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Saccharomyces pastorianus: genomic insights inspiring innovation for industry

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Abstract

A combination of biological and non-biological factors has led to the interspecific hybrid yeast species *Saccharomyces pastorianus* becoming one of the world's most important industrial organisms. This yeast is used in the production of lager-style beers, the fermentation of which requires very low temperatures compared to other industrial fermentation processes. This group of organisms has benefited from both the whole-genome duplication in its ancestral lineage and the subsequent hybridization event between *S. cerevisiae* and *S. eubayanus*, resulting in strong fermentative ability. The hybrid has key traits, such as cold tolerance and good maltose- and maltotriose-utilizing ability, inherited either from the parental species or originating from genetic interactions between the parent genomes. Instability in the nascent allopolyploid hybrid genome may have contributed to rapid evolution of the yeast to tolerate conditions prevalent in the brewing environment. The recent discovery of *S. eubayanus* has provided new insights into the evolutionary history of *S. pastorianus* and may offer new opportunities for generating novel industrially-beneficial lager yeast strains.

Keywords: ***Saccharomyces pastorianus*; *S. eubayanus*; lager beer; Saaz; Frohberg; brewing; hybrid; heterosis**

Emergence of lager beer

Beer has played an integral role in most human societies since at least the Neolithic period. In addition to its current status as a globally popular beverage, beer has historically had diverse societal functions. It has, for example, been used as an offering in religious ceremonies, been prescribed by medical practitioners to cure various ailments, been paid as a salary or tithe and has constituted an important part of the human diet, particularly in the absence of safe supplies of drinking water (Cornell, 2003; Gately, 2008; Hornsey, 2012). For the greater part of its history, beer brewing has involved the production of ales, i.e. beer brewed at a relatively high temperature with 'top-fermenting' *S. cerevisiae* strains. These yeast, so called because of their tendency to become buoyant after flocculation and rise to the surface of fermenting wort, produced beers with pronounced floral and fruit flavour notes. Lager has existed as a beer style at least since the fifteenth century, when it was produced in the German state of Bavaria and was at the time brewed as a dark brown beer (Meussdoerffer, 2009). Lager or 'bottom-fermented' beer is brewed at low temperatures (5–15°C) and does not have the pronounced fruit/floral notes typical of ales. The yeasts involved in lager fermentation, in addition to being cryotolerant, have a tendency to sediment after flocculation and sink to the bottom of the fermentation vessel (for review, see Vidgren and Londesborough, 2011). Louis Pasteur, in his *Études sur la Bière*, was one of the first to appreciate that the top- and bottom-fermenting yeasts were two

inherently distinct groups (Pasteur, 1876). The rise in popularity of lager beer in the nineteenth century was due to a number of factors, not the least of which was the development of Pilsner-style lager beer, which was paler and more heavily hopped than traditional lagers. This popular beer, first brewed in Plzeň in Bohemia (today the Czech Republic), was quickly adopted by German brewers. Its production and export was aided by the industrialization of German brewing and advances in refrigeration technology, which allowed lager beers to be brewed all year round. Scientific principles were also being applied to the brewing process to ensure quality and consistency. In particular, the realization that a living organism was responsible for the fermentation led to a greater focus on brewery hygiene and the maintenance of viable yeast stocks. This culminated in the isolation of the first pure brewing yeast cultures by Emil Hansen of the Carlsberg brewery in 1883 (Moritz and Morris, 1891).

