

## MINIREVIEW

# The complex and dynamic genomes of industrial yeasts

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## Introduction

The *Saccharomyces sensu stricto* complex (Vaughan-Martini & Martin, 1995), recently raised to the level of genus (Kurtzman, 2003), currently includes the species *Saccharomyces cerevisiae*, *Saccharomyces bayanus* and *Saccharomyces pastorianus*, associated with anthropic environments due to their high fermenting capabilities; *Saccharomyces paradoxus*, mainly isolated from natural habitats; the species *Saccharomyces cariocanus* and *Saccharomyces mikatae* (Naumov *et al.*, 2000b); *Saccharomyces kudriavzevii*, first isolated from decayed leaves and soils in Japan, and from oaks in Portugal (Sampaio & Goncalves, 2008); and the recently described *Saccharomyces arboricolus*, isolated from oak trees in China (Wang & Bai, 2008). This genus contains many species that are industrially important.

While *S. cerevisiae* is the predominant species responsible for alcohol fermentation, *S. bayanus* var. *uvarum* (or simply *S. uvarum*; Pulvirenti *et al.*, 2000) has been described as adapted to low-temperature fermentations during wine-making (Naumov, 2000a; Naumov *et al.*, 2002) and cider production (Naumov *et al.*, 2001; Coton *et al.*, 2006). *Saccharomyces paradoxus* has been isolated from Croatian wines (Redzepović *et al.*, 2002). The species associated with the brewing of beers such as lagers and ales display distinct physiological characteristics that reflect their different gen-

## Abstract

The *Saccharomyces sensu stricto* genus contains many species that are industrially important for fermentation of wines, beers and ales. The molecular characterization of the genomes of yeasts involved in these processes reveals that the majority arose from interspecific hybridization between two and sometimes three yeast species. The hybridization events generated allopolyploid genomes, and subsequent recombination events between the parental genomes resulted in the formation of mosaic chromosomes. The polyploid and hybrid nature of the genomes confers robust characteristics such as tolerance to environmental stress to these industrial yeasts and provides a means for adaptive evolution.

ome make-up. The lager yeasts, originally referred to as *Saccharomyces carlbergensis*, are now classified as *S. pastorianus* (Rainieri *et al.*, 2006). Ale yeasts are predominantly *S. cerevisiae*; however, *S. bayanus* strains have also been isolated from beer. Ale yeasts are often referred to as top-fermenters due to the fact that they tend to float on the top of the vat at the end of the fermentation while the lager yeasts are classified as bottom-fermenters as they flocculate and accumulate at the bottom of the vat.

Taxonomic classification within the genus *Saccharomyces* is complicated by the fact that the majority of the strains display common physiological and morphological characteristics. Additionally, the biological definition of speciation is difficult to apply to strains within the genus as many natural isolates show poor mating ability and sporulation and in many cases contain complex hybrid genomes containing genetic information from other species within the genus.

## The genomes of brewing yeasts

### Lager yeasts

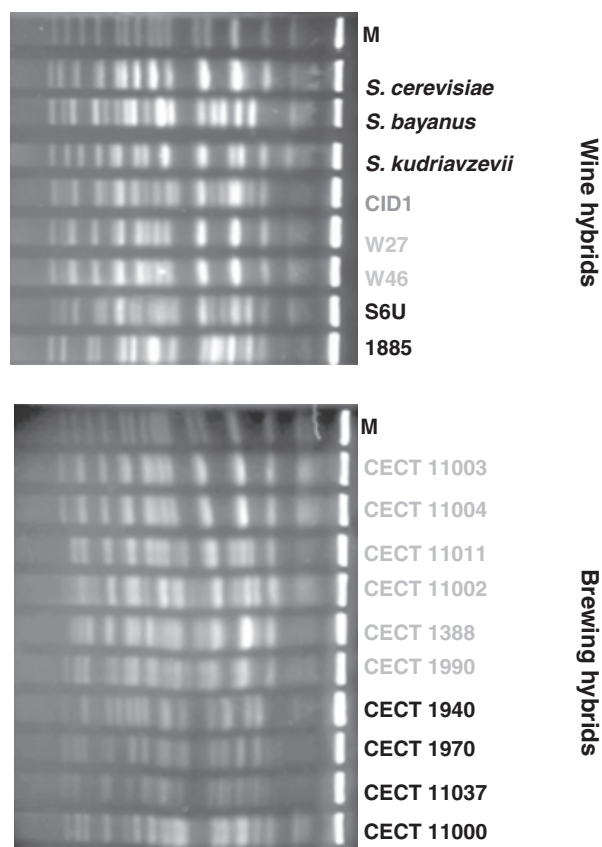
Molecular characterization of lager yeasts indicates that all strains contain complex polyploid genomes that are generally believed to have arisen from an interspecific

