

# A new hypothesis for the origin of the lager yeast *Saccharomyces pastorianus*

Mathias Hutzler<sup>1,\*</sup>, John P. Morrissey<sup>2</sup>, Andreas Laus<sup>1</sup>, Franz Meussdoerffer<sup>1,†</sup>, Martin Zarnkow<sup>1</sup>

<sup>1</sup>Research Center Weihenstephan for Brewing and Food Quality, Technische Universität München, Alte Akademie 3, 85354 Freising, Germany

<sup>2</sup>School of Microbiology, SUSPERM Research Centre, Environmental Research Institute, APC Microbiome Institute, University College Cork, Western Road, T12 K8AF, Ireland

\*Corresponding author. Mathias Hutzler, Research Center Weihenstephan for Brewing and Food Quality, Technische Universität München, Alte Akademie 3, 85354 Freising, Germany. E-mail: [m.hutzler@tum.de](mailto:m.hutzler@tum.de)

†Author note: deceased

Editor: Isak Pretorius

## Abstract

*Saccharomyces pastorianus*, which is responsible for the production of bottom-fermented lager beer, is a hybrid species that arose from the mating of the top-fermenting ale yeast *Saccharomyces cerevisiae* and the cold-tolerant *Saccharomyces eubayanus* around the start of the 17th century. Based on detailed analysis of Central European brewing records, we propose that the critical event for the hybridization was the introduction of top-fermenting *S. cerevisiae* into an environment where *S. eubayanus* was present, rather than the other way around. Bottom fermentation in parts of Bavaria preceded the proposed hybridization date by a couple of hundred years and we suggest that this was carried out by mixtures of yeasts, which may have included *S. eubayanus*. A plausible case can be made that the *S. cerevisiae* parent came either from the Schwarzbach wheat brewery or the city of Einbeck, and the formation of *S. pastorianus* happened in the Munich Hofbräuhaus between 1602 and 1615 when both wheat beer and lager were brewed contemporaneously. We also describe how the distribution of strains from the Munich Spaten brewery, and the development by Hansen and Linder of methods for producing pure starter cultures, facilitated the global spread of the Bavarian *S. pastorianus* lineages.

**Keywords:** *S. pastorianus*, *S. cerevisiae*, *S. eubayanus*, lager yeast, ale, wheat beer, history, hybridization, top-fermenting, bottom-fermenting, brewing

## Franz Meussdoerffer (1949–2019)—an appreciation

In the summer of 2019, shortly after we had completed the basic framework of this publication, our friend and idea provider Franz Meussdoerffer unfortunately passed away. We first had to cope with this sad event. Then came the Corona pandemic and we had more or less forgotten about this article until we picked it up again in 2022 and completed it as a dedication to Franz. Born in 1948, Franz Meussdoerffer came from a Franconian family of brewers and maltsters. After graduating from high school, he studied chemistry at the Technical University of Aachen and did his doctorate at the Biochemical Institute of the University of Freiburg (topic: studies on inhibitors of proteinase A from yeast). After a scholarship (1980–82) at Cornell University in Ithaca, USA, with Gerald Fink (Methods in Yeast Genetics) and intermediate stations at the ETH Zurich and at the Henkel Group, he was managing partner of the Meußdoerffer Mälzerei in Kulmbach (now IREKS). He completed his habilitation in 1989 and was an associate professor in Dortmund from 1996. From 2003, he taught on the history of beer brewing at the TUM campus in Weihenstephan together with Martin Zarnkow and from 2009, he was head of the Bioanalytics working group of the Research Centre for Food Quality at the Kulmbach site of the University of Bayreuth. Franz's passion was brewing and yeast history, which he conveyed with great

