey and Freeman 1980) |
| | S. eubayanus | – | RBS cultivations on synthetic medium with maltose and traces of glucose | Maltose utilization | (Baker and Hittinger 2018) |
| | S. pastorianus | – | Chemostat cultivation on maltotriose enriched mock-wort | Maltotriose utilization | (Brickwedde et al. 2017) |
| | S. eubayanus | – | RBS cultivations on synthetic medium with maltotriose and traces of glucose | Maltotriose utilization | (Baker and Hittinger 2018) |
| | S. eubayanus | UV mutagenesis | RBS cultivations on synthetic medium with maltotriose and chemostat cultivation on maltotriose-enriched wort | Maltotriose utilization | (Brouwers et al. 2019b) |
| | S. cerevisiae × S. uvarum | – | Chemostat cultivation under ammonium limitation | Increased fitness under nitrogen limitation | (Dunn et al. 2013) |
| | S. cerevisiae | – | Chemostat cultivation under nitrogen limitation | Increased fitness under nitrogen limitation | (Hong and Gresham 2014) |
| | S. cerevisiae × S. uvarum | – | Chemostat cultivation under carbon-, phosphate- and sulfate limitation | Increased fitness under nutrient limitation | (Smukowski Heil et al. 2017) |
| | S. cerevisiae | – | Chemostat cultivation under carbon-, phosphate- and sulfate limitation | Increased fitness under nutrient limitation | (Gresham et al. 2008) |
| Industrial performance | S. pastorianus | UV mutagenesis | RBS cultivations on high-gravity wort | High gravity fermentation | (Blieck et al. 2007) |
| | S. pastorianus | EMS mutagenesis | Fed-batch cultivation on high-gravity wort | High gravity fermentation | (Huuskonen et al. 2010) |
| | S. pastorianus | UV and EMS mutagenesis | Growth on solid medium with high ethanol concentrations | High gravity fermentation | (Yu et al. 2012) |
| | S. cerevisiae × S. eubayanus | – | RBS cultivation with high ethanol concentrations | High gravity fermentation | (Krogerus, Holmström and Gibson 2018) |
| | S. cerevisiae | MBC mutagenesis | Batch cultivation in high gravity medium in the presence of ethanol | High gravity fermentation | (Zheng et al. 2014) |
| Application | Strain      | Mutagenesis | Selection and/or laboratory evolution                                                                 | Selected phenotype                                                                                                               | Reference                        |
|-------------|-------------|-------------|--------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|----------------------------------|
|             | *S. cerevisiae* | –           | Turbidostat cultivation with increasing ethanol concentrations                                          | Increased ethanol tolerance                                                                                                     | (Voordeckers et al. 2015)        |
|             | *S. cerevisiae* | –           | RBS cultivations with increasing ethanol concentrations                                                 | Increased ethanol tolerance                                                                                                     | (Dinh et al. 2008)              |
|             | *S. cerevisiae* | EMS mutagenesis | Turbidostat cultivation with increasing ethanol concentrations                                          | Increased ethanol tolerance                                                                                                     | (Stanley et al. 2010)            |
|             | *S. uvarum* | EMS mutagenesis | Turbidostat cultivation with increasing ethanol concentrations                                          | Increased ethanol tolerance                                                                                                     | (Brown and Oliver 1982)          |
|             | *S. cerevisiae* | –           | Batch cultivations with intermittent exposure to 0.3-4.4 M of H$_2$O$_2$, 52 °C, 20-55 % ethanol and freeze/thawing cycles | Increased tolerance to oxidative-, temperature-, ethanol- and freezing-thawing stress                                           | (Çakar et al. 2005)             |
| *S. pastorianus* | EMS mutagenesis | –           | Repeated heat shocks at 55 °C                                                                             | Increased heat shock tolerance                                                                                                  | (James et al. 2008)              |
| *S. cerevisiae* | UV mutagenesis | –           | Subjection to 200 freeze-thaw cycles                                                                      | Increased freeze tolerance                                                                                                     | (Teunissen et al. 2002)          |
| *S. cerevisiae × S. eubayanus* | – | RBS cultivations on wort |                                                                                                              | Increased flocculation                                                                                                          | (Gorter de Vries et al. 2019b)    |
| *S. cerevisiae* | – | Chemostat cultivation | Increased flocculation                                                                                       | (Hope et al. 2017)                                                                                                              |                                  |
| *S. cerevisiae* | MNNG mutagenesis | – | RBS cultivation enriching for slow-sedimenting cells                                                      | Loss of flocculation                                                                                                            | (Holmberg and Kießling-Brandt 1978) |
| *S. pastorianus* | – | Batch cultivation in the presence of Ethydium Bromide                                                    | Loss of respiratory capacity                                                                                                    | (Holmberg and Kießling-Brandt 1978) |
| *S. cerevisiae × S. uvarum* | – | Chemostat cultivation at 15 °C                                                                            | Increased growth at low temperatures                                                                                             | (Heil et al. 2019)               |
| *S. cerevisiae* | – | RBS cultivation with sulfate                                                                              | Increased glycerol production                                                                                                   | (Kutyna et al. 2012)             |
| *S. cerevisiae* | – | Batch cultivation with S-methyl-L-cysteine                                                                | Increased thiol production                                                                                                      | (Belda et al. 2016)             |
| *S. cerevisiae* | UV mutagenesis | – | Screening for lack of coloration on lead plates                                                            | Decreased H$_2$S production, increased SO$_2$ production                                                                         | (Chen et al. 2012)              |
| *S. pastorianus* | – | Growth on solid medium with ethionine, screening for coloration on lead plates                            | Increased SO$_2$ production                                                                                                      | (Yoshida et al. 2008)           |
| *S. cerevisiae* | UV mutagenesis | – | Growth on solid medium with cadmium                                                                       | Increased glutathione production                                                                                                 | (Chen et al. 2012)              |
| *S. pastorianus* | UV mutagenesis | – | Growth on solid medium with disulfiram                                                                     | Decreased acetaldehyde production                                                                                               | (Shen et al. 2014)              |
| *S. pastorianus* | EMS mutagenesis | – | RBS cultivation in the presence of chlorsulfuron                                                           | Decreased diacetyl production                                                                                                   | (Gibson et al. 2018)            |
| *S. eubayanus* | UV mutagenesis | – | Screening for insensitivity to cinnamic acid                                                              | Decreased 4-vinyl guaiacol production                                                                                             | (Diderich et al. 2018)          |
| Application | Strain | Mutagenesis | Selection and/or laboratory evolution | Selected phenotype | Reference |
|-------------|--------|-------------|----------------------------------------|--------------------|-----------|
| Flavor       | S. cerevisiae | UV mutagenesis | Growth on solid medium with cerulenin | Increased fatty-acid synthesis | (de Araújo Vicente et al. 2006) |
| modulation | S. pastorianus | – | Growth on solid medium with 5,5,5-trifluoro-DL-leucine | Increased isoamyl alcohol and isoamyl acetate production | (Strejc et al. 2013) |
| | S. cerevisiae | EMS mutagenesis | Growth on solid medium with isoamyl monochloroacetate | Increased isoamyl acetate production | (Watanabe, Nagai and Kondo 1995) |
| | S. cerevisiae | – | RBS cultivation in the presence of 1-famesilypridinium | Increased isoamyl acetate production | (Hirooka et al. 2005) |
| | S. cerevisiae | EMS mutagenesis | Growth on solid medium with econazole | Increased isoamyl acetate production | (Asano et al. 1999) |
| | S. cerevisiae | – | Batch cultivation in the presence of 8 mM Cu²⁺ | Increased isoamyl acetate production | (Hirooka et al. 2010) |
| | S. cerevisiae | EMS mutagenesis | Growth on solid medium with isoamyl monofluoracetate | Increased isoamyl acetate production | (Watanabe et al. 1993) |
| | S. uvarum | – | Growth on solid medium with 5,5,5-, trifluoro-DL-leucine and fluoro-dl-phenylalanine | Increased isoamyl acetate and phenylethyl acetate | (Lee, Villa and Patino 1995) |
| | S. cerevisiae | – | Growth on solid medium with p-Fluoro-DL-phenylalanine | Increased β-phenethyl alcohol and β-phenethyl acetate production | (Fukuda et al. 1991) |
| | S. pastorianus | MNNG mutagenesis | Growth on solid medium with thiaisoleucine | Increased 2-methyl-1-butanol production | (Kielland-Brandt, Petersen and Mikkelsen 1979) |
mutations by enabling rapid reverse engineering (Gorter de Vries et al. 2019b).

