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Research review paper

Brewing up a storm: The genomes of lager yeasts and how they evolved

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ABSTRACT

Yeasts used in the production of lager beers belong to the species *Saccharomyces pastorianus*, an interspecies hybrid of *Saccharomyces cerevisiae* and *Saccharomyces eubayanus*. The hybridisation event happened approximately 500–600 years ago and therefore *S. pastorianus* may be considered as a newly evolving species. The happenstance of the hybridisation event created a novel species, with unique genetic characteristics, ideal for the fermentation of sugars to produce flavoursome beer. Lager yeast strains retain the chromosomes of both parental species and also have sets of novel hybrid chromosomes that arose by recombination between the homeologous parental chromosomes. The lager yeasts are subdivided into two groups (I and II) based on the *S. cerevisiae*: *S. eubayanus* gene content and the types and numbers of hybrid chromosomes. Recently, whole genome sequences for several Group I and II lager yeasts and for many *S. cerevisiae* and *S. eubayanus* isolates have become available. Here we review the available genome data and discuss the likely origins of the parental species that gave rise to *S. pastorianus*. We review the compiled data on the composition of the lager yeast genomes and consider several evolutionary models to account for the emergence of the two distinct types of lager yeasts.

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1. Introduction

1.1. Anthropogenic and human cultural influences on the evolution of S. pastorianus

The history of the art of brewing is intimately associated with the social and cultural development of the human race, and has been

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influenced by human migration and the ancient customs and practices of brewing. Beer has been brewed for several millennia and most likely evolved independently in many societies during human development. Archaeological findings show evidence of brewing in China as early as 7000 BCE and in Mesopotamia and Egypt in the period 4000–3500 BCE (Hornsey, 2012).

Modern day brewing owes its origins to brewing practices in Medieval Europe. The temperate climate of Northern Europe and the British Isles provided ideal conditions for the cultivation of grains such as wheat, oats and barley, from which sugars such as maltose, the main ingredient in beer, could be extracted (Hornsey, 2003). Spontaneous fermentation by wild yeasts converted the sugars into alcohol. As brewing practices developed from a cottage industry to a skilled craft, the nature of the "substance" responsible for the fermentation remained elusive but was called yeast, a word deriving from the German word *Gischt* or the Dutch *Gist*, referring to the froth or foam on the top of the vat of beer at the end of the fermentation process. This froth was scooped off the top of the vat and used to "inoculate" the next batch of beer, thus continuing the propagation of the "ferment". These yeasts are referred to as "Top Fermenters" as the yeast floated to the top of the vessel at the end of the fermentation and are associated with Ale production (Barnett, 1998).

In Medieval times, the stability and purity of beer was a constant problem. As the brewing industry grew, laws were enacted to control the purity and the price of beer. The *Reinheitsgebot*, introduced into Bavaria in 1516, restricted brewing of beer between St. Michael's Day (September 29) and St. George's Day (April 23) and also restricted the ingredients of beer to barley, water and hops. The law did not mention yeast, which was considered a by-product of the process. To preserve the beer during the warm summer months, beer was stored in cool caves often packed with ice (Hornsey, 2012). This storing (lagering) process produced a more stable beer and over time it was noted that the yeasts rather than floating to the top of the vat, sank to the bottom at the end of the fermentation and thus were called "Bottom fermenters". The shift to cold-temperature brewing, together with the practice of lagering, favoured the emergence of the "bottom fermenting" lager yeasts that we know of today. The bottom fermenters were given a taxonomical classification, *Saccharomyces pastorianus* by Max Reess in 1870. In 1890, while working as a mycologist at the Carlsberg Laboratory, Emil Christian Hansen developed techniques to separate and culture yeasts. One isolate, which he named "Unterhefe Nr. I" (bottom-fermenting yeast no. 1) was a particularly good fermenter and was named as *Saccharomyces carsbergensis*. Hansen subsequently identified several other yeast isolates from beer vats, and based on their fermentation characteristics and physiological properties, classified the isolates as separate species. One isolate was classified as *Saccharomyces pastorianus*, in deference to Reese (Barnett and Lichtenthaler, 2001). The Unterhefe Nr. I strain, which displayed very different physiological properties to the *S. pastorianus* strain was named *Saccharomyces carlsbergensis* while a third isolate Unterhefe Nr. II was designated as *Saccharomyces monacensis*.