hybridization event between two different yeast species (Kielland-Brandt *et al.*, 1995; Bond & Blomberg, 2006; Rainieri *et al.*, 2006; Caesar *et al.*, 2007; Smart, 2007; Dunn & Sherlock, 2008). Following the species fusion, the genomes appear to have undergone a duplication event, leading to the formation of the allotetraploid strain (Kielland-Brandt *et al.*, 1995). Alternatively, the parental strains may have been diploids, which fused to generate a tetraploid. Subsequent genome changes, such as chromosome loss and/or duplications, have resulted in unequal numbers of chromosomes in the present-day strains (Fig. 1), a state referred to as aneuploidy.

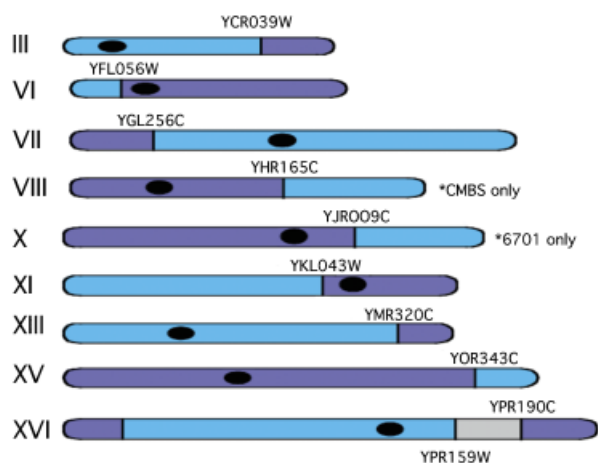
Early genetic characterization of lager yeasts (Nilsson-Tillgren *et al.*, 1981) provided evidence for the hybrid nature and general allotetraploid DNA content of the genome. Using the technique of single chromosome transfer into *kar1* mutants of *S. cerevisiae*, several individual chromosomes from lager strains were isolated and genetically

analysed. The first such analysis, of chromosome III from an *S. carlsbergensis* strain, indicated that the lager yeast chromosome was functionally equivalent to the *S. cerevisiae* chromosome (Nilsson-Tillgren *et al.*, 1981). However, analysis of the rates of recombination between genetic markers on chromosome III identified a structural difference between the chromosomes. This and subsequent genetic analyses of chromosomes V, VII, X, XII and XIII provided evidence for three types of chromosomes in the lager yeasts: (1) homologous chromosomes that readily recombined with *S. cerevisiae* chromosomes, (2) homeologous chromosomes that rarely recombine and (3) mosaic chromosomes that recombined only with certain regions of the *S. cerevisiae* chromosome and were assumed to be composed to regions of the homologous and homeologous chromosomes (Casey, 1986a; Nilsson-Tillgren *et al.*, 1986; Kielland-Brandt *et al.*, 1995). Molecular analysis of chromosome composition by pulse field gel electrophoresis (Casey, 1986a; Tamai *et al.*, 1998), together with DNA sequence analysis of individual genes, confirmed the composite nature of the lager yeast chromosomes (Holmberg, 1982; Pedersen, 1985; Casey, 1986b; Hansen & Kielland-Brandt, 1994; Hansen *et al.*, 1994). Finally, data from the complete sequencing of the whole genome of one strain of *S. pastorianus* (Weihenstephan 34/70) suggest that the parental strains contributing to the lager yeasts closely resemble *S. cerevisiae* and *S. bayanus*. *Saccharomyces cerevisiae*-like genes showed 99% identity to the published *S. cerevisiae* sequence (Kodama *et al.*, 2005), while the other homeologues were 75–85% identical to *S. cerevisiae* and 93–99% identical to the published *S. bayanus* gene sequence (Casaregola *et al.*, 2001; Kodama *et al.*, 2005).