Regardless of recent advances in genetic accessibility, the lager-brewing industry does not currently use GM yeast for lager beer brewing. Many countries and trade blocks, including important beer markets such as the EU and the USA, tightly regulate use of GM technology in the food and beverages industry (Sprink et al. 2016). Historically, regulation was technology based: methods to modify genomes by non-targeted methods such as UV mutagenesis and chemical mutagenesis were not regulated, while any mutation introduced by targeted genetic engineering was subject to specific legislation (Nevoigt 2008). Recently, regulation appeared to be moving toward product- and risk-based evaluation, in which the type of mutation introduced determines regulatory status (Conko et al. 2016; Sprink et al. 2016). For example, Japan regulates genetic engineering less strictly when no foreign DNA is introduced (‘self-cloning’). Similarly, in the USA, GM foods which only harbor single-nucleotide changes that might also have arisen after non-targeted mutagenesis, have been introduced into the market (Hino 2002; Ledford 2016; Waltz 2016). However, similar developments toward product- and risk-based regulation were recently blocked by legislative courts in the European Union. As a consequence, updating the GM regulations in the EU will now require a considerable political process (Erikkson et al. 2018).

Since, in the EU, food products only need to be labeled and regulated as GM if they contain >0.9% GM biomass, removal of GM yeast by filtration could, in principle, obviate the need for labeling the resulting beer as a GM product (Pérez-Torrado, Querol and Guillamón 2015). Moreover, already in 1990, a lager-brewing strain engineered for dextrin utilization was approved and used to brew a low-caloric beer in the UK (Hammond 1995; Akada 2002). As illustrated by the commercial failure of this GM beer, the application of GM yeasts for beer brewing is precluded primarily by customer acceptance—or by producers’ concerns about consumer acceptance—rather than by insurmountable regulatory hurdles (Ishii and Araki 2016). However, recent regulatory developments have resulted in successful commercialization of foods based on targeted genetic modification, particularly on the US market (Waltz 2016; Ishii and Araki 2017). Moreover, Lallemand (Montreal, Canada) is currently concluding trials with a brewing yeast engineered to produce lactic acid, called Sourvisiae (Rice 2019).

Despite the current absence of large-scale industrial application, many possible genetic engineering strategies for lager-brewing yeasts are available, based on insights gained from laboratory studies and from analysis of strains obtained by classical strain improvement. Such strategies could rapidly and efficiently improve a vast array of yeast characteristics, including substrate utilization, general brewing performance and energy requirements for cooling, off-flavor and flavor profiles and, moreover, enable the introduction of novel flavors (Table 2). The relatively permissive legislation and relatively high consumer acceptance in countries such as Brazil, USA, Japan and Argentina may enable industrial application of GM yeast for lager beer brewing in the near future (Mertens et al. 2019).

OUTLOOK

Recent progress in genome sequencing and genome editing technologies has yielded chromosome-level genome assemblies and improved our understanding of the complex hybrid genomes of S. pastorianus. Ongoing developments in chromosome copy haplotyping and emerging assembly algorithms for haplotype phasing will further clarify the role of aneuploidy and heterozygosity in such genomes (Chin et al. 2016; He et al. 2018; Wenger et al. 2019). Furthermore, analogous to recent developments in S. cerevisiae and S. eubayanus, chromosome-level reference genomes will contribute to improved understanding of the complexity and plasticity of S. pastorianus genomes, and to simplifying and accelerating strain improvement strategies by mutagenesis and selection and/or laboratory evolution (Brickwedde et al. 2018; Mans, Daran and Pronk 2018; Brouwers et al. 2019b; Gorter de Vries et al. 2019b).

While the genetic diversity of S. pastorianus is limited by its reproductive isolation and, probably, by population bottlenecks during domestication, non-GM methods for the generation of interspecies hybrids can create new opportunities to expand the diversity of lager-brewing strains (Mallet 2007; Mertens et al. 2015; Gallone et al. 2016; Nikulin, Krogerus and Gibson 2018; Salazar et al. 2019). Moreover, the emergence of Cas9 genome editing tools compatible with S. pastorianus enables the use of high-quality genome assemblies for functional characterization of genes (Gorter de Vries et al. 2017a), determination of targets for non-GM techniques (Diderich et al. 2018) and reverse engineering after non-GM strain improvement methods (Gorter de Vries et al. 2019b). Current developments in GM regulation outside the EU may lead to the direct applicability of genetically engineered strains, particularly when no heterologous DNA is introduced (Waltz 2016; Ishii and Araki 2017). Consolidations in the brewing industry during the 20th century have transformed brewing companies into international conglomerates with broad portfolios of beer brands (Poelmans and Swinnen 2011b; Howard 2014). Such conglomerates are unlikely to adopt GM yeasts for brewing, as customer acceptance backlash may not be restricted to a specific beer brand or customer market, but could result in decreasing sales of their entire brand portfolio over all markets. However, the 21st century saw a revitalization of the declining beer market, resulting in the emergence of many small new breweries, commonly referred to as craft and micro-breweries (Carroll and Swaminathan 2000; Ellis and Bosworth 2015). Due to their small volumes and the presence of numerous competing beer brands, microbreweries generally strive toward clearly defined product identity to target highly specific customer segments (Thurnell-Read 2014; Maier 2016). GM-technology could be used to obtain characteristics which are popular in the microbrewery customer market, such as environmental sustainability and product uniqueness (Williams and Mekonen 2014; Carr 2017). For example, the use of GM yeast without diacetyl production could reduce the energy requirements of lager brewing by alleviating the need for lagering, which typically requires cooling during time periods of about two weeks (Duong et al. 2011). Similarly, introduction of genes for the production of hop flavors, could strongly reduce water, land and energy usage for hop production (Denby et al. 2018). The introduction of genes for the production of novel flavor compounds can generate novel products clearly distinct from other brands (Hansen et al. 2009), and fits into the recent commercial success of beers with fruity flavor additives, such as Radler or Shandy, which consist of beer mixed with non-alcoholic fruit-flavored beverages (Paixão 2015). While GM microbreweries could theoretically target progressive market segments with high GM acceptance specifically, technological and financial hurdles to generate and implement genetically modified yeast have been prohibitive. However, the development of efficient gene-editing tools has considerably lowered such hurdles and popularized genome editing, as illustrated in the extreme by the biohacking movement (Bennett et al. 2009; Yetisen 2018).
Table 2. Genetic engineering strategies that were successfully applied in Saccharomyces yeasts with potential application for the lager-brewing industry.