1.2. The genomes of lager yeasts

It would not be till the end of the 20th Century that an understanding of the composition of the genomes of lager yeasts would emerge. Genetic analysis of the yeast isolates originally identified by Hansen led to a reclassification of the original three species into a single species, designated *S. pastorianus* (Vaughan Martini and Martini, 1987). Using the technique of single chromosome transfer into *kar1* mutants of *Saccharomyces cerevisiae*, Morten Kielland-Brand, at the Carlsberg Laboratory, demonstrated that certain regions of chromosomes from the lager yeasts recombined readily with *S. cerevisiae* chromosomes but other regions did not, leading to the hypothesis that sections of some chromosomes in the lager yeasts were *S. cerevisiae*-like while other sections were derived from a non-*S. cerevisiae* origin (Nilsson-Tillgren et al., 1986; Nilsson-Tillgren et al., 1981). Two different sets of chromosomes in *S. pastorianus* were later identified by Southern blot hybridisation. One set were defined as *S. cerevisiae*-like while the other was tentatively assigned as *S. bayanus*-like due to the conservation of the reciprocal translocation between chromosomes IV and II in *S. bayanus* and *S. pastorianus* (Tamai et al., 1998). Analysis of individual genes confirmed the presence of different gene alleles, with one allele being similar to *S. cerevisiae* and the other allele more similar to *S. bayanus* and *S. uvarum* (Hansen and Kielland-Brandt, 1994). At the end of the 20th century it was believed *S. pastorianus* was an allopolyploid interspecies hybrid between *S. cerevisiae* and most likely *S. bayanus* or a closely related species, containing at least three types of chromosomes; *S. cerevisiae*-like, *S. bayanus*-like and hybrid chromosomes consisting of part *S. cerevisiae* and part *S. bayanus* (Nilsson-Tillgren et al., 1981, 1986; Rainieri et al., 2006; Tamai et al., 1998). The composition of the lager yeast genomes was later assessed using the technique of competitive genomic hybridisation (CGH), which allowed for the estimation of the copy number of *S. cerevisiae*-like and *S. bayanus*-like genes and the genome locations where recombination between the homeologous chromosomes had occurred (Bond et al., 2004; Dunn and Sherlock, 2008). The analysis of the recombination sites and chromosome copy number indicated that the strains clearly segregated into two distinctive groups (Table 1). The Group I, or Saaz-type strains, included several strains isolated from breweries in the Czech Republic and Germany as well as strain CBS1513, a descent of the first pure culture of Unterhefe Nr. I, strain CBS1503, originally designated Unterhefe II (*S. monacensis*) and strain CBS1538, formerly designated *S. pastorianus*. The Group II, or Frohberg-type strains, included strains from Dutch, Danish and North American breweries. The Group I and II strains differ in their *S. cerevisiae* DNA content, with Group I strains having a lower *S. cerevisiae* content than Group II strains (Dunn and Sherlock, 2008).

Table 1
General recombination sites in Group I and II lager yeasts.

Chr	ORF	Gene	Group I CBS1513	Group I CBS1503	Group II[b]
II	YBR289W	SNF5	■		
III	MAT[a]	MAT	■		■
IV	YDR324C	UTP4	■	■	
V	YER164W	CHD1		■	
VII	YGL173C[a]	XRN1	■	■	■
VII	YGR285C	ZUOI	■	■	■
VIII	YHR165C[a]	PRP8	■		■
X	YJR009C[a]	TDH2			■
XI	YKL203C	TOR2		■	
XI	YKL080W	VMA5		■	
XI	YKL045W	PRI2			■
XIII	YML074C-YML073C	Intergenic	■		
XIII	YML051W	GAL80		■	
XIII	YMR287C	DSS1	■		
XIII	YMR302C	YME2			■
XIII	YMR306W	FKS3		■	
XV	YOR092W	ECM3		■	
XV	YOR109W	INP53		■	
XV	YOR127W	RGA1		■	
XV	YOR133W	EFT1	■		
XVI	YPL240C	HSP82	■	■	■
XVI	YPL036W	PMA2	■		
XVI	YPR160W[a]	GPH1			■
XVI	YPR184W-YPR185W	Intergenic	■		
XVI	YPR191W	QCR2			■

Shaded region, detected recombination site
[a]Recombination site induced under heat shock stress (James, Usher, 2008)
[b]Recombination sites found in at least two Group II *S. pastorianus* genomes (WS34/70 and CBS1260)