The nature of the chromosomal composition of *S. pastorianus* strains has been addressed using competitive genomic hybridization (CGH) (Bond *et al.*, 2004) and DNA sequencing (Kodama *et al.*, 2005) technologies. CGH analysis of two different lager yeast strains, 6701 and CMBS, identified recombination events between the two parental genomes at specific locations on chromosomes III, VI, VII, X, XI, XIII, XV and XVI, in both strains, leading to the generation of mosaic or hybrid chromosomes. The exact location of recombination sites can be clearly defined at a single gene resolution using the CGH approach (Fig. 2). Most recombination sites are proximal to Ty elements, tRNA gene clusters or in regions of highly repetitive DNA sequences. Certain recombination sites are unique to individual strains (see Fig. 2). One striking discovery from the CGH analysis was the identification of a region on chromosome XVI, from YRP160 to YPR190, which appears to exclusively contain *S. bayanus*-like alleles in all strains (Fig. 2). Subsequent DNA sequence analysis of one *S. pastorianus* strain (Weihenstephan) (Kodama *et al.*, 2005) confirmed the presence of three types of chromosomes, referred to as (1)



**Fig. 1.** Electrophoretic karyotyping of wine and brewing yeasts: chromosomal profiles of the parental strains *Saccharomyces cerevisiae* (FY 1679), *Saccharomyces bayanus* (CECT 1969) and *Saccharomyces kudriavzevii* (IFO 1802<sup>T</sup>) and a number of *S. cerevisiae* × *S. kudriavzevii* (grey) and *S. cerevisiae* × *S. bayanus* (*Saccharomyces pastorianus*) hybrids (black), isolated from wine or beer fermentations. The cider strain triple hybrid CID1 is also shown. Lane m corresponds to the standard marker strain *S. cerevisiae* YNN295 (BioRad).



**Fig. 2.** *Saccharomyces pastorianus* strains possess mosaic chromosomes resulting from recombination between the parental homoeologous chromosome (depicted by blue and navy sections). The locations of the sites of recombination are shown in relation to the centromeres (black dots). The grey region on XVI depicts a region consisting of *Saccharomyces bayanus* genes only. Certain rearrangements are unique to specific strains\*. Generated from data presented in Bond *et al.* (2004).

*S. cerevisiae*-like, (2) *S. bayanus*-like and (3) mosaics resulting from recombination events between the homoeologous chromosomes.

Since the initial analysis (Bond *et al.*, 2004), several more strains or substrains of *S. pastorianus* have been analysed using CGH. Dunn & Sherlock (2008) used CGH to characterize 17 strains of *S. pastorianus* from a variety of geographic locations and representing different beer types. These authors concluded that all lager strains can be classified into two distinctive groups (Group I and Group II) based on the number of copies of the parental chromosomes, the location of genome rearrangements and DNA polymorphisms. DNA sequence analysis of several *S. cerevisiae*-like genes within the *S. pastorianus* genome predicts that the parental strain most likely was an ale-type *S. cerevisiae*, confirming earlier genotype analysis (Legras *et al.*, 2007). The *S. cerevisiae* content of the Groups I and II lager yeasts is almost identical, with only a 0.3% nucleotide variation between the two groups. The original production strains analysed by Bond *et al.* (2004) and Kodama *et al.* (2005) most closely resemble the newly identified Group II strains.

### Ale yeasts

Ale brewing strains constitute a broad variety of *Saccharomyces* species, most of which seem to be closely related to *S. cerevisiae*, but some of the ale strains also appeared to be hybrids (Rainieri *et al.*, 2006). Genotype analysis of 651 different *S. cerevisiae* strains, using 12 microsatellite loci,

revealed a complex relationship between beer, bread and wine yeasts. As mentioned above, ale and lager yeasts clustered together in this analysis, but tetraploid ales most closely resembled tetraploid bread strains (Legras *et al.*, 2007). González demonstrated the presence among ale strains of a new type of hybrid between *S. cerevisiae* and *S. kudriavzevii* (González *et al.*, 2008). Some of these hybrids were isolated as predominant in Trappist beer bottles, where a secondary fermentation takes place. These strains were originally misidentified as *S. cerevisiae*, which suggests that an important fraction of ale strains classified as *S. cerevisiae* may correspond to hybrids contributing to the complexity of the *Saccharomyces* diversity in brewing environments. To date, there have been no CGH studies carried out on ale yeasts.