Emergence of *Saccharomyces pastorianus*

The production of lager-style beers is dependent on low-temperature fermentation, which, in turn, is dependent on the ability of yeast to survive and remain metabolically active at such temperatures. The lager-brewing yeast *S. pastorianus* is now understood to be an interspecific hybrid involving *S. cerevisiae* and *S. eubayanus* (Libkind *et al*., 2011). While it was known for some time that the lager yeast was a hybrid organism, the exact parentage was a matter of dispute (Vaughan-Martini and Kurtzman, 1985; Kielland-Brandt *et al*., 1995; Kodama *et al*., 2005). The hybrid appeared to involve *S. cerevisiae* (possibly an ale strain) and a strain somewhat related to the genetically complex 'species' *S. bayanus* (Rainieri *et al*., 2006; Nakao *et al*., 2009), which actually comprises a heterogeneous group of interspecific hybrids. The discovery of a new free-living diploid species, *S. eubayanus*, which is an almost exact genetic match of the non-*S. cerevisiae*-type subgenome of lager yeast, appears to have resolved the issue (Libkind *et al*., 2011). The authors suggest a possible scenario in which the initial hybridization event between a diploid *S. cerevisiae* cell and a diploid *S. eubayanus* cell led to the formation of an allotetraploid yeast. This was followed by extensive genome reorganization by mitotic recombination, resulting in loss of heterozygosity and recombinant chimeric chromosomes. This genomic reorganization was probably constrained and shaped by differences in chromosomal organization (Fischer *et al*., 2000) and genetic incompatibilities (Lee *et al*., 2008) that resulted in lethal genetic combinations.

When and under what circumstances the hybridization event(s) between *S. cerevisiae* and *S. eubayanus* occurred is not known. It may be speculated that *S. eubayanus* initially occurred as a wild yeast contaminant in the brewing process, possibly with a selective advantage over the native ale yeast when fermentations were carried out under cooler temperatures (prior to refrigeration, brewing was often performed only during winter and the cooler months of fall and spring, because beer quality could not be controlled easily in the higher summer temperatures). Hybridization of the two species (presumably the original *S. cerevisiae* ale strain and the *S. eubayanus* contaminant) would have resulted in the creation of a hybrid with the strong fermentative ability of the former and the cold tolerance of the latter. This interspecific hybrid would have had a competitive advantage over both parents, leading to a rapid numerical domination of the fermenting yeast population. Assuming this new organism did not have a major detrimental effect on beer quality, it would then have been unwittingly selected by the brewer. If an improvement in fermentation or product quality was apparent, then the brewer may have preferentially used this yeast to ferment subsequent batches of beer.

Saccharomyces eubayanus isolates were originally discovered in South America (Libkind *et al*., 2011), with rare isolates later found in North America (Peris *et al*., 2014). The abundance of *S. eubayanus* in Patagonia suggested a South American origin for the *S. eubayanus* component of the lager yeast genome (Libkind *et al*., 2011), possibly occurring after the advent of transatlantic travel between Europe and the Americas. However, recent field surveys have also recovered *S. eubayanus* isolates in different geographic areas of China (Bing *et al*., 2014). Phylogenetic analysis indicates that the Asian subpopulations are distinct from the South American strains, and apparently more closely related to the *S. eubayanus* portion of the modern *S. pastorianus* genome, with as much as 99.82% sequence similarity. Bing *et al*. (2014) therefore suggest that the

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non-*cerevisiae* subgenome of lager yeast is of Asian origin, specifically from the Tibetan plateau. This contention may be more feasible than the Patagonian hypothesis, considering the more well-established historic trade links between East Asia and Europe compared to South America and Europe. *Saccharomyces eubayanus* has, to date, not been found in Europe. It is possible, however, that the species inhabits specific niche environments and awaits discovery. Future population genomics studies of subpopulations of *S. eubayanus* and *S. uvarum* have the potential to trace back the exact *S. pastorianus* genomic parentage, with the possibility of inferring the geographic origin of the hybridization event.

It appears that the *S. pastorianus* hybrid strains may also have served as progenitors of the various *S. bayanus* hybrid strains, often found as brewing contaminants, through additional hybridization events involving strains belonging to yet another closely related species, *S. uvarum* (Libkind *et al*., 2011; Nguyen *et al*., 2011); this has resulted in strains with varying genomic compositions. For example, genome analysis of two well-known *S. bayanus* strains reveals that the contribution of the two non-*S. cerevisiae* species varies greatly within the genome: in CBS380^{T} this subgenome is 67% *S. uvarum* and 33% *S. eubayanus*, whereas in NBRC1948 it is 37% *S. uvarum* and 63% *S. eubayanus* (Libkind *et al*., 2011).

