Adventures in Yeast

Or

How a Medieval Yeast Hybridization Changed the World of Beer

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History and Genetics

Several years ago, I became fascinated by the transition from the production of ales to the production of lager beer in Bavaria in the 15-16th centuries. Many sources suggested that Bavarian brewers were the first to discover that beer could ferment in the cool storage of caves and cellars. It is difficult to say when this cold fermented beer first appeared. In fact, it may be that there is no single point in time at which lager beer appeared. Several sources cite a 1420 Munich Town Council record as mentioning "cold fermented" beer that is assumed to be a predecessor of what we call lager beer today (German Beer Institute, 2006; Jackson, 1999). On the other hand, at least one author suggested that lager beer didn't emerge until 1500 (Mosher, 2004), yet another suggested that the mid-19th century ushered lager into Munich and Vienna (Noonan, 1996).

So, we do not have unequivocal evidence of a point in time when lager beer emerged. We also do not know the process by which these lager yeasts came into being. Modern genetic investigation and knowledge about the effects of selective pressure on yeast strains by brewers may give us some possible explanations. Recently, some investigators suggested that lager yeasts emerged over a transition period during which yeast hybridized to enable previously thermotolerant yeast (ale yeasts) to become cryrotolerant. Today we dub these cryrotolerant species lager yeast and they are able to ferment in colder temperatures. The distinction between the two types of yeast is somewhat artificial. There is considerable overlap in some yeast species' ability to tolerate warmer or cooler temperatures.

A little terminology is in order. While there are hundreds of species of yeasts that have been described in scientific literature, only a few have been specifically linked to the production of quality drinks and breads. *Saccharomyces cerevisiae* is the most frequently used yeast species in baking and wine-making (although many strains of this species exist). It is also a thermotolerant yeast that is used to produce ales at higher fermentation temperatures and is sometimes called a top-fermenting yeast. *S. pastorianus* (sometimes called *S. carlsbergensis*) is a cryrotolerant yeast that can ferment at cooler temperatures and is often referred to as a lager or bottom-fermenting yeast.

Early research suggested that the cold-fermenting *S. pastorianus* was a hybrid of *S. cerevisiae* and another species, *S. bayanus* (Martini & Kurtzman, 1985; Rainieri, 2006). *S. bayanus* has often been used as a synonym for *S. uvarum*, which is a dubious assumption based on the most modern genetic analysis of the *S. uvarum* genome (Rainieri, 2006). I won't get into that controversy here! The view taken by most researchers was that the non-*S.cerevisiae* component of *S. pastorianus'* genotype was accounted for by the genes contributed by *S. bayanus*.

This view, that *S. cerevisiae* and *S. bayanus* hybridized to produce *S. pastorianus*, was questioned by Libkind, et al. (2011) in recent research. They isolated a yeast from Patagonia, *S. eubayanus*, that is a 99.56% match to the part of the genome of *S. pastorianus* that is not attributable to *S. cerevisiae*. Essentially, these authors suggested that today's major lager yeast is a hybrid of *S. cerevisiae*, the traditional ale yeast, and a yeast that has never been isolated in Europe, but hails from the New World, namely *S. eubayanus*.

This poses some temporal problems. If lager beer was being produced in 15th century Europe, it could not have been a *S. cerevisiae/S. eubayanus* hybrid since a potential carrier of *S. eubayanus* from the New World would have to pre-date known voyages from South America, which would be well into the 16th century.

Several possibilities may account for this (and this list is not exhaustive).

1. Lager beer as we know it today actually emerged later than the 1420 reference cited above.

2. Some hybrid, yet unidentified, may have produced lager beer in the 15th century.

3. *S. eubayanus* may have been transported to Europe sometime in the 16th century and hybridized with *S. cerevisiae* to become the preferred yeast for producing lager beer, making the 1420 date dubious.

4. *S. eubayanus* existed in Medieval Europe and hybridized with *S. cerevisiae* even though the former has never been isolated in the wild in Europe.