## The genomes of wine hybrids

### Hybrids between *S. bayanus* and *S. cerevisiae*

Several *S. bayanus* var. *uvarum* × *S. cerevisiae* hybrid strains have been isolated from Italian wines, S6U (Masneuf *et al.*, 1998), Hungarian Tokaj wine (Antunovics *et al.*, 2005) and Alsatian wines in France (Demuyter *et al.*, 2004; Le Jeune *et al.*, 2007). An *S. bayanus* var. *bayanus* × *S. cerevisiae* hybrid was isolated from a wine fermentation in Valladolid, Spain (González *et al.*, 2006). However, most of these hybrids have scarcely been characterized. Some other strains have only been characterized by restriction fragment length polymorphism (RFLP) analysis of a few genes. Additional studies, similar to those performed for lager hybrid yeasts, are required to understand their genome constitution and their origins.

### Hybrids between *S. cerevisiae* and *S. kudriavzevii*

Hybrids resulting from hybridization between *S. cerevisiae* and *S. kudriavzevii* have been described among wine yeast (González *et al.*, 2006). The hybrid nature of some of these wine strains was unknown due to the similarity of their chromosomal profiles to that of the *S. cerevisiae* parent species (Fig. 1). These strains, misidentified as *S. cerevisiae*, appeared to be predominant during spontaneous wine fermentations in the wine region of Zurich, Switzerland (Schütz & Gafner, 1994), and some of them were selected as commercial strains adapted to fermentations in cold areas of Central Europe (strains W27 and W46 from Lallemant Inc.). Wine strains of a similar hybrid nature were also predominant in some wine regions of Austria (Lopandic *et al.*, 2007). González *et al.* (2006) also found a triple hybrid *S. bayanus* var. *uvarum* × *S. cerevisiae* × *S. kudriavzevii* among wine strains from Switzerland.

The genetic characterization of the wine *S. cerevisiae* and *S. kudriavzevii* hybrids by restriction analysis of five nuclear genes located in different chromosomes, the 5.8S-internal transcribed spacer (ITS) rRNA gene region and the mitochondrial COX2 gene revealed the presence of three types of hybrids in Swiss wines (González *et al.*, 2006). Belloch and Querol (unpublished data) have characterized the genomic constitution of several wine *S. cerevisiae* × *S. kudriavzevii* strains using a combined approach based on the RFLP analysis of gene regions, comparative genome hybridizations with *S. cerevisiae* DNA arrays, ploidy analysis and gene dose determination by quantitative real-time (RT)-PCR. This analysis confirmed the presence of different, but related, genome types among wine hybrids that contain putative chimerical chromosomes in the cases of chromosomes IV, V, IX, XIV, XII and XV and also in shorter subtelomeric regions in several chromosomes, for example, chromosomes VII and XVI (Fig. 3). These hybrids appeared as almost diploid (*c.* 2.3*n*), with two copies of most chromosomes, estimated by quantitative RT-PCR analysis, and three for chromo-



**Fig. 3.** Genome composition in wine hybrids of *Saccharomyces cerevisiae* × *S. kudriavzevii*. *Saccharomyces cerevisiae* chromosomes are represented as black lines and *S. kudriavzevii* by white lines. Chimerical chromosomes IV, V, IX, XIV, XII and XV are indicated by black and white segments. Centromeres are shown as grey lines.

somes V and XIV. The recombination sites in the chimerical chromosomes XIV and XV are located between genes flanking large regions containing Ty1 delta, Ty3 sigma, Ty4 tau elements and tRNA genes. In chromosome XII, recombination occurs in the large cluster of 100–200 tandem repeats containing the highly conserved rRNA (*RDN*) genes. However, on chromosomes IV, V and IX, recombination sites appear to be located within protein-coding genes. Molecular analyses of natural wine hybrids revealed an extensive variation in genome organization.