Similar genomic analysis of yeasts used for fermentation of other alcoholic beverages has led to the realization that many commercially important strains are in fact natural yeast hybrids. A number of *Saccharomyces* interspecific hybrid combinations have been identified, associated with cider fermentation and in European vineyards (Masneuf *et al*., 1998; Groth *et al*., 1999; Marinoni *et al*., 1999; Sipiczki, 2008; Borneman *et al*., 2012; Erny *et al*., 2012), including *S. cerevisiae* × *S. uvarum* and *S. cerevisiae* × *S. kudriavzevii* and even triple hybrids involving all three of the aforementioned species (Peris *et al*., 2012). The genetic diversity of natural hybrids used in the wine industry is high (Peris *et al*., 2012), suggesting that hybridization events are common and that several hybrids have been retained for industrial use. The hybrid condition of many wine yeast strains has proffered an evolutionary advantage in the vineyard environment and has led to the expression of properties with distinct biotechnological benefit. Examples include low production of acetic acid and high production of glycerol (Caridi *et al*., 2002), adaptation to stress (Belloch *et al*., 2009), cold (Peris *et al*., 2012) and novel flavour profiles (Masneuf *et al*., 1998; Bellon *et al*., 2011, 2013; Bizaj *et al*., 2012; Gamero *et al*., 2013). The ubiquity of interspecific hybrids in industrial processes suggests that this state confers benefits to the organism that outweigh the potential disadvantages of reproductive isolation.

Recent genomic analysis has revealed multiple *S. eubayanus* introgressions in European strains of *S. uvarum* associated with wine and cider fermentations (although not in wild strains) (Almeida *et al*., 2014). The genetic contribution of *S. eubayanus* suggests that this species may have a special, as-yet undefined, role in yeast domestication, possibly conferring evolutionary advantage through changes in nitrogen metabolism or sulphite resistance (Almeida *et al*., 2014). The origins of these introgressions have yet to be elucidated, i.e. whether they were introduced via interactions with *S. pastorianus* or by a more direct interaction between *S. uvarum* and *S. eubayanus*.

Heterosis and cooperation of hybrid subgenomes

Charles Darwin envisaged evolution as a tree with multiple branches, each representing a unique genetically isolated species (Darwin, 1859). However, modern genomic analysis suggests that an interconnected network with branches diverging and reconverging over time may be a more accurate representation of species relationships (Bapteste *et al*., 2013). Such convergence can occur due to hybridization between different species, once thought to be rare in fungi but now recognized as quite common (Brasier, 2000; Morales and Dujon, 2012). The process can confer distinct advantages to the nascent hybrid organism. Heterosis (also called hybrid vigour) is the tendency of a hybrid individual to show qualities superior to those of either parent. Agriculturalists exploited this phenomenon to significantly improve animal and crop yields, and in modern times hybrid versions of many important crops (maize, sorghum, sunflower, wheat)

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constitute a major part of the human diet (Lippman and Zamir, 2006). Heterosis also occurs in microorganisms such as yeasts (Zörgö *et al*., 2012; Plech *et al*., 2013) and has considerable potential for industrial exploitation.