What seems reasonable to assume is that at some point in history, a hybridization occurred such that previously thermotolerant yeast evolved into a cryrotolerant species under the selective pressure of brewers who observed and tasted the benefits of cold fermentation. These benefits go beyond merely the fermentation temperatures. Genes in lager yeasts code for different proteins that affect specific metabolic processes of the yeasts and determine which, and at what levels, the yeast can process different types of sugars (but that is an issue for another paper).

A Possible Resolution?

I described the contention by some researchers that during the Middle Ages lager yeasts emerged from a hybridization of ale yeast and an "alien" yeast from the New World. However, the scenario that a yeast found in Patagonia made its way to Europe does not mesh with reports of cold-fermented beer as early as 1420. Regular trade upon which a yeast could have hitched a ride from the New World to Europe came somewhat later.

A possible resolution may reside in the results of a study by Bing and his colleagues (2014). The Bing, et al. report is a short one and not particularly detailed unless you access the supplemental materials. Their work involved the isolation of strains of *S. eubayanus* from several regions in China and Tibet (not getting into the political/boundary issues here!). The researchers claimed that a Tibetan strain of *S. eubayanus* had an average non-ale whole genome sequence identity of 99.82% with the Weihenstephan 34/70 *S. pastorianus* strain (the major lager yeast used in Europe). This is only a marginally stronger match than the Patagonian sample reported by Libkind et al (99.56%). What is more important, however, is the fact that trade with Europe and this Eastern region of the world pre-dated the emergence of lager beer, unlike trade with the New World. This fact, certainly makes the Tibetan strain of *S. eubayanus* a more likely candidate for hybridization than the Patagonian strain.

But, there are other possibilities. Maybe modern lager yeasts resulted from two separate hybridization events involving the Patagonian and the Chinese/Tibetan strains of *S. eubayanus*. Or, it could be that *S. eubayanus* existed in the New World in places other than Patagonia. In fact, Peris, et al. (2014) isolated strains of S. eubayanus in Wisconsin that were closely related to those isolated in Patagonia. More research is needed to clarify these issues.

Single- or Dual-Hybridization?

Cryrotolerant yeasts that we call lager yeasts likely resulted from a hybridization of older ale yeasts (*S. cerevisiae*) and an "alien" yeast that allowed for this fermentation temperature difference. So far, the best candidate appears to be *S. eubayanus* that has, to date, been isolated in Patagonia, Wisconsin (U.S.) and China/Tibet. The China/Tibet version of this yeast seems to be the most likely to have hybridized with the European ale yeasts because trade from that geographical area pre-dates the discovery and use of cryrotolerant yeasts.

If we investigate the most widely used lager yeasts, we find two major groupings: Group I and Group II. Both are hybrids, presumably between *S. cerevisiae* and *S. eubayanus*.

Group I (Saaz) yeasts are more cold tolerant than Group II and can ferment down to 5 degrees. They are triploid (3 sets of chromosomes) and they seem to have retained proportionately more DNA from their *S. eubayanus* ancestor as compared to Group II yeasts. Group I yeasts do not utilize maltotriose during fermentation.

Group II (Frohberg) yeasts are less cryrotolerant and retain proportionately more of the DNA from *S. cerevisiae* as compared to the Group I yeasts and they are tetraploids (4 sets of chromosomes). Group II yeasts can utilize maltotriose.

There are still major questions to be answered.

- Have the two groups of lager yeasts resulted from independent hybridization events? Walther, et al. (2014) argue that they share hybridization events and ancestry.

- Are these or their ancestors the "original" lager yeasts that ushered in the lager brewing era in Germany in the 15/16th century or were the brewers of those days able, via selective pressure, to produce *S. cerevisiae* strains that fermented well in colder temperatures?

- Was there a yeast that existed in Europe during the 15th/16th century that combined with *S. cerevisiae* to form these lager yeasts? To date, none has been isolated in Europe.

Much still needs to be determined so that we may better understand how these yeasts developed into the most widely used in the world's beer markets today. As Boynton and Greig (2014) suggest:

Further archaeological and historical study, paired with DNA analysis, is needed to definitively identify the yeast responsible for brewing the first lagers and the circumstances surrounding the S. pastorianus hybridization events.