### Triple hybrids

Yeast strains arising from triple hybridization events between *S. cerevisiae* × *S. uvarum* × *S. kudriavzevii* have been identified, the most well characterized being the CID1 strain isolated from cider in Brittany, France (Masneuf *et al.*, 1998) (Fig. 1). Flow cytometry analysis of DNA isolated from the CID1 strain revealed a near-triploid amount of DNA, suggesting that at a minimum, this strain is triploid in nature (Naumova *et al.*, 2005). These strains appear to be alloaneuploids based on analysis of the parental alleles for a number of nuclear genes (Masneuf *et al.*, 1998; González *et al.*, 2006). The CID1 genome appears to lack a copy of the *S. kudriavzevii*-type MET2 allele, and both the *S. cerevisiae*-type and the *S. kudriavzevii*-type ITS1–5.8S–ITS2 sequences are also missing. On the other hand, all three parental alleles of the ACT1 gene are present.

### The mitochondrial genomes of lager and wine yeasts

It is generally accepted that the mitochondrion genomes (mtDNA) in hybrid yeasts are homoplasmic and uniparentally inherited. Restriction analysis of mtDNA from *S. pastorianus* type strains CBS 1538 and CBS 1153 (*S. carlsbergensis*) revealed a high degree of similarity to that of *S. bayanus* (Piškur *et al.*, 1998). This was later confirmed from whole-genome sequencing of the Weihenstephan 34/70 strain (Kodama *et al.*, 2005). That study determined that the mitochondrial genome was smaller than that of *S. cerevisiae* containing only 70 kbp compared with 85.8 kbp in the latter. A recent comprehensive analysis of the inheritance of mtDNA from 22 lager yeast strains revealed that the *S. cerevisiae* mtDNA is never inherited in these hybrids and that the COX2 gene sequence was identical to *S. bayanus* strain NBRC 1948. Surprisingly, hybrids constructed *in vitro* inherited equally the mtDNA from the parental strains, implying a selective advantage to acquiring the *S. bayanus* mtDNA in the natural lager yeast hybrids (Rainieri *et al.*, 2008).

Likewise, it appears that the mtDNAs of all wine yeast hybrids are homoplasmic; however, it appears that they can be inherited from either parent under some circumstances.

For example, a number of *S. cerevisiae* × *S. uvarum* strains had either *S. cerevisiae*-like or *S. uvarum*-like COX2 alleles, whereas hybrids resulting from *S. kudriavzevii* hybridizations all inherited the *S. kudriavzevii*-like COX2 sequences (González *et al.*, 2006; Lopandic *et al.*, 2007). Surprisingly, analysis of the COX2 gene from the CID1 strain differed significantly from that of the type strain of *S. kudriavzevii* and the other *S. kudriavzevii* hybrids, suggesting a different origin for the mtDNA in this triple hybrid.

## The polyploid nature of lager and wine yeast genomes

### Lager yeasts

Early genetic studies described lager yeasts as being allotetraploid, possessing two copies of each of two homoeologous alleles of most genes (Hansen & Kielland-Brandt, 1994; Hansen *et al.*, 1994; Borsting *et al.*, 1997). This is borne out by DNA content determination and flow cytometry analysis that identified a broad  $4n$  peak in a variety of lager yeasts (J. Usher, T.C. James & U. Bond, unpublished data). RT-PCR has been used to determine, at the individual gene resolution, the number of copies of *S. cerevisiae* genes in *S. pastorianus* strains (Bond *et al.*, 2004). Based on this analysis, the gene copy number of individual *S. cerevisiae* genes varies from one to six, indicating the true aneuploid nature of the genome. By combining CGH data with RT-PCR analysis, it is possible to deduce the minimum number and composition of each chromosome in *S. pastorianus* strains (Bond & Blomberg, 2006).

As mentioned above, Dunn & Sherlock (2008) distinguished two broad classes of lager yeasts (Groups I and II). The CGH data suggest that these groups differ in the *S. cerevisiae* genome content, with Group I containing one (or less) *S. cerevisiae* genome equivalents and Group II containing two genome equivalents. Both groups contain one *S. bayanus* genome equivalent. Presumably, these ratios reflect the minimum chromosome content, and multiple copies of the *S. cerevisiae*, *S. bayanus* and hybrid chromosomes must be present to generate the allotetraploid genome. Taken together, it appears that lager strains vary in the copy number of the parental chromosomes and the number and type of hybrid chromosomes. Ultimate determination of the DNA content of individual strains will require complete genome sequencing. Currently, only one lager yeast strain (Weihenstephan 34/70) has been sequenced, but these sequence data are not publically available to date.