| Application                     | Modification                          | Phenotype                                           | Organism    | Reference                      |
|---------------------------------|---------------------------------------|-----------------------------------------------------|-------------|--------------------------------|
| Substrate utilization           | AGT1 overexpression                   | Increased maltose and maltotriose utilization       | S. pastorianus | (Vidgren et al. 2009)          |
|                                 | Heterologous gene expression          | Increased β-glucan degradation                      | S. pastorianus | (Penttilä et al. 1987)         |
|                                 | Heterologous gene expression          | Increased dextrin utilization                       | S. pastorianus | (Cole et al. 1988; Perry and Meaden 1988; Sakai et al. 1989) |
|                                 | PUT4 overexpression                   | Increased proline assimilation                      | S. pastorianus | (Omura et al. 2005)            |
|                                 | FLO1, FLOS or FLO11 overexpression    | Increased flocculation                              | S. cerevisiae | (Govender et al. 2008)         |
|                                 | Stationary-phase FLO1 overexpression  | Stationary-phase flocculation                       | S. pastorianus | (Verstrepen et al. 2001)       |
|                                 | PEP4 disruption                       | Improved foam stability                             | S. cerevisiae | (Liu et al. 2009)              |
|                                 | LEU1 overexpression                   | Improved high gravity fermentation                  | S. pastorianus | (Blick et al. 2007)            |
|                                 | FKS1 disruption                       | Improved anti-staining of beer due to reduced yeast autolysis | S. pastorianus | (Wang et al. 2014)             |
|                                 | MET10 disruption                      | Increased SO₂                                        | S. pastorianus | (Hansen and Kielland-Brandt 1996) |
|                                 | MET14 and SSU1 overexpression         | Increased SO₂                                        | S. cerevisiae | (Donalies and Stahl 2002)      |
|                                 | HOM3 overexpression, SKP2 disruption  | Increased SO₂ and decreased H₂S production         | S. pastorianus | (Yoshida et al. 2008)          |
|                                 | CYS4 overexpression                   | Decreased H₂S production                            | S. cerevisiae | (Tezuka et al. 1992)           |
|                                 | NHS5 overexpression                   | Decreased H₂S production                            | S. pastorianus | (Tezuka et al. 1992)           |
|                                 | MXR1 disruption                       | Decreased dimethylsulfide production               | S. cerevisiae | (Hansen 1999)                  |
|                                 | ILV5 overexpression                   | Decreased diacetyl production                      | S. cerevisiae | (Omura 2008)                   |
|                                 | ILV6 disruption                       | Decreased diacetyl production                      | S. pastorianus | (Duong et al. 2011)            |
|                                 | Heterologous gene expression          | Increased diacetyl degradation                     | S. pastorianus | (Sone et al. 1988; Fujii et al. 1990; Blomqvist et al. 1991; Yamano et al. 1994a; Yamano, Tanaka and Inoue 1994b) |
|                                 | FDC1 disruption                       | Decreased 4-vinyl guaiacol production              | S. pastorianus | (Mertens et al. 2019)          |
|                                 | LEU4 overexpression                   | Increased isoamyl acetate production                | S. cerevisiae | (Hirata et al. 1992)           |
|                                 | ATF1 and ATF2 overexpression          | Increased acetate ester production                  | S. pastorianus | (Verstrepen et al. 2003)       |
|                                 | ATF1 and ATF2 disruption              | Decreased acetate ester production                  | S. pastorianus | (Verstrepen et al. 2003)       |
|                                 | ALD3 disruption, AR09 and AR010 overexpression | Increased 2-phenylethanol production                        | S. cerevisiae | (Kim, Cho and Hahn 2014)       |
|                                 | Heterologous gene expression          | Increased ethyl hexanoate production                | S. cerevisiae | (Han et al. 2009)              |
|                                 | Heterologous gene expression          | Hop monoterpenes                                    | S. cerevisiae | (Denby et al. 2018)            |
|                                 | Heterologous gene expression          | Hop lupulone production                             | S. cerevisiae | (Guo et al. 2019)              |
|                                 | Heterologous gene expression          | β-ionone production                                 | S. cerevisiae | (Beekwilder et al. 2014)       |
|                                 | Heterologous gene expression          | Vanillin production                                 | S. cerevisiae | (Hansen et al. 2009; Brochado et al. 2010) |
|                                 | Heterologous gene expression          | Valencene production                                | S. cerevisiae | (Asadollahi et al. 2008)       |
|                                 | Heterologous gene expression          | Nootkatone production                               | S. cerevisiae | (Gavira et al. 2013)           |
|                                 | Heterologous gene expression          | Raspberry ketone production                         | S. cerevisiae | (Beekwilder et al. 2007; Lee et al. 2016) |
Overall, ongoing developments in genome sequencing, genome editing and interspecies hybridization methods are giving a new impulse to lager yeast strain improvement, and are likely to shape the lager beer market in the coming years.