In the case of *S. pastorianus*, where the hybridization event probably occurred quite recently (possibly about 500 years ago), the orthologous genes would have been separated by a period lasting tens of millions of years, i.e. since the divergence *of S. cerevisiae* and *S. eubayanus*. During this time, a considerable amount of sequence and functional divergence may have occurred. A number of recent studies have shown differential functionality in *S. pastorianus* orthologues (note that genes derived from the *S. cerevisiae* or *S. eubayanus* lineage can be differentiated by comparison with published *S. cerevisiae* and *S. pastorianus* genome sequences). For example, the *KEX2* gene (encoding kexin protease) derived from *S. eubayanus* has a role in supporting cryotolerance in *S. pastorianus*, while the *S. cerevisiae* form of the gene has no influence on this characteristic (Yamagishi *et al*., 2010). A similar functional divergence has been noted for a number of genes that directly impact on brewing yeast properties, including beer flavour and stability (Iijima and Ogata, 2010; Duong *et al*., 2011; Bolat *et al*., 2013; Ogata *et al*., 2013; He *et al*., 2014). In addition to functional differences exhibited by the diverged *S. pastorianus* orthologues, there exist considerable differences between the two subgenomes in their protein levels and transcription patterns. For certain orthologous gene pairs, differences in protein abundance are seen in lager strains grown under different conditions (Caesar *et al*., 2007). Similarly, differences in gene expression levels between the two subgenomes of *S. pastorianus* are seen when using microarrays that can differentiate between the subgenomes (Yoshida *et al*., 2007; Minato *et al*., 2009; Horinouchi *et al*., 2010). These gene expression differences can be temporal as well; initiation of transcription of genes involved in α-glucoside utilization can differ by as much as 12 h during wort fermentation, depending on the lineage to which the gene belongs (Gibson *et al*., 2013).

Gene expression is known to be dependent on a number of factors, including environmental signalling within the cell, the presence and abundance of certain transcriptional regulators and the abundance and sequence of promoter regions and transcription factor binding sites upstream of a particular gene. Differences in gene transcription between the two subgenomes of *S. pastorianus* suggest differences in regulation for each of the subgenomes. An interesting question is how orthologous genes are regulated in hybrid species, i.e. whether the regulatory machinery for one orthologue also controls expression of the other, or whether regulatory mechanisms remain functionally separate. Tirosh *et al*. (2009) used laboratory-created interspecific hybrids to investigate differential regulation of allelic genes, by comparing expression within either parent alone (*S. cerevisiae* or *S. paradoxus*) to that within the interspecies hybrid (*S. cerevisiae* × *S. paradoxus*). It was shown that differential expression in the hybrid was influenced both by divergence in promoter regions of the genes (*cis* effects) as well as by divergence of upstream regulators (*trans* effects); which effect was most pronounced was influenced by environmental conditions (Tirosh *et al*., 2009). Bias in allelic expression may be regulated at the level of transcription or translation, as shown by Artieri and Fraser (2014) in a study involving an interspecific hybrid of *S. cerevisiae* and *S. paradoxus*. Experimental interspecific hybrids have also been crucial for our understanding of how protein complexes interact in hybrid yeasts (Piatkowska *et al*., 2013). Protein interactions appear largely conserved within the *Saccharomyces* species (Leducq *et al*., 2012) and experimental evidence illustrates that chimeric protein complexes can be formed and perhaps constitute an additional source of phenotypic variation and plasticity (Piatkowska *et al*., 2013).

Regulation of gene expression in the *S. pastorianus* hybrid has received little attention. Bolat *et al*. (2013) observed that deletion of the *S. eubayanus* form of *ARO80* (the regulator of *ARO10* expression) did not alter expression of the *S. eubayanus* form of *ARO10*, suggesting that, at least in this case, the regulators are interchangeable. Further investigation of functional divergence of orthologues in *S. pastorianus*, including divergence in RNA/protein levels and in protein–protein interactions, may help to explain the competitive advantage shown by the hybrid species relative to its parents and also help to identify the determinants of important biological characteristics, such as cold tolerance and flocculation.