### Wine yeasts

The hybrid genomes, from hybridization among different *Saccharomyces* species and containing complete sets of

chromosomes from the partners, can be allodiploid or allotetraploid. Other hybrids have only portions of the partner genomes in the form of extra (supernumerary) chromosomes and mosaic or chimerical chromosomes generated by recombination between homeologous chromosomes.

The wine *S. cerevisiae* × *S. bayanus* var. *bayanus* hybrid strain, CECT 1885, appears to be an alloaneuploid based on data showing that it lacks both parental alleles of six nuclear genes analysed. In fact, it seems more similar to lager strains due to the absence of *S. cerevisiae* alleles (González *et al.*, 2006). The strain S6U appeared as a perfect allotetraploid, according to its DNA content per cell measured by flow cytometry (Naumov *et al.*, 2000c).

The *S. cerevisiae* × *S. kudriavzevii* hybrids characterized so far appear as alloaneuploids. Both the intricate electrophoretic karyotypes (Fig. 1) exhibited by these hybrids as well as the molecular characterization of their genes by PCR/RFLP analysis are indicative of the presence of strain-specific gross chromosomal rearrangements resulting in the loss of chromosomal regions from the *S. kudriavzevii* parent (González *et al.*, 2008). A similar situation was observed in wine *S. cerevisiae* × *S. kudriavzevii* hybrids from Switzerland (González *et al.*, 2008). These strains are almost diploid and contain chimerical recombinant chromosomes (Belloch and Querol, unpublished data). It appears that *S. cerevisiae* × *S. kudriavzevii* hybrids exhibit a trend to maintain the *S. cerevisiae* genome and to reduce the *S. kudriavzevii* fraction, while preserving the *S. kudriavzevii* mitochondrial genome (González *et al.*, 2008).

## Consequences of hybrid genomes

The polyploid and aneuploid nature of the lager and wine yeast genomes pose both unique opportunities and problems for these organisms. It has been argued that allopolyploid genomes are in an evolutionary flux and can accommodate a higher rate of evolutionary change than their haploid counter parts, thus allowing for increased opportunities for adaptive evolution and providing possibilities for long-term diversification and evolutionary success. The extensive and variable allopolyploidy found in the genomes of hybrid strains of *Saccharomyces* species, as exemplified by the hybrid strains of lager and wine yeasts, is suggestive of such ongoing evolutionary fluxes.

Polyploidy is believed to reduce the fitness of an organism (Mable & Otto, 2001; Thorpe *et al.*, 2007). Studies have shown that autopolyploid yeasts show a marked decrease in survival during the stationary phase (Andalis *et al.*, 2004). Analysis of the effects of aneuploidy on cellular physiology identified a number of phenotypes such as defects in cell cycle progression, increased glucose uptake and alterations in protein synthesis, folding and degradation (Torres *et al.*,

2007). Taken together, the data suggest that polyploidization (and specifically aneuploidy) can lead to changes in cellular physiology that affect the viability and the subsequent survival of an organism. Polyploidization also leads to increased cell size, which can adversely affect the stoichiometric relationship between various architectural components within the cells (Andalis *et al.*, 2004). In the case of lager yeasts, the aneuploid nature of the chromosomes is believed to account for the inability of these strains to mate and their poor spore viability, leading to genetically isolated species. Yet, despite these apparent defects, the allopolyploid lager yeasts show characteristics of increased fitness under certain environmental conditions such as their ability to grow at low temperatures and to survive under the harsh environmental conditions experienced during industrial fermentations.

A few wine *S. cerevisiae* × *S. bayanus* var. *uvarum* hybrids have been characterized genetically. Hybrid S6U, an allotetraploid (Naumov *et al.*, 2000c) that contains the complete genomes of *S. cerevisiae* and *S. uvarum* (A. Querol, unpublished data), can produce viable F1 spores (Johnston *et al.*, 2000). The F1 clones formed by the F1 spores also sporulate, but the spores are nonviable, which indicates that S6U segregates into allodiploids during meiosis (Naumov *et al.*, 2000c). The nonviability of the F2 spores further indicates that the F1 spores are heterozygous at the mating type locus *MAT*. The hybrids RC1-1, RC1-11, RC2-12, RC2-19, RC4-87, RP1-4, RP2-5, 191 RP2-6 and RP2-17 isolated in an Alsace winery have diploid or near-diploid amounts of DNA and produce nonviable F1 spores (Le Jeune *et al.*, 2007).