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REFERENCES

Akada R. Genetically modified industrial yeast ready for application. J Biosci Bioeng 2002;94:536–44.
Alexander WC, Peris D, Pfannenstiel BT et al. Efficient engineering of marker-free synthetic allotetraploids of Saccharomyces. Fungal Genet Biol 2016;89:10–7.
Alsammar HF, Naseeb S, Brancia LB et al. Targeted metagenomics approach to capture the biodiversity of Saccharomyces genus in wild environments. Environ Microbiol Rep 2018;11:206–14.
Appel SK. Artificial refrigeration and the architecture of 19th-century American breweries. IA J Soc Indust Archol 1990;16:21–38.
Arnold ML. Natural hybridization and the evolution of domesticated, pest and disease organisms. Mol Ecol 2004;13:997–1007.
Asadollahi MA, Maury J, Möller K et al. Production of plant sesquiterpenes in Saccharomyces cerevisiae: effect of ERG9 repression on sesquiterpene biosynthesis. Biotechnol Bioeng 2008;99:666–77.
Asano T, Inoue T, Kurose N et al. Improvement of isoamyl acetate productivity in sake yeast by isolating mutants resistant to econazole. J Biosci Bioeng 1999;87:69–79.
Bachmann H, Starrenburg MJ, Molenaar D et al. Microbial domestication signatures of Lactococcus lactis can be reproduced by experimental evolution. Genome Res 2012;22:115–24.
Baker E, Wang B, Bellora N et al. The genome sequence of Saccharomyces eubayanus and the domestication of lager-brewing yeasts. Mol Biol Evol 2015;32:2818–31.
Baker EP, Hittinger CT. Evolution of a novel chimeric maltotriose transporter in Saccharomyces eubayanus from parent proteins unable to perform this function. PLoS Genet 2018;15:e1007786.
Baker EP, Peris D, Moriarty RV et al. Mitochondrial DNA and temperature tolerance in lager yeasts. Sci Adv 2019;5:eaaav1869.
Barney M, Jansen G, Helbert J. Use of spheroplast fusion and genetic transformation to introduce dextrin utilization into Saccharomyces uvarum. J Am Soc Brew Chem 1980;38:1–5.
Beekwilder J, van Der Meer IM, Sibbesen O et al. Microbial production of natural raspberry ketone. Biotechnol J 2007;2:1270-9.
Beekwilder J, van Rossum HM, Koopman F et al. Polycistronic expression of a β-carotene biosynthetic pathway in Saccharomyces cerevisiae coupled to β-ionone production. J Biotechnol 2014;192:383–92.
Belda I, Ruiz J, Navascués E et al. Improvement of aromatic thiol release through the selection of yeasts with increased β-lyase activity. Int J Food Microbiol 2016;225:1–8.
Belloch C, Orlic S, Barrio E et al. Fermentative stress adaptation of hybrids within the Saccharomyces sensu stricto complex. Int J Food Microbiol 2008;122:188–95.
Bennett G, Gilman N, Stavrianakis A et al. From synthetic biology to biohacking: are we prepared? Nat Biotechnol 2009;27:1109.
Bergström A, Simpson JT, Salinas F et al. A high-definition view of functional genetic variation from natural yeast genomes. Mol Biol Evol 2014;31:872–88.
Bilinski CA, Casey GP. Developments in sporulation and breeding of brewer’s yeast. Yeast 1989;5:429–38.
Bing J, Han P-J, Liu W-Q et al. Evidence for a Far East Asian origin of lager yeast. Curr Biol 2014;24:R380–1.
Bleich L, Toye G, Dumortier F et al. Isolation and characterization of brewer’s yeast variants with improved fermentation performance under high-gravity conditions. Appl Environ Microb 2007;73:815–24.
Blomqvist K, Suihko M-L, Knowles J et al. Chromosomal integration and expression of two bacterial α-acetolactate decarboxylase genes in brewer’s yeast. Appl Environ Microb 1991;57:2796–803.
Boeke JD, Trueheart J, Natsoulis G et al. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. Methods Enzymol 1987;154:164–75.
Böker-Schmitt E, Francischi S, Schwreyen R. Mutations releasing mitochondrial biogenesis from glucose repression in Saccharomyces cerevisiae. J Bacteriol 1982;151:303–10.
Bolat I, Romagnoli G, Zhu F et al. Functional analysis and transcriptional regulation of two orthologs of ARO10, encoding broad-substrate-specificity 2-oxo-acid decarboxylases, in the brewing yeast Saccharomyces pastorianus CBS1483. FEMS Yeast Res 2013;13:505–17.
Brickwede A, Brouwers N, van den Broek M et al. Structural, physiological and regulatory analysis of maltose transporter genes in Saccharomyces eubayanus CBS 12357T. Front Microbiol 2018;9:1786.
Brickwede A, van den Broek M, Geertman J-MA et al. Evolutionary engineering in chemostat cultures for improved maltotriose fermentation kinetics in Saccharomyces pastorianus lager brewing yeast. Front Microbiol 2017;8:1690.
Brochado AR, Matos C, Möller BL et al. Improved vanillin production in baker’s yeast through in silico design. Microb Cell Fact 2010;9:84.
Brouwers N, Brickwede AGdV, Arthur R et al. The genome sequences of Himalayan Saccharomyces eubayanus revealed genetic markers explaining heterotic maltotriose consumption by hybrid Saccharomyces pastorianus. Appl Environ Microb 2019a;EEM:01516–19.
Brouwers N, Gorter de Vries AR, van den Broek M et al. In vivo recombination of Saccharomyces eubayanus maltose-transporter genes yields a chimeric transporter that enables maltotriose fermentation. PLoS Genet 2019b;15:e1007853.
Brown CA, Murray AW, Verstrepen KJ. Rapid expansion and functional divergence of subtelomeric gene families in yeasts. Curr Biol 2010;20:895–903.
Brown S, Oliver S. Isolation of ethanol-tolerant mutants of yeast by continuous selection. Appl Microbiol Biotechnol 1982;16:119–22.
Çakar ZP, Seker OU, Tamerler C et al. Evolutionary engineering of multiple-stress resistant Saccharomyces cerevisiae. FEMS Yeast Res 2005;5:569–78.

Carr AM. Microbrewery consumer behavior. Ph.D. Thesis. University of Alabama Libraries 2017.

Carroll GR, Swaminathan A. Why the microbrewery movement? Organizational dynamics of resource partitioning in the US brewing industry. Am J Socio 2000;106:715–62.

Chattoo BB, Sherman F, Azubalis DA et al. Selection of his2 mutants of the yeast Saccharomyces cerevisiae by the utilization of a-aminoadipate. Genetics 1979;93:51–69.

Chen Y, Yang X, Zhang S et al. Development of Saccharomyces cerevisiae producing higher levels of sulfur dioxide and glutathione to improve beer flavor stability. Appl Biochem Biotechnol 2012;166:402–13.

Chen ZJ. Genomic and epigenetic insights into the molecular bases of heterostis. Nat Rev Genet 2013;14:471.

Chin C-S, Peluso P, Sedlacek FJ et al. Phased diploid genome assembly with single-molecule real-time sequencing. Nat Methods 2016;13:1050.

Cole GE, McCabe PC, Inlow D et al. Stable expression of Aspergillus awamori glucoamylase in distiller’s yeast. Nat Biotechnol 1988;6:417.

Conko G, Kershen DI, Miller H et al. A risk-based approach to the regulation of genetically engineered organisms. Nat Biotechnol 2016;34:493.

de Araújo Vicente M, Fietto LG, de Miranda Castro I et al. Isolation of Saccharomyces cerevisiae strains producing higher levels of flavoring compounds for production of ‘cachaca’ the Brazilian sugarcane spirit. Int J Food Microbiol 2006;108:51–9.

Delneri D, Colson I, Grammenoudi S et al. Engineering evolution to study speciation in yeasts. Nature 2003;422:68.

Denayrolles M, de Villechenon EP, Lonvaud-Funel A et al. Incidence of SUC-RTM telomeric repeated genes in brewing and wild wine strains of Saccharomyces. Curr Genet 1997;31:457–61.

Denby CM, Li RA, Vu VT et al. Industrial brewing yeast engineered for the production of primary flavor determinants in hopped beer. Nat Commun 2018;9:965.

DiCarlo JE, Norville JE, Mali P et al. Genome engineering in Saccharomyces cerevisiae using CRISPR-Cas systems. Nucleic Acids Res 2013;41:4336–43.

Diderich JA, Weening SM, van den Broek M et al. Selection of Pof-Saccharomyces eubayanus variants for the construction of S. cerevisiae x S. eubayanus hybrids with reduced 4-vinyl guaia-col formation. Front Microbiol 2018;9:1640.

Dinh TN, Nagahisa K, Hirasawa T et al. Adaptation of Saccharomyces cerevisiae cells to high ethanol concentration and changes in fatty acid composition of membrane and cell size. PLoS One 2008;3:e2623.

Donalies UE, Stahl U. Increasing sulphite formation in Saccharomyces cerevisiae by overexpression of MET14 and SSU1. Yeast 2002;19:475–84.