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Saaz and Frohberg yeasts

The combination of alleles within the lager yeast *S. pastorianus* derived from the two parental species has undoubtedly led to its success as a fermentative organism and consequently to the success of the lager-brewing industry. The hybridization event that led to the creation of *S. pastorianus* possibly occurred numerous times, leading to an initially high level of diversity in the brewing industry. It is now known that the *S. pastorianus* group encompasses at least two distinct lineages, referred to as Saaz and Frohberg, which may have arisen independently (Liti *et al*., 2005; Dunn and Sherlock, 2008), as suggested by differences in genome sequence and in ploidy levels between the two groups. The two yeast types, named after the regions in Bohemia and Germany where they were first utilized, were isolated by Paul Lindner at the end of the nineteenth century (Lindner, 1909). This isolation occurred in a period when the importance of pure yeast cultures was beginning to be recognized as essential for successful brewing. The Saaz group strains, also referred to as Hybrid Group 1, were used in the area that is now the Czech Republic and also by the Carlsberg brewery in Denmark. The Frohberg, or Hybrid Group 2, strains were commonly used in other Danish breweries and in The Netherlands. These two groups, despite sharing common features that support lager fermentation (good fermentation potential at low temperature and sedimentation of flocculent yeast), differ functionally in a number of respects. The Saaz yeast group has historically been considered to be strongly flocculent but less able to fully utilize sugars during fermentation compared to the less flocculent but strongly fermenting Frohberg yeasts (Guilliermond, 1920). Early research showed that the two yeast groups had similar capacities for utilization of glucose and maltose but differed in their ability to use maltotriose (referred to as 'low-type maltodextrin', 'achrodextrin' or simply 'dextrin' in the original literature) (Morris, 1895; Prior, 1896; Glendinning, 1899; Johnson, 1904; Siau, 1906).

The realization that the Saaz and Frohberg groups of lager yeasts represent yeast with distinct genomes has revived interest in the functional differences between the groups. The different hybrid group types are also readily distinguishable, due to the almost complete absence of *S. eubayanus* rDNA in the Frohberg strains (Nakao *et al*., 2009; Pham *et al*., 2011). Layfield *et al*. (2011) noted that the Frohberg yeasts appeared to have a greater sensitivity to desiccation and rehydration than the Saaz yeasts. Powell *et al*. (2012) showed that while the strains of the two groups did not differ in their tolerance to stress, there was a clear difference in their optimal temperature growth, with Saaz yeasts growing better at low temperatures than Frohberg yeasts (Powell *et al*., 2012). Differences in tolerance to cold were confirmed by Gibson *et al*. (2013), although these differences appeared to have no bearing on overall fermentation performance at low temperature. Frohberg yeasts invariably fermented faster than Saaz yeasts, due to a faster and more complete utilization of maltose in the former and an inability to utilize maltotriose in the latter (Gibson *et al*., 2013). The authors suggested that differences in maltotriose utilization may relate to differential transmembrane transport of maltotriose, rather than differences in intracellular enzyme activity, as suggested by earlier researchers (the α-glucosidase that hydrolyses maltose also hydrolyses maltotriose). Other differences observed by Gibson *et al*. (2013) and Walther *et al*. (2014) were a generally lower concentration of certain higher alcohols and esters in beers produced by the Saaz yeast relative to the Frohberg yeast. Melibiose-hydrolysing ability, which is a defining characteristic of lager yeast and readily detected (Box *et al*., 2012), has been observed in both hybrid groups (our own unpublished data). A greater tendency of the Saaz yeast CBS1513 population to generate respiratory-deficient 'petite' mutants compared to the Weihenstephan W34/70 strain was also noted (Walther *et al*., 2014).