### Genome instability in polyploid yeasts: the influence of environmental stress

Polyploidy and aneuploidy are associated with increased genome instability (Storchova & Pellman, 2004). High levels of chromosome instability have been observed previously in autopolyploid strains of *S. cerevisiae* (Mayer & Aguilera, 1990). Tetraploid strains of *S. cerevisiae* display an *c.* 400-fold increase in chromosome loss compared with isogenic diploid strains (Andalis *et al.*, 2004). In a recent study to identify the genes required for viability in polyploids, a set of 39 'polyploidy-specific lethality' genes have been identified (Storchova *et al.*, 2006). The majority encoded for proteins involved in the maintenance of genome stability, involving functions related to homologous recombination, sister chromatid cohesion or mitotic spindle function.

Lager and wine yeasts are subjected to a variety of environmental stresses during the process of fermentation. Lager yeasts are exposed to high osmotic and hydrostatic pressure, anaerobiosis, low pH, low temperature, high alcohol concentrations and high cell density (Attfield, 1997;

Brosnan *et al.*, 2000; Carrasco *et al.*, 2001; Zuzuarregui & del Olmo, 2004; Zuzuarregui *et al.*, 2005; Gibson *et al.*, 2008). The environment of wine fermentations poses different, but no less stressful challenges to the yeast. Here, yeasts are exposed to very high ethanol concentrations, strong acidity, high sugar and the presence of sulphites. One may speculate that the combined stresses might pose a life-threatening challenge to these yeasts. However, the hybrid genome of lager and wine yeasts appears to confer a very high degree of resistance to these stresses in comparison with their haploid parents. For example, the parental species of the lager yeasts, *S. cerevisiae* and *S. bayanus*, do not survive well under industrial fermentation conditions and are much less capable of metabolizing the available sugars to ethanol. Such an adaptation may be anticipated because these strains have been selected over many hundreds of years, thereby enriching for stress-tolerant variants.

The adaptation of wine to the harsh conditions prevailing in grape musts and wines is also shaped by their genomes (Pretorius, 2000; Querol *et al.*, 2003). The two main wine species, *S. cerevisiae* and *S. bayanus* var. *uvarum* (*S. uvarum*), are able to grow in substrates characterized by high sugar and ethanol contents, and low pH, demonstrating that their genomes are well adapted to oenological conditions. Some wine hybrids have been characterized for their physiological properties of enological interest (Gonzalez *et al.*, 2007; Belloch *et al.*, 2008). These hybrid strains retained the ethanol tolerance and ability to grow in media with a high sugar content of the *S. cerevisiae* parent, but clearly grew better at low temperatures, a trait inherited from the non-*S. cerevisiae* parent (Belloch *et al.*, 2008).

As mentioned above, the genetic analysis of autopolyploid strains of *S. cerevisiae* has revealed a general trend of genome instability, rearrangements and chromosome loss. Additionally, autopolyploid strains of *S. cerevisiae* appear to show reduced fitness compared with haploids. The question arises therefore as to why lager and wine yeast strains retain their allopolyploid genomes and appear to derive selective advantages from this status. Polyploidy in industrial yeast strains raises the possibility that genome instability may occur in these species, influenced by the environmental stresses encountered during fermentations.

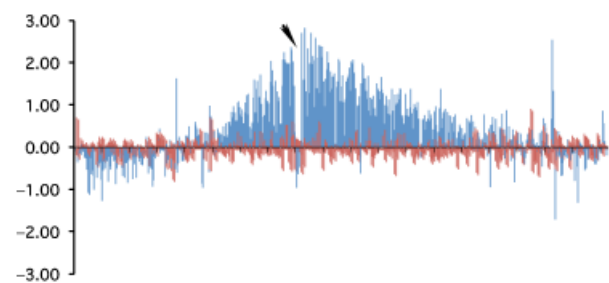
A recent analysis of clonal isolates of 'stress-tolerant' lager yeasts, isolated following exposure to severe high temperatures and then selection for growth under high specific gravity stress conditions (22% maltose), revealed that each had undergone gross rearrangements, small deletions and regional amplifications, some of which were specific to each isolate (James *et al.*, 2008). The majority of the rearrangements mapped to the previously identified recombination sites (refer to Fig. 2). These experiments showed for the first time that genome rearrangements can be induced in allopolyploid yeast cells by exposure to environmental stress. This

finding is of significance to the brewing industry as the stability of proprietary strains is of utmost importance to the industry. This observation also raised the question 'Are the genomes of lager yeasts stable under fermentation conditions and secondly, can changes in the environmental conditions experienced by the cells influence their genome stability?'