Dunn B, Paulish T, Stanbery A et al. Recurrent rearrangement during adaptive evolution in an interspecific yeast hybrid suggests a model for rapid introgression. PLoS Genet 2013;9:e1003366.

Dunn B, Sherlock G. Reconstruction of the genome origins and evolution of the hybrid lager yeast Saccharomyces pastorianus. Genome Res 2008;18:1610–23.

Durong C, Strack L, Futschik M et al. Identification of Sc-type ILV6 as a target to reduce diacetyl formation in lager brewers’ yeast. Metab Eng 2011;13:638–47.

Ellis V, Bosworth G. Supporting rural entrepreneurship in the UK microbrewery sector. Br Food J 2015;117:2724–38.

Eriksson D, Harwood W, Hofvander P et al. A welcome proposal to amend the GMO legislation of the EU. Trends Biotechnol 2018;36:1100–03.

Fujita T, Kondo K, Shimizu F et al. Application of a ribosomal DNA integration vector in the construction of a brewer’s yeast having alpha-acetolactate decarboxylase activity. Appl Environ Microb 1990;56:997–1003.

Fukuda K, Watanabe M, Asano K et al. Isolation and genetic study of p-fluoro-dl-phenylalanine-resistant mutants over-producing β-phenethyl-alcohol in Saccharomyces cerevisiae. Curr Genet 1991;20:449–52.

Fukunaga M, Yielding LW, Firth WJ, III et al. Petite induction in Saccharomyces cerevisiae by ethidium analogs: distinction between resting and growing cells. Mutat Res 1980;78:151–7.

Gallone B, Steensels J, Prahl T et al. Domestication and divergence of Saccharomyces cerevisiae beer yeasts. Cell 2016;166:1397–1410.

Gavira C, Höfer R, Lesot A et al. Challenges and pitfalls of P450-dependent (+)-valencene biocconversion by Saccharomyces cerevisiae. Metab Eng 2013;18:25–35.

Gayevskiy V, Goddard MR. Saccharomyces eubayanus and Saccharomyces arboricola reside in North Island native New Zealand forests. Environ Microbiol Rep 2016;8:1137–47.

Gibbons JG, Salichos L, Slot JC et al. The evolutionary imprint of domestication on genome variation and function of the filamentous fungus Aspergillus oryzae. Curr Biol 2012;22:1403–09.

Gibbons JG, Rinker DC. The genomics of microbial domestication in the fermented food environment. Curr Opin Genet Dev 2015;35:1–8.

Gibson B, Londoño E, Rautio J et al. Transcription of α-glucoside transport and metabolism genes in the hybrid brewing yeast Saccharomyces pastorianus with respect to gene provenance and fermentation temperature. J I Brewing 2013a;119:23–31.

Gibson B, Geertman J, Hittinger C et al. New yeasts—new brews: modern approaches to brewing yeast design and development. FEMS Yeast Res 2017;17:fox038.

Gibson B, Vidgren V, Peddinti G et al. Diacetyl control during brewery fermentation via adaptive laboratory engineering of the lager yeast Saccharomyces pastorianus. J Ind Microbiol Biotechnol 2018;45:1103–12.

Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Mol Cell 2000;58:61–8.

Goldstein AL, McCusker JH. Thirty new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Mol Cell 2000;58:61–8.

Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae. Mol Cell 2000;58:61–8.

Gjerde B, Aigrain L, Quail MA et al. De novo yeast genome assemblies from MinION, PacBio and MiSeq platforms. Sci Rep 2017;7:3935.

Giordano F, Aigrain L, Quail MA et al. De novo yeast genome assemblies from MinION, PacBio and MiSeq platforms. Sci Rep 2017;7:3935.

Gjerde B, Aigrain L, Quail MA et al. De novo yeast genome assemblies from MinION, PacBio and MiSeq platforms. Sci Rep 2017;7:3935.

Gjerde B, Aigrain L, Quail MA et al. De novo yeast genome assemblies from MinION, PacBio and MiSeq platforms. Sci Rep 2017;7:3935.
Gorter de Vries AR, Pront JT, Daran J-MG. Industrial relevance of chromosomal copy number variation in Saccharomyces yeasts. Appl Environ Microb 2017b;83:e03206–16.

Gorter de Vries AR, Koster CC, Weening SM et al. Phenotype-independent isolation of interspecies Saccharomyces hybrids by dual-dye fluorescent staining and fluorescence-activated cell sorting. Front Microbiol 2019a;10:871.

Gorter de Vries AR, Voskamp MA, van Aalst ACA et al. Laboratory evolution of a Saccharomyces cerevisiae × S. eubayanus hybrid under simulated lager-brewing conditions. Front Genet 2019b;10:242.

Govender P, Domingo JL, Bester MC et al. Controlled expression of the dominant flocculation genes FLO1, FLO5, and FLO11 in Saccharomyces cerevisiae. Appl Environ Microb 2008;74:6041–52.

Gresham D, Desai MM, Tucker CM et al. The repertoire and dynamics of evolutionary adaptations to controlled nutrient-limited environments in yeast. PLoS Genet 2008;4:e1000303.

Gritz L, Davies J. Plasmid-encoded hygromycin B resistance: the sequence of hygromycin B phosphotransferase gene and its expression in Escherichia coli and Saccharomyces cerevisiae. Gene 1983;25:179–88.

Gunge N, Nakatomi Y. Genetic mechanisms of rare matings of the yeast Saccharomyces cerevisiae heterozygous for mating type. Genetics 1972;70:41–58.

Guo X, Shen H, Liu Y et al. Enabling heterologous synthesis of lupulones in the yeast Saccharomyces cerevisiae. Appl Biochem Biotechnol 2019;188:1–11.

Hammond JR. Genetically modified brewing yeasts for the 21st century. Progress to date. Yeast 1995;11:1613–27.

Han S-Y, Pan Z-Y, Huang D-F et al. Highly efficient synthesis of ethyl hexanoate catalyzed by CALB-displaying Saccharomyces cerevisiae whole-cells in non-aqueous phase. J Mol Catal B Enzym 2009;59:168–72.

Hansen EC. Recherches sur la physiologie et la morphologie des fermentes alcooliques. V. methodes pour obtenir des cultures pures de Saccharomyces et de microorganismes analogues. Compt Rend Trau Lab Carlsberg 1883;2:92–105.

Hansen EH, Møller BL, Kock GR et al. Stable overproduction of isoamyl alcohol by Saccharomyces cerevisiae with chromosome-integrated multicopy LEU4 genes. Biosci Biotechnol Biochem 1992;56:1682–3.

Hirooka K, Yamamoto Y, Tsutsui N et al. Improved production of isoamyl acetate by a sake yeast mutant resistant to an isoamyl alcohol and its dependence on alcohol acetyltransferase activity, but not on isoamyl alcohol production. J Biosci Bioeng 2005;99:125–29.

Hirooka K, Ogita A, Fujita KI et al. Isolation of a copper-resistant sake yeast mutant with improved flavour compound production. J Brewi ng 2010;116:261–4.

Hockney RC, Freeman RF. Gratuitous catabolite repression by glucosamine of maltose utilization in Saccharomyces cerevisiae. Microbiology 1980;121:479–82.