More Evidence on the Course of Hybridization

As more research emerged, the differences between the two yeast groups (Saaz & Frohberg) proved to be more numerous than originally suspected.

Saaz. Some researchers refer to this lineage as Group I (some also prefer to refer to Saaz as *S. carlsburgensis*). Saaz yeasts can ferment at lower temperatures (5 degrees) and, generally, have less efficient fermentation properties due to reduced maltose and maltotriose utilization (Gibson, et al., 2013). When we look at the genotype of Saaz we find it to be an allotriploid (3 sets of chromosomes). This triploidy is represented by one full diploid *S. eubayanus* and one haploid *S. cerevisiae* (Walther, et al., 2014). This configuration makes the Saaz lineage more like *S. eubayanus* than like *S. cerevisiae*, which may account for its ability to ferment at lower temperatures, a characteristic of *S. eubayanus* (Gibson, et al., 2013). There is also some evidence that the Saaz yeasts produce lower levels of several flavor compounds (Gibson, et al., 2013).

Frohberg. Some researchers refer to this lineage as Group II (some also prefer to refer Frohberg as *S. pastorianus*). Frohberg yeasts ferment at higher temperatures in comparison to Saaz (14 degrees). Maltose and maltotriose are utilized much more efficiently by the Frohberg yeasts, which may account for the fact that they are used much more frequently in commercial brewing (Baker, et al., 2015). The ability to ferment at higher temperatures may be due to Frohberg's genotype. It is an allotetraploid (4 sets of chromosomes), constructed of one full diploid *S. eubayanus* and one full diploid *S. cerevisiae* (Walther, et al., 2014). The latter may be responsible for Frohber's ability to ferment at higher temperatures more closely associated with *S. cerevisiae* strains.

One question that remained unanswered since the discovery of the *S. eubayanus* hybrid is whether the two lineages of modern lager yeasts developed independently as a result of two separate hybridization events or if both are linked to a single event (Dunn and Sherlock, 2008; Walther, et al., 2014; Wendland, et al., 2014). Some researchers have argued that the Frohberg lineage appeared first and that the Saaz lineage is an offshoot of the Frohberg (Walther, et al., 2014; Wendland, et al., 2014). However, studies up through mid-2015 were based on incomplete *S. eubayanus* genome assemblies. In August, 2015, Baker, et al. (2015) reported support for separate hybridization events based on a nearly complete genome assembly of *S. eubayanus*. They suggested that the two hybrid events involved nearly identical *S. eubayanus* strains but different *S. cerevisiae* strains. Furthermore, Baker, et al. suggest that the diversity observed between the *S. cerevisiae* components of the Saaz and Frohberg lineages need more than the 500 years to account for the differences, making a single hybrid event unlikely.

Baker, et al. (2015) also discussed some of the specific genes that encode for the utilization of maltose and maltotriose as well as some of the possible changes in lager yeasts due to selective pressure by brewers. This publication, and many others over the last 3-4 years, has ushered in an exciting time for researchers and brewers interested in the origins of cold fermentation in the Middle Ages. While there are still many unknowns, this yeast archaeology may give us greater insights into how 15th/16th century brewers came to use cryrotolerant yeasts and how they may have begun to shape the evolution of these hugely commercially valuable microorganisms. The research also may give us more insight into possible flavor profiles that are possible with new hybrids (Krogerus, et al., 2015).

Summary and Future Directions

So, what do we know at this point in time regarding the development of cold fermentation? The preponderance of evidence suggests that traditional ale yeasts (*S. cerevisiae*) were hybridized by a yeast that was likely carried to Europe from the Far East and/or the New World. That yeast, *S. eubayanus*, has not yet been isolated in the wild in Europe. This hybridization produced a yeast that was significantly more cryrotolerant and fermented wort in temperatures as low as 5 degrees. The hybrid has been called *S. pastorianus* or *S. carlsbergensis*; the former being the preferred moniker.