Analysis of lager yeasts grown in either 16% (low specific gravity) or 22% (high specific gravity) maltose medium at either the standard low temperatures of brewery fermentations (13 °C) or at room temperature (20 °C) revealed that growth of cells in the higher sugar concentration and at room temperature as opposed to growth at the low temperature and low specific gravity resulted in greater genome instability in a number of defined regions. The areas of greatest instability were located at the telomeres of a subset of chromosomes and at specific regions on chromosomes I and XII (James *et al.*, 2008). The latter site was centred at the major rRNA gene cluster (Fig. 4), while the amplification on chromosome I was located in a region referred to as DUP240, which contains genes belonging to one of the largest gene families in yeast. The multigene family consists of at least 10 genes with a high level of nucleotide identity (from 50% to 98%), scattered on four chromosomes and arranged either as tandem repeats or as isolated genes (Despons *et al.*, 2006). Short repetitive DNA sequences identified in this cluster have been implicated in large chromosomal rearrangements observed at the tandem DUP240 loci on chromosome I through nonallelic recombination events (Leh-Louis *et al.*, 2004).

## Conclusions

The diversity of *Saccharomyces* hybrids from different species, their different origins and their presence in diverse fermenta-



**Fig. 4.** CGH analysis of a lager yeast strain fermented in high specific gravity wort (22% maltose). DNA was isolated on day 1 and day 8 of fermentation and differentially labelled with Cy5 and Cy3. Copy number variations, as depicted by changes in ratio of hybridization (y-axis, log<sub>2</sub> scale), are observed for genes surrounding the rRNA gene locus (arrow) on chromosome XII when comparing DNA from day 1 and day 8 (blue). CGH of DNA from day 1 differentially labelled with Cy5 and Cy3 shows no variation (red). Adapted from Fig. 3, James *et al.* (2008), with permission from Springer.

tion processes indicate that interspecific hybridization is not such a rare event in the *Saccharomyces* genus, in spite of the homothallic character of most natural *Saccharomyces* strains and the persistence of their asci. The finding that *S. bayanus* and *S. cerevisiae* species coexist during winemaking, brewing, cider production, etc., has led some authors to propose that hybrids could be generated in these environments by rare-mating between diploid strains (de Barros Lopes *et al.*, 2002). In the case of *S. cerevisiae* × *S. kudriavzevii* hybrids, there are several evidences suggesting that the hybridization events likely occurred in the wild and not in fermentation environments. *Saccharomyces cerevisiae* wine strains, better adapted to grow at higher temperatures, have problems in performing fermentations at a low temperature. Under such climate conditions, cryotolerant species, such as *S. bayanus* var. *uvarum* or *S. kudriavzevii* may outcompete *S. cerevisiae*. Under such circumstances, however, hybrids will have clear advantages over the parental species.

In the case of lager yeasts, Dunn & Sherlock (2008) argue for separate origins for Groups I and II lager yeasts and hypothesize that Group 1 strains arose as a hybrid between a haploid ale *S. cerevisiae* yeast spore and a haploid *S. bayanus* spore, followed by loss of large portions of the *S. cerevisiae* DNA content, while Group II strains arose from a fusion between a diploid *S. cerevisiae* with a haploid *S. bayanus*. The large variations of *S. cerevisiae* DNA content in lager strains and the relative stability of the cryotolerant *S. bayanus* DNA content may reflect adaptive pressures to cold fermentations and other environmental stresses experienced during the brewing process. The finding that the allopolyploid genomes of lager yeasts are dynamic and can undergo rearrangements, gene amplification and general genome instability in response to exposure to environmental stress (James *et al.*, 2008) may point to the molecular mechanisms for adaptive evolution of yeast species driven by environmental influences (Hittinger & Carroll, 2007; Coyle & Kroll, 2008).

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