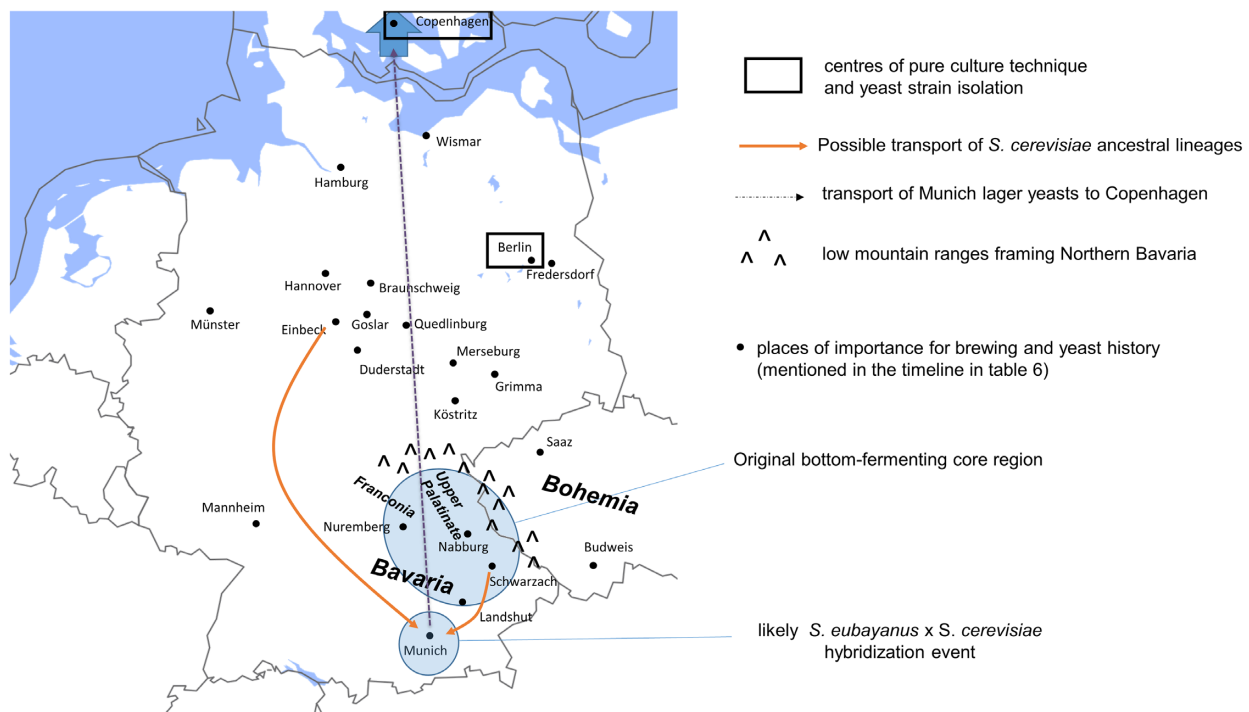
enthusiasm in various publications and lectures. Most of the figures and tables with accurate facts and data in this article come from Franz's detailed research (data on historical beers, historical beer locations combined with annual data, and yeast distribution paths over time). Many of the conclusions in this article come from his astute mind, such as the probable *Saccharomyces cerevisiae* ancestor of *Saccharomyces pastorianus* from the Schwarzbach wheat beer brewery. We miss Franz and his inspiration and hope that this article is a fitting memorial to him.

## Historical development of beer styles in Central Europe

Main locations of the following paragraphs are shown in Fig. 1 and major events are listed in a timeline in Table 6. Beer has been made since ancient times with recent archaeobotanical investigations uncovering evidence of brewing in the Levant 13 000 years ago (Liu et al. 2018). Even without the insights of modern microbiology and science, yeasts were known to play a role in fermentation and medieval glossaries list several terms for yeast (Steinmeyer et al. 1895). However, a greater interest in yeasts and beer fermentation ensued only after urban professional brewing came of age in Central and Northern Europe in the 12th and 13th centuries (Meussdoerffer 2009, Meussdoerffer and Zarnkow

Received: January 23, 2023. Revised: March 29, 2023. Accepted: March 31, 2023

© The Author(s) 2023. Published by Oxford University Press on behalf of FEMS. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<http://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)



**Figure 1.** Map showing the regions of Europe relevant for the emergence and distribution of *S. pastorianus*. The regions shown should be understood in their historical context. Bavaria denotes the original duchy/electorate between the Alps, the Danube, the Salzach, and the Lech. Franconia comprises the territories in the northwest of Bavaria, bound by the Danube to the south and the low mountain range (Frankenwald) to the north. The Upper Palatinate comprises the region between the Böhmerwald (low mountain range Bohemian Forest), the Danube and the low mountain ranges (Fichtelgebirge). Bohemia describes the former kingdom with its capital, Prague. Top-fermentation was the primary brewing method in most of Europe, including Bohemia, but bottom-fermentation became common in the 14th century in Franconia and the Upper Palatinate. Once the Beer Purity Law (*Reinheitsgebot*) was introduced in 1516, bottom-fermentation became universal in Bavaria, with few exceptions. One exception was Schwarzach, which had a famous wheat-beer brewery. Brewing of this beer transferred to the Munich Hofbräuhaus in 1602. Top-fermenting yeast from Einbeck also came to the Hofbräuhaus in 1615. The hypothesis put forward in this paper is that one of these events introduced the *S. cerevisiae* parent of *S. pastorianus* to the Munich Hofbräuhaus. Here, it hybridized with the *S. eubayanus* that was already used there as part of the bottom-fermenting ‘Stellhefen’ (pitching yeast) mix. From the early 19th century, yeasts from the Munich Spaten brewery (Spaten owner Sedlmayer previously worked at Hofbräuhaus) were widely distributed, including to the Carlsberg brewery in 1845. Copenhagen and Berlin became the major centres for the cultivation of pure *S. pastorianus* from Bavarian bottom-fermenting Stellhefen and Grimma (home of the Froberg Brewery) and Saaz (home of the Bürgerliches Brauhaus) are key locations that are also described in this text and in Table 6.