Genetic analysis suggests that the most popular lager yeasts resulted from two distinct hybridization events producing two distinct genotypes and some phenotypic differences as well. One strain, called Saaz (Group I) is an allotriploid (3 sets of chromosomes) that can ferment at low temperatures but is a bit less efficient in its utilization of some fermentables compared to the other strain. That other strain called Frohberg (Group II) is an allotetraploid (4 sets of chromosomes) and it ferments at slightly higher temperatures, utilizes some fermentable sugars more efficiently, and is the most commonly used lager yeast by commercial brewers After the initial hybridizations, these yeasts were subjected to selective pressures by brewers whereby shaping these hybrids into the yeasts we used today.

Complete genetic sequencing of these lager yeast strains provides more insight into their genotypes (genetic makeup) and phenotypes (fermenting characteristics). It appears that over the 500 or so years since the original hybridizations considerable variation in genotype has occurred. A very recent publication by Marcel van den Broek and his colleagues (2015) attempted to demonstrate the genotype diversity of some lager yeasts and to link this diversity to differences in yeast behavior (phenotype). What did van den Broek et al. find?

The strains tested by van den Broek et al. showed considerable aneuploidy (missing components of chromosomes). In humans, this condition would usually result in fetal miscarriage or significant physical and/or psychological abnormalities. Yeast, however, seem to be able to tolerate this condition to a greater degree. What causes this aneuploidy? Van den Broek, et al. suggested that it is related to the selective pressures applied by brewers over the centuries. Because lager yeasts have never been isolated in the wild, they are mostly products of human activity, in this case, the variety of brewing practices to which the yeast were subjected.

Van den Broek, et al. investigated possible phenotypic and taxonomic implications of their work. They found that the genotype of some lager yeast have connections to diacetyl production and flocculation properties; two characteristics of yeast that interest brewers. Additionally, significant differences emerged in their study with respect to chromosome and gene copy numbers of lager yeasts. The differences were consistent with the two lager yeast groups (Group I vs Group II). Van den Broek, et al. suggested that these quantifiable differences might be the basis for different species names (*S. pastorianus* and *S. carlsbergensis*. We'll see what happens in future research.

Finally, in what may be an important point of interest to those of us who would like more information on the early history of lager yeast, van den Broek, et al. (2015) postulated that research such as theirs provides insight into how lager yeasts developed such diversity and the role of human domestication during their evolution.

What does this mean for the homebrewer and craft brewing industry? Home brewers and the craft brewing industry tend to focus on the production of ales. Commercially available ale yeasts outnumber lager yeasts by quite a bit. The Wyeast Labs website lists about 3 times as many ale yeasts as compared to the number of lager yeasts. Will our emerging knowledge of lager yeasts lead to development of new strains that will provide us with the ability to explore previously untapped beer characteristics? It's hard to say at this point. But, new studies may give us some hints.

Krogerus, et al., (2015) and Mertens, et al. (2015) provide some data that may foretell future directions for brewers. Lager beer has long been prized for its crisp, clean, malt-forward characteristics as compared to ales that tend to derive many flavors from esters and phenols produced by yeast. Is it possible to develop interspecific hybrid lager yeasts that ferment at lower temperatures, but also impart desirable flavor components? Mertens, et al. did just that.

In their study, Mertens, et al. developed 31 interspecific hybrids and tested them on a variety of dimensions including cold tolerance, fermentation capacity, and aroma profiles. To make a long story short, they found that many of these hybrids had fermentation capacities and cold tolerances that exceeded their parental strains. A small number of the hybrids were used to ferment a standard wort and were then tasted by an expert panel. Some hybrids produced fruity aromas and flavors that the panel deemed pleasant. Chemical analyses verified the tasters' impressions of these lagers showing enhanced levels of esters such as isoamyl acetate.

While this research on the biodiversity of lager yeasts is still in the laboratory, it presents of picture of what might happen in the future. Up to the present, brewers used a limited set of lager yeasts available to them. Most likely this was driven by the economics of lager production. Lagers, particularly Pilsners, dominate the worldwide beer market. Apparently, the vast majority of consumers of these beers desire a uniformity and consistency of flavor/aroma. Consequently, the big brewers have no particular stake in producing "new" flavors from newly developed yeast hybrids. The burgeoning craft brewing and home brewing movement has no such motivation. In fact, craft and home brewers are well known for their innovation and creativity. Imagine what they would do with new commercially available lager yeasts that deviate in character from the limited set now available. We may be on the verge of some major changes in the way that we define "lager." We may need to develop new "hybrid" categories. :-)

What began as a discovery of the origins of lager beer yeasts in central Europe in the Middle Ages (Libkind, et al., 2011) has led us to some exciting possibilities for the future production of beer. Will a "revolution" in beer-making in Medieval Europe underlie a similar "revolution" in the 21st century? I hope so!