Interestingly, the functional differences observed between the two lager yeast hybrid groups appear to parallel differences in their genomes. Dunn and Sherlock (2008) proposed that the ancestor of the Group 1 (Saaz) yeast was the result of a cross between a haploid *S. cerevisiae* ale strain spore and a haploid *S. eubayanus* spore, producing a diploid hybrid of *S. pastorianus*. Saaz yeasts have lost a considerable portion of their *S. cerevisiae* DNA (chromosomes VI and VIII as well as parts of IV, XIII and XV) and the extant strains, which have proportionally more DNA derived from the *S. eubayanus* parent, appear to have retained mainly characteristics of this yeast. By contrast, the Group 2 (Frohberg) ancestor may have formed after a cross

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occurred between a diploid *S. cerevisiae* cell and a haploid *S. eubayanus* cell, thereby creating a hybrid genome containing more *S. cerevisiae* than *S. eubayanus* DNA. Modern Frohberg strains still retain proportionally more DNA from the *S. cerevisiae* parent, even after considerable loss of DNA from this subgenome. Thus, while both groups inherited their cryotolerant phenotype from the *S. eubayanus* parent (Walsh and Martin, 1977; Kishimoto, 1994; Sato *et al*., 2002; Dunn and Sherlock, 2008; Libkind *et al*., 2011), their respective sensitivities to cold, with Saaz yeasts more cold-tolerant than Frohberg yeasts, is consistent with the proportional amount of *S. eubayanus* DNA retained in their respective genomes. Interestingly, recent genome sequencing of three *S. pastorianus* strains revealed that a large fraction of recombination events between the *S. cerevisiae* and *S. eubayanus* genomes have occurred in intragenic regions, resulting in a large number of chimeric genes (Hewitt *et al*., 2014). One such gene may be the *S. pastorianus*-specific *lg-FLO1*, an important determinant of the flocculation properties that are characteristic of lager-brewing yeast (Kobayashi *et al*., 1998). These new combinations, which can also influence regulatory regions, can have high adaptive potential, offering new ground for expanding the phenotypic landscape. A recent example illustrates this point: a chimeric gene repeatedly arose in laboratory-created interspecific hybrids (intended to be similar to lager yeast hybrids) during selective growth in low nitrogen, implying that the chimeric gene is strongly adaptive (Dunn *et al*., 2013). It appears that at least some of the genome rearrangements that occurred during the evolution of lager yeasts may have been driven by selective pressures encountered in the brewing environment.

Recent publication of the Carlsberg strain's (CBS 1513) genome sequence has shown that the Saaz yeast genome, despite being quite distinct from that of the Frohberg genome, shares a number of conserved translocation events (Walther *et al*., 2014). These data suggest that both lager groups could have a common ancestor and that the major chromosomal differences observed between the two groups may have occurred post-hybridization, possibly because of different selection pressures in the breweries in which they were employed. Further investigation into chromosomal loss and translocation within the hybrid *S. pastorianus* yeast is required and may be facilitated by the creation of novel *S. cerevisiae* × *S. eubayanus* hybrids and observation of the genomic changes that occur under different environmental conditions.

Novel lager-brewing yeast

The naturally-occurring hybrids of *S. pastorianus* have been successful in the brewing environment, presumably due to a combination of superior fermentation performance and cryotolerance, derived from their *S. cerevisiae* and *S. eubayanus* progenitors, respectively (Sato *et al*., 2002). But the genetic diversity of lager brewing strains is poor, with the existence of only two genetically distinct groups (and derived variants) available to brewers (Dunn and Sherlock, 2008). Due to limited sporulation and the low viability of spores, classical genetic approaches to improving the brewing properties of *S. pastorianus* have been limited (Gjermansen and Sigsgaard, 1981). However, rare viable spore clones of *S. pastorianus* can be produced, and crossing these strains with either laboratory or ale strains of *S. cerevisiae* appears to improve their resistance to certain stresses, including high temperature, ethanol toxicity and osmotic stress (Sanchez *et al*., 2012). These artificial hybrid strains also show improved fermentation properties, including improved performance under the stressful conditions of high sugar fermentation. It has been shown that poor sporulation due to aneuploidy can be overcome by overexpression of the *IME1* gene involved in the induction of meiosis (Smith *et al*., 1990; Ogata *et al*., 2011). Resultant meiotic segregants have potential for use in breeding programmes (Ogata *et al*., 2011). The use of genetic modification (GM) technology may, however, preclude the use of this approach in commercial lager brewing.