Holmberg S, Kielland-Brandt MC. A mutant of Saccharomyces cerevisiae temperature sensitive for flocculation. Influence of oxygen and respiratory deficiency on flocculence. Carlsberg Res Commun 1978;43:37–47.

Holt S, Miks MH, de Carvalho BT et al. The molecular biology of fruity and floral aromas in beer and other alcoholic beverages. FEMS Microbiol Rev 2019;43:fuy041.

Hong J, Gresham D. Molecular specificity, constraint and constraint shape adaptive evolution in nutrient-poor environments. PLoS Genet 2014;10:e1004041.

Hope EA, Amorosi CJ, Miller AW et al. Experimental evolution reveals favored adaptive routes to cell aggregation in yeast. Genetics 2017;206:1153–67.

Hornesey IS, A History of Beer and Brewing. Cambridge: Royal Society of Chemistry, 2003.

Howard PH. Too big to ale? Globalization and consolidation in the beer industry. The Geography of Beer. New York: Springer, 2014, 155–65.

Huuskonen A, Markkula T, Vidgren V et al. Selection from industrial lager yeast strains of variants with improved fermentation performance in very-high-gravity worts. Appl Environ Microb 2010;76:1563–73.

Ishii T, Araki M. Consumer acceptance of food crops developed by genome editing. Plant Cell Rep 2016;35:1507–18.

Ishii T, Araki M. A future scenario of the global regulatory landscape regarding genome-edited crops. GM Crops Food 2017:8:44–56.

Istance B, Friedrich A, d’Agata L et al. De novo assembly and population genomic survey of natural yeast isolates from the Oxford Nanopore MinION sequencer. Gigascience 2017;6:1–13.

Jakočiūnas T, Jensen MK, Keasling JD. CRISPR/Cas9 advances engineering of microbial cell factories. Metab Eng 2016;34:44–59.

James TC, Usher J, Campbell S et al. Lager yeasts possess dynamic genomes that undergo rearrangements and gene amplification in response to stress. Curr Genet 2008;53:139–52.

Jimenez A, Davies J. Expression of a transposable antibiotic resistance element in Saccharomyces. Nature 1980;287:869.

Jones R, Russell I, Stewart G. The use of catabolite derepression as a means of improving the fermentation rate of brewing yeast strains. J Am Soc Brew Chem 1986;44:161–6.

Jordan P, Choe J-Y, Boles E et al. Hxt13, Hxt15, Hxt16 and Hxt17 from Saccharomyces cerevisiae represent a novel type of polyol transporters. Sci Rep 2016;6:23502.

Kielland-Brandt MC, Petersen JGL, Mikkelsen JD. Mutants in the biosynthesis of isoleucine in a non-mating, non-sporulating
brewing strain of Saccharomyces carlsbergensis. Carlsberg Res Commun 1979;44:27–36.
Kim B, Cho BR, Hahn JS. Metabolic engineering of Saccharomyces cerevisiae for the production of 2-phenylethanol via Ehrlich pathway. Biotechnol Bioeng 2014;111:115–24.
Kim JM, Vanguri S, Boeke JD et al. Transposable elements and genome organization: a comprehensive survey of retrotransposons revealed by the complete Saccharomyces cerevisiae genome sequence. Genome Res 1998;8:464–78.
Kobayashi O, Hayashi N, Kuroki R et al. Region of Flo1 proteins responsible for sugar recognition. J Bacteriol 1998;180:6503–10.
Kodama Y, Omura F, Ashikari T. Isolation and characterization of a gene specific to lager brewing yeast that encodes a branched-chain amino acid permease. Appl Environ Microbiol 2001;67:3455–62.
Krogerus K, Magalhães F, Vidgren V et al. New lager yeast strains generated by interspecific hybridization. J Ind Microbiol Biotechnol 2015;42:769–78.
Krogerus K, Arvas M, De Chiara M et al. Ploidy influences the functional attributes of de novo lager yeast hybrids. Appl Microbiol Biotechnol 2016;100:7203–22.
Krogerus K, Magalhães F, Vidgren V et al. Novel brewing yeast hybrids: creation and application. Appl Microbiol Biotechnol 2017;101:65–78.
Krogerus K, Holmström S, Gibson B. Enhanced wort fermentation with de novo lager hybrids adapted to high-ethanol environments. Appl Environ Microbiol 2018;84:e02302–317.
Kunze W. Technology Brewing & Malting. Berlin: VLB, 2004.
Kutyna DR, Varela C, Stanley GA et al. Adaptive evolution of Saccharomyces cerevisiae to generate strains with enhanced glycerol production. Appl Microbiol Biotechnol 2011;89:1175–84.
Lancaster SM, Payen C, Heil CS et al. Fitness benefits of loss of heterozygosity in Saccharomyces hybrids. Genome Res 2019;29:1685–92.
Ledford H. Gene-editing surges as US rethinks regulations. Nature 2016;532:158.
Lee D, Lloyd ND, Pretorius IS et al. Heterologous production of raspberry ketone in the wine yeast Saccharomyces cerevisiae via pathway engineering and synthetic enzyme fusion. Microb Cell Fact 2016;15:49.
Lee S, Villa K, Patino H. Yeast strain development for enhanced production of desirable alcohols/esters in beer. J Am Soc Brew Chem 1995;53:153–6.
Libkind D, Hittinger CT, Valério E et al. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc Natl Acad Sci USA 2011;108:201105430.
Lippman ZB, Zamir D. Heterosis: revisiting the magic. Trends Genet 2007;23:60–6.
Liti G, Peruffo A, James SA et al. Inferences of evolutionary relationships from a population survey of LTR-retrotransposons and telomeric-associated sequences in the Saccharomyces sensu stricto complex. Yeast 2005;22:177–92.
Liti G, Barton DB, Louis EJ. Sequence diversity, reproductive isolation and species concepts in Saccharomyces. Genetics 2006;174:839–50.
Liu L, Wang J, Rosenberg D et al. Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: investigating Natufian ritual feasting. J Archaeol Sci 2018;81:783–93.
Liu XF, Wang ZY, Wang JJ et al. Expression of GAI gene and disruption of PEP4 gene in an industrial brewer’s yeast strain. Lett Appl Microbiol 2009;49:117–23.
Lockhart B. The origins and life of the export beer bottle. Origins 2007;49:49–58.
Lodolo EJ, Kock JL, Axcell BC et al. The yeast Saccharomyces cerevisiae—the main character in beer brewing. FEMS Yeast Res 2008;8:1018–36.
Magalhães F, Krogerus K, Vidgren V et al. Improved cider fermentation: performance and quality with newly generated Saccharomyces cerevisiae × Saccharomyces eubayanus hybrids. J Ind Microbiol Biotechnol 2014;41:1203–13.
Maier T. Sources of microbrewery competitiveness in the Czech Republic. Agris 2016;8:14.
Mallet J. Hybrid speciation. Nature 2007;446:279.
Mans R, van Rossouw HM, Wijman M et al. CRISPR/Cas9: a molecular Swiss army knife for simultaneous introduction of multiple genetic modifications in Saccharomyces cerevisiae. FEMS Yeast Res 2015;15:fov004.
Mans R, Daran J-MG, Pronk JT. Under pressure: evolutionary engineering of yeast strains for improved performance in fuels and chemicals production. Curr Opin Biotechnol 2018;50:47–56.
Matheson K, Parsons L, Gammie A. Whole-genome sequence and variant analysis of W303, a widely-used strain of Saccharomyces cerevisiae. G3 (Bethesda) 2017;7:2219–26.
Mendlik F. Some aspects of the scientific development of brewing in Holland. J I Brewing 1937;43:294–300.
Mertens S, Steensels J, Saels V et al. A large set of newly created interspecific yeast hybrids increases aromatic diversity in lager beers. Appl Environ Microbiol 2015;81:8202–14.
Mertens S, Gallone B, Steensels J et al. Reducing phenolic off-flavours through CRISPR-based gene editing of the FDC1 gene in Saccharomyces cerevisiae × Saccharomyces eubayanus hybrid lager beer yeasts. PLoS One 2019;14:e0209124.
Meesdoerffer FG. A comprehensive history of beer brewing. Handbook of Brewing: Processes, Technology, Markets. Weinheim: Wiley-VCH, 2009, 1–4.
Michel C. Geschichte des Bieres von der ältesten Zeit bis zum Jahre. Ausburg: Verlagsbuchhandlung Gebrüder Reichel, 1899.
Michel RH, McGovern PE, Badler VR. Chemical evidence for ancient beer. Nature 1992;360:24.
Monerawela C, James TC, Wolfe KH et al. Loss of lager specific genes and subtelomeric regions define two different Saccharomyces cerevisiae lineages for Saccharomyces pastorianus Group I and II strains. FEMS Yeast Res 2015;15:fou008.
Moritz ER, Morris GH. A Text-Book of the Science of Brewing. London: Spon, 1891.
Murakami N, Miyoshi S, Yokoyama R et al. Construction of a URA3 deletion strain from the allotetraploid bottom-fermenting yeast Saccharomyces pastorianus. Yeast 2012;29:155–65.
Nakao Y, Kamamori T, Itoh T et al. Genome sequence of the lager brewing yeast, an interspecies hybrid. DNA Res 2009;16:115–29.
Nevoigt E, Pilger R, Mast-Gerlach E et al. Genetic engineering of brewing yeast to reduce the content of ethanol in beer. FEMS Yeast Res 2002;2:225–32.
Nevoigt E. Progress in metabolic engineering of Saccharomyces cerevisiae. Microbiol Mol Biol Rev 2008;72:379–412.
Nielsen J, Larsson C, van Maris AJA et al. Metabolic engineering of yeast for production of fuels and chemicals. Curr Opin Biotechnol 2013;24:398–404.
Nielsen J, Keasling JD. Engineering cellular metabolism. Cell 2016;164:1185–97.
Nikulin J, Krogerus K, Gibson B. Alternative Saccharomyces interspecies hybrid combinations and their potential for low-temperature wort fermentation. Yeast 2018;35:113–27.