References

Baker, E. C., et al. (2015). The Genome Sequence of *Saccharomyces eubayanus* and the domestication of lager-brewing yeasts. *Molecular Biology and Evolution*, DOI: 10.1093/molbev/msv168, Accessed online, August 27, 2015.

Bing, J., Han, P.J., Liu, W.Q., Wang, Q.M., & Bai, F.Y. (2014). Evidence for a Far East Asian origin of lager beer yeast. *Current Biology, 24*(10). R380-381.

Boynton, P. J., & Greig, D. (2014). The ecology and evolution of non-domesticated Saccharomyces species. *Yeast, 20,* 449-462.

Dunn, B., & Sherlock, G. (2008). Reconstruction of the genome origins and evolution of the hybrid lager yeast *Saccharomyces pastorianus*. *Genome Research, 18*, 1610-1623.

German Beer Institute. (2006). Retrieved from http://www.germanbeerinstitute.com/history.html.

Gibson, B, R., Storgards, E., Krogerus, K., & Vidgren, V. (2013). Comparative physiology and fermentation performance of Saaz and Frohberg lager yeast strains and the parental species *Saccharomyces eubayanus*. *Yeast, 30*, 255-266.

Jackson, M. (1999). *The birth of lager*. Retrieved from http://www.beerhunter.com/documents/19133-000255.html.

Krogerus, K., Magalhaes, F., Vidgren, V., & Gibson, B. (2015). New lager yeast strains generated by interspecific hybridization. Journal of Industrial Microbiology and Biotechnology, 42, 769-778.

Libkind, D., Hittinger, C. T., Valerio, E., Goncalves, C., Dover, J, Johnston, M., Goncalves, P., & Sampaio, J. P. (2011). Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proceedings of the National Academy of Sciences, 108*, 14539-14544.

Martini, A. V., & Kurtzman, C. P. (1985). Deoxyribonucleic acid relatedness among species of the genus *Sensu Stricto*. *International Journal of Systematic Bacteriology. 35*, 508-511.

Mertens, S., Steensels, J., Saels, V., De Rouck, G., Aerts, G., & Verstrepen, K. J. (2015). A large set of newly created interspecific *Saccharomyces* hybrids increases aromatic diversity in lager beer. *Applied and Environmental Microbiology, 81*, 8202-8214.

Mosher, R. (2004). *Radical brewing*. Boulder, CO: Brewers Publications.

Noonan, G. J. (1999). *New brewing lager beer*. Boulder, CO: Brewers Publications.

Peris, D., Sylvester, K., Libkind, D., Goncalves, P., Sampaio, J. P., Alexander, W. G., & Hittinger, C. T. (2014). Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Molecular Ecology, 23,* 2031–2045

Rainieri, S., Kodama, Y., Kaneko, Y., Mikata, K., Nakao, Y., & Ashikari, T. (2006). Pure and Mixed Genetic Lines of *bayanus* and *pastorianus* and Their Contribution to the Lager Brewing Strain Genome. *Applied and Environmental Microbiology, 72*, 3968–3974.

Walther, A., Hesselbart, A., & Wendland, J. (2014). Genome sequence of Saccharomyces carlsbergensis, the world's first pure culture lager yeast. *G3: Genes* **|** *Genomes* **|** *Genetics, 4*, 783-793.

Wendland J. (2014). Lager yeast comes of age. *Eukaryotic Cell, 13*, 1256–1265.

van den Broek, M., et. al. (2015). Chromosomal copy number variation in *Saccharomyces pastorianus* is evidence for extensive genome dynamics in industrial lager brewing strains. *Applied and Environmental Microbiology, 81*, 6253-6267.