The recent discovery of *S. eubayanus* raises the possibility of this species being used as a lager-brewing strain in its own right. The species is tolerant to cold (Libkind *et al*., 2011) and, like all members of the genus *Saccharomyces*, has a natural ability to ferment sugars to ethanol (Piškur *et al*., 2006). To date, only one study has investigated the functional brewing properties of *S. eubayanus*, and only the type strain of this species was investigated (Gibson *et al*., 2013). The *S. eubayanus* strain was found to perform poorly at

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higher temperature (22°C) compared to other lager strains. At lower temperature (10°C), growth and fermentation performance were, however, relatively good and, while *S. eubayanus* could not ferment as well as the high-yielding Frohberg yeast included in the study, its performance was similar to that of the Saaz yeast strains. Poor attenuation in this strain (and in certain Saaz strains) may relate to a limited ability to utilize maltotriose, possibly due to a low level of transmembrane transport activity (Gibson *et al*., 2013), as mentioned above. Relatively poor fermentation performance may explain why the species had previously only been found in the brewing environment within the *S. cerevisiae* × *S. eubayanus* hybrid complex. Further research is required to determine the industrial potential of *S. eubayanus* and greater availability of strains will aid in this endeavour. It will be interesting to see how other geographic subpopulations of *S. eubayanus* compare with those found in South America, particularly in relation to brewing-relevant functions, such as α-glucoside use or flocculation. It is likely that distinct geographic subpopulations of *S. eubayanus* vary in terms of genome content (presence and absence of genes), with this kind of variation having a large effect on phenotypic variability (Bergström *et al*., 2014).

Although the use of *S. eubayanus* alone as a novel lager-brewing yeast appears infeasible at this time, it can be used for the creation of novel artificial hybrids of *S. pastorianus* (Pérez-Travéz *et al*., 2012). Selection of individual *S. eubayanus* or *S. cerevisiae* strains with specific desirable characteristics, and hybridization of these strains, can now be carried out for tailored construction of non-GM production yeast strains immediately available for industrial use. These designed strains could even incorporate properties not typical of lager yeast. For example, 4-vinylguaiacol is formed by decarboxylation of (free) ferulic acid, catalysed by phenylacrylic decarboxylase (encoded by the gene *PAD1*). *PAD1* is not found in lager strains of brewer's yeast (Coghe *et al*., 2004) but is present in some ale strains, including all tested strains used for production of wheat beers, and its action imparts a distinctive clove-like taste to these beers (Vanbeneden *et al*., 2008). This approach to generating new *S. pastorianus* strains does not have the same problem of intractability found when creating and crossing rare meiotic segregants of existing lager yeast strains, and is expected to greatly increase the phenotypic diversity of strains available for brewing in the future. Mimicking fermentative stress conditions (e.g. limited nitrogen source) in designed *S. cerevisiae* × *S. uvarum* hybrids resulted in recombinant chimeric chromosomes (Dunn *et al*., 2013), suggesting that this method could be used with novel *S. cerevisiae* × *S. eubayanus* hybrids to generate even more genomic diversity and possibly to induce adaptive mutations and rearrangements, while at the same time avoiding a sporulation step, with its intrinsic gamete viability problems.