Ogata T, Shikata-Miyoshi M, Tadami H et al. Isolation of meiotic segregants from a bottom fermenting yeast. J I Breving 2011;117:199–205.

Pirttinen M, Suihko M, Lehtinen U et al. Multiple pathwaysof recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol Mol Biol Rev 2008;72:303–10.

Painter W. Bottle-Sealing Device. Specification Forming Part of Letters Patent No. 468,226. Washington, DC: US Patent Office. http://www.uspto.gov, USA, 1892.

Oliver G, Colicchio T. The Oxford Companion to Beer. New York: Oxford University Press, 2011.

Patnaik R. Engineering complex phenotypes in industrial brewer’s yeasts secreting fungal endo-ß-glucanase. Biocatal Biotechnol Biochem 2005;69:1162–71.

Peças F, Haber JE. Multiple pathways of recombination induced by double-strand breaks in Saccharomyces cerevisiae. Microbiol Mol Biol Rev 1999;63:349–404.

Pastoureau L. Études sur la Bière: ses Maladies, Causes qui les Provoquent, Procédé Pour la Rendre Inaltérable. Paris: Gauthier-Villars, 1876.

Patnaik R. Engineering complex phenotypes in industrial strains. Biotechnol Prog 2008;24:38–47.

Pennill M, Suikko M, Lehtinen U et al. Construction of brewer’s yeasts secreting fungal endo-ß-glucanase. Curr Genet 1987;12:413–20.

Pérez-Torrado R, Querol A, Guillamón JM. Genetic improvement of non-GMO wine yeasts: strategies, advantages and safety. Trends Food Sci Technol 2015;45:1–11.

Pérez Través L, Lopes CA, Barrio E et al. Study of the stabilization process in Saccharomyces intra-and interspecific hybrids in fermentation conditions. Int Microbiol 2014;17:213–24.

Peris D, Sylvester K, Libkind D et al. Population structure and reticulate evolution of Saccharomyces eubayanus and its lager-brewing hybrids. Mol Ecol 2014;23:2031–45.

Peris D, Moriarty RV, Alexander WG et al. Hybridization and adaptive evolution of diverse Saccharomyces species for cel lulolic biofuel production. Biotechnol Biofuels 2017;10:78.

Perry C, Meaden P. Properties of a genetically-engineered dextrin-fermenting strain of brewer’s yeast. J I Breving 1988;94:64–7.

Peter J, De Chiara M, Friedrich A et al. Genome evolution across 1,011 Saccharomyces cerevisiae isolates. Nature 2018;556:339.

Piatkowska EM, Naseeb S, Knight D et al. Chimeric protein complexes in hybrid species generate novel phenotypes. PLoS Genet 2013;9:e1003836.

Poelmans E, Swinnen JF. From monasteries to multinationals (and back): a historical review of the beer economy. J Wine Econ 2011a;6:196–216.

Poelmans E, Swinnen JF. A Brief Economic History of Beer. Oxford, UK: Oxford University Press, 2011b.

Pryde FE, Huckle TC, Louis EJ. Sequence analysis of the right end of chromosome XV in Saccharomyces cerevisiae: an insight into the structural and functional significance of sub-telomeric repeat sequences. Yeast 1995;11:371–82.

Rainieri S, Kodama Y, Kaneko Y et al. Pure and mixed genetic lines of Saccharomyces bayanus and Saccharomyces pastorianus and their contribution to the lager brewing strain genome. Appl Environ Microb 2006;72:3968–74.

Rainieri S, Kodama Y, Nakao Y et al. The inheritance of mtDNA in lager brewing strains. FEMS Yeast Res 2008;8:586–96.

Rice C. Sourvisiae: a bioengineered brewing yeast for easy, fast, reproducible sour beer production. ASBC-AEV Joint Symposium: Yeast and Fermented Beverage Flavor. Sonoma County, CA, 2019.

Sakai K, Fukui S, Yabuuchi S et al. Expression of the Saccharomyces diastaticus STA1 gene in brewing yeasts. J Am Soc Brew Chem 1989;47:87–91.

Salazar AN, Gorter de Vries AR, van den Broek M et al. Nanopore sequencing enables near-complete de novo assembly of Saccharomyces cerevisiae reference strain CEN.PK113-7D. FEMS Yeast Res 2017;17:foz074.

Sanchez RG, Solodovnikova N, Wendland J. Breeding of lager yeast with Saccharomyces cerevisiae improves stress resistance and fermentation performance. Yeast 2012;29:343–55.