Adaptive evolution of *S. pastorianus*

The whole-genome duplicaton (WGD) that occurred in the shared *Saccharomyces* ancestor ca. 100 million years ago (Wolfe and Shields, 1997) was followed by rapid gene loss and major chromosomal rearrangement (Scannell *et al*., 2006), a process that appears to be universal, as it occurs also in polyploid and hybrid plants and animals (Albertin and Marullo, 2012). The genome doubling that occurs when interspecific hybrids, such as *S. pastorianus*, form has likewise resulted in major genomic changes. The sequenced strain W34 (a Frohberg type) contains 36 chromosomes, with eight-chromosome translocations occurring between the two subgenomes, as well as multiple internal rearrangements (Nakao *et al*., 2009). The resultant *S. pastorianus* chromosomes are therefore *S. cerevisiae*-type, *S. eubayanus*-type or mosaics containing regions derived from both parents. In the majority of cases, divergent *S. cerevisiae* and *S. eubayanus* orthologues of each gene are present. However, in a number of these pairs, one orthologue is non-functional due to truncation as a result of frame-shift mutation or presence of a premature stop codon. It is believed that these mutations occurred post-hybridization, due to the fact that several of the genes are believed to be essential for survival and therefore were likely to be functional in both parental strains (Nakao *et al*., 2009). Interestingly, many of these mutations occur in genes associated with lager yeast fermentation characteristics, such as sulphite transport and metabolism. Genes related to maltose and maltotriose transport, including *S. cerevisiae AGT1* and *S. eubayanus MALx1*, have been subject to this loss of function.

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The *S. cerevisiae* Agt1 transporter is known to be cold-sensitive and it may be speculated that its loss may have freed space in the cell membrane for the less sensitive *S. eubayanus* version of the transporter (Vidgren and Londesborough, 2012; Cousseau *et al*., 2013), thereby potentially increasing the uptake of α-glucoside sugars from wort during lager fermentation (Vidgren *et al*., 2010).

The inherent instability of their genomes appears to promote evolutionary adaptation of yeast hybrids to their respective environments and also helps to explain their increased fitness relative to the parent species (Antunovics *et al*., 2005; James *et al*., 2008; Kunicka-Styczyńska and Rajkowska, 2011; Louis *et al*., 2012; Piotrowski *et al*., 2012; Dunn *et al*., 2013). A hybrid may initially inherit genes and properties of both parents equally, but over time the characteristics of one parent may become dominant, depending on the evolutionary pressures to which the hybrid is exposed. A number of investigations have demonstrated the feasibility of directing evolution in natural *S. pastorianus* hybrid stains in order to create variant strains with improved functional properties. Such investigations have focused on adaptation to very high-gravity brewing conditions (Blieck *et al*., 2007; Huuskonen *et al*., 2010; Yu *et al*., 2012) or associated stresses, such as osmotic stress and ethanol toxicity (James *et al*., 2008; Ekberg *et al*., 2013). Adaptive evolution has also been utilized to modify the production of flavour compounds (Strejc *et al*., 2013). To what extent the success of artificial evolution in *S. pastorianus* has been due to hybrid genome instability is not known and the relationship between instability and adaptation requires further study. If the genomic instability that follows interspecies hybridization confers added genotypic and phenotypic malleability to the newly-formed hybrid, then this property could be exploited in artificial *S. pastorianus* hybrids (see above) to create tailored variant strains with desired phenotypes. Experimental evolutionary approaches offer a valid alternative to the difficulties of targeted genetic manipulation (Murakami *et al*., 2012) and do not have the same commercial restrictions as GM organisms.

Summary

Previous research on the biology of *S. pastorianus* had been impeded somewhat by a lack of basic knowledge about its genome. Recent advances in genomic technologies have helped to clarify the nature of this complex organism. It is now recognized that the taxon may not be derived from a single lineage, but could include descendants of at least two separate and independent interspecific hybridization events involving *S. cerevisiae* and *S. eubayanus*. Typical lager yeast characteristics appear to be due to a combination of traits inherited from both parents, as well as a considerable amount of postzygotic chromosomal loss and rearrangement. Until now, brewers have relied on a relatively small number of lager yeast strains exhibiting limited phenotypic diversity, in contrast to an often wide variety of strains available in other fermentation industries. Techniques are now available that permit meiotic segregation and mating of lager yeast segregants, thereby allowing the design and creation of tailor-made production lager strains. In addition, the availability of *S. eubayanus* isolates for creation of novel artificial *S. pastorianus* hybrids has the potential to greatly increase the genotypic and phenotypic diversity of strains available for use in the lager-brewing industry, without recourse to genetic modification.

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