Santaguida S, Amon A. Short-and long-term effects of chromosome mis-segregation and aneuploidy. Nat Rev Mol Cell Biol 2015;16:473.

Sato M, Kishimoto M, Watari J et al. Breeding of brewer’s yeast by hybridization between a top-fermenting yeast Saccharomyces cerevisiae and a cryophilic yeast Saccharomyces bayanus. J Biosci Bioeng 2002;93:509–11.

Shapira R, Levy T, Shaked S et al. Extensive heterosis in growth of yeast hybrids is explained by a combination of genetic models. Heredity 2014;113:316.

Shen N, Wang J, Liu C et al. Domesticating brewing yeast for decreasing acetaldehyde production and improving beer flavor stability. Eur Food Res Technol 2014;238:347–55.

Sicard D, Legras J-L. Bread, beer and wine: yeast domestication in the Saccharomyces sensus stricto complex. C R Biol 2011;334:229–36.

Smukowski Heil CS, DeSevo CG, Pai DA et al. Loss of heterozygosity drives adaptation in hybrid yeast. Mol Biol Evol 2017;34:1596–612.

Sone H, Fujii T, Kondo K et al. Nucleotide sequence and expression of the Enterobacter aerogenes alpha-acetolactate decarboxylase gene in brewer’s yeast. Appl Environ Microb 1988;54:38–42.

Sprink T, Eriksson D, Schiemann J et al. Regulatory hurdles for genome editing: process-vs. product-based approaches in different regulatory contexts. Plant Cell Rep 2016;35:1493–506.

Stanley D, Fraser S, Chambers PJ et al. Generation and characterisation of stable ethanol-tolerant mutants of Saccharomyces cerevisiae. J Ind Microbiol Biotechnol 2010;37:139–49.

Steensels J, Meersman E, Snoek T et al. Large-scale selection and breeding to generate industrial yeasts with superior aroma production. Appl Environ Microb 2014a;80:6965–75.
Steensels J, Snoek T, Meersman E et al. Improving industrial yeast strains: exploiting natural and artificial diversity. FEMS Microbial Rev 2014b;38:947–95.

Strejc J, Šišišová L, Karabin M et al. Production of alcohol-free beer with elevated amounts of flavouring compounds using lager yeast mutants. J Brew 2013;119:149–55.

Struyk AP. Onderzoekingen over de alcoholsche gisting. Ph.D. Thesis. University of Technology Delft, Delft 1928.

Teste M-A, François JM, Parrou J-L. Characterization of a new multigene family encoding isomaltases in the yeast Saccharomyces cerevisiae: the IMA family. J Biol Chem 2010;285:26815–24.

Teunissen A, Dumortier F, Gorwa M-F et al. Characterization of a freeze-tolerant diploid derivative of an industrial baker's yeast strain and its use in frozen doughs. Appl Environ Microbiol 2002;68:4780–7.

Tezuka H, Mori T, Okumura Y et al. Cloning of a gene suppressing hydrogen sulfide production by Saccharomyces cerevisiae and its expression in a brewing yeast. J Am Soc Brew Chem 1992;50:130–3.

Thurnell-Read T. Craft, tangibility and affect at work in the microbrewery. Emot Space Soc 2014;13:46–54.

Tirosh I, Reikhav S, Levy AA et al. A yeast hybrid provides insight into the evolution of gene expression regulation. Science 2009;324:659–62.

Unger RW. Beer in the Middle Ages and the Renaissance. Philadelphia: University of Pennsylvania Press, 2004.

Van den Broek M, Bolat I, Nijkamp J et al. Production of alcohol-free beer with elevated amounts of flavouring compounds using lager yeast mutants. J Brew 2013;119:149–55.

Van den Broek M, Bolat I, Nijkamp J et al. Production of alcohol-free beer with elevated amounts of flavouring compounds using lager yeast mutants. J Brew 2013;119:149–55.

Voordeckers K, Kominek J, Das A et al. Adaptation to high ethanol reveals complex evolutionary pathways. PLoS Genet 2015;11:e1005635.

Walther A, Hesselbart A, Wendland J. Genome sequence of Saccharomyces carlsbergensis, the world's first pure culture lager yeast. G3 (Bethesda) 2014;4:783–93.

Waltz E. Gene-edited CRISPR mushroom escapes US regulation. Nature 2016;532:293.

Wang J-J, Xu W-N, Li J et al. Absence of fks1p in lager brewing yeast results in aberrant cell wall composition and improved beer flavor stability. World J Microbiol Biotechnol 2014;30:1901–8.

Watanabe M, Tanaka N, Mishima H et al. Isolation of sake yeast mutants resistant to isoamyl monofluoracetate to improve isoamyl acetate productivity. J Biosci Bioeng 1993;76:229–31.

Watanabe M, Nagai H, Kondo K. Properties of sake yeast mutants resistant to isoamyl monochloroacetate. J Biosci Bioeng 1995;80:291–3.

Wenger AM, Peluso P, Rowell WJ et al. Highly-accurate long-read sequencing improves variant detection and assembly of a human genome. bioRxiv 519025, 2019.

Williams AG, Mekonen S. Environmental performance of traditional beer production in a micro-brewery. In: Proceedings of the 9th International Conference on Life Cycle Assessment in the Agri-Food Sector (LCA Food 2014), San Francisco, CA, 8–10 October, 2014. pp. 1535–1540. American Center for Life Cycle Assessment, 2014.

Yamagishi H, Ohnuki S, Nomura T et al. Role of bottom-fermenting brewer’s yeast KEX2 in high temperature resistance and poor proliferation at low temperatures. J Gen Appl Microbiol 2010;56:297–312.

Yamano S, Kondo K, Tanaka et al. Construction of a brewer's yeast having α-acetolactate decarboxylase gene from Acetobacter actei ssp. xylinum integrated in the genome. J Biotechnol 1994a;32:173–8.

Yamano S, Tanaka J, Inoue T. Cloning and expression of the gene encoding α-acetolactate decarboxylase from Acetobacter actei ssp. xylinum in brewer’s yeast. J Biotechnol 1994b;32:165–71.

Yao H, Gray AD, Auger DL et al. Genomic dosage effects on heterosis in triploid maize. Proc Natl Acad Sci USA 2013;110:2665–9.

Yetisen AK. Biohacking. Trends Biotechnol 2018;36:744–7.

Yoshida S, Imoto J, Minato T et al. Development of bottom-fermenting Saccharomyces strains that produce high SO2 levels, using integrated metabolome and transcriptome analysis. Appl Environ Microbiol 2008;74:2787–96.

Yoshimoto H, Fujiiwara D, Momma T et al. Characterization of the ATF1 and Lg-ATF1 genes encoding alcohol acetyltransferases in the bottom fermenting yeast Saccharomyces pastorianus. J Biosci Bioeng 1998;86:15–20.

Yu Z, Zhao H, Li H et al. Selection of Saccharomyces pastorianus variants with improved fermentation performance under very high gravity wort conditions. Biotechnol Lett 2012;34:365–70.

Zheng D-Q, Chen J, Zhang K et al. Genomic structural variations contribute to trait improvement during whole-genome shuffling of yeast. Appl Microbiol Biotechnol 2014;98:3059–70.