2016). During this time, the spread of single-storey houses with stonewalled ovens, the increased use of hops and barley, and the separation of boiling and mashing processes resulted in a new beer type, the hopped lager. This beer style most likely took advantage of mixed yeast cultures that harboured cryotolerant *Saccharomyces* species (pre-*S. pastorianus* modern lager yeast time). This ‘original’ lager beer was so special because an elongated fermentation took place during the so-called lager process in rock cellars at a low average ambient temperature. These ‘Märzen’ (beer brewed in March before spring and the summer brewing break)—or ‘overseas’—beers (more stable and stronger beers for transport and export) replaced older beer types like Gose, Gruit, or Gräuwwing. Soon, the new beers were produced by professionals at a scale >100 000 hl p.a. and were traded over long distances. Technological changes had consequences for beer styles and the ‘beer wort’ niche as the microbicidal effect of hops on lactic acid bacteria (LAB) would have altered the synergetic metabolism of LAB and yeast (Sieuwerds et al. 2018). Furthermore, from the 14th century, barley increasingly superseded oats as the major brewing grain. This would have increased the 4-vinyl guaiacol production, as barley extracts contain 10- to 100-fold more ferulic acid than whole oats, oat bran, or wheat (Zhao and Moghadasian 2008). Later, during the 15th and 16th centuries, beer consumption in Central Europe increased dramatically while the long-distance trade of beer decreased. This development has been attributed

to several factors including changes in nutritional habits (Wiegmann and Mohrmann 1996), economics (Huntemann 1970), and climate change. Regarding the economic aspect, when brewing became a profitable business, local beer production flourished and cities applied tolls and taxes to exclude foreign beers and protect local brewers (Huntemann 1970). While climate change is also a concern of the modern wine industry, it is also thought to have contributed to the reduction of wine consumption in the Middle Ages as cheap local products disappeared, which meant that making wine was comparatively more expensive than beer. The cold/wet period of 1430s (Camenisch et al. 2016) has been directly correlated to the spread of the Dutch (kuyte) beer type in northwestern Germany (Irsigler 1996). Certainly, the substitution of wine by beer occurred at different times at different places, but by the beginning of the 17th century, cheap local wine had become rare in many regions of Central Europe (Landsteiner 1999). Thus, by 1600 Central Europe was home to many local brewing centres, each providing a distinct niche for independent yeast domestication. There were differences in grain usage (oats, barley, and wheat), the use of unmalted grain and mashing technologies, and of course the regional yeasts. The tremendous variety of beers might be deduced from Tables 1–3. For example, for wheat-beer types, also called ‘white beer’ and defined as beers that contained more wheat than other starch-containing grains, the tables illustrate that the ratio of barley, wheat, and oats varied substantially

**Table 1.** Top-fermented beer types including some ‘white beer/wheat beer types’ with different raw material compositions around 1600 AD (Meussdoerffer and Zarnkow 2018).

Origin	Designation	Barley:wheat:oats) [%]	Hops	Malt	Comments
Hamburg	White beer	60:40	+	Kilned malt	Lübeck, Hannover, and Netherlands
Goslar	Gose	0:100	–/(±)	Air-dried malt	Quedlinburg, Halberstadt, Aschersleben, and so on
Hannover	Broyhan	19:76:5* 33:66* 50:50*	(+)	Air-dried malt/oven dried	Halberstadt, Quedlinburg, Wolfenbüttel, Hildesheim, Braunschweig, and so on
Holland	Kuyt, koyt (Cervisia Batavica)	19:30:51	+	Kilned malt, unmalted grain	Münster, Dortmund, and Cologne
Münster	Koit	85:15:(10)	++	Kilned malts	
England	Ale	100:0:0	–/(±)	Kilned malt, malted grain	Unmalted grain: Harrison, in his preface to Hollinshed’s Chronicles 1587
Poland, Bohemia, Bavaria	Weißbier	0:100* 30:70*	–/(±)	Kilned malt	Bavaria

\*Different recipes/ratios depending on source/location.

**Table 2.** Raw material concentrations of different ‘Brown Beers’ (Herbststadt 1814).

Beer	Diverse Malts combined kg/hl	Hops kg/hl
Double beer Köstritz	64.5	1.2
Good wheat beer	39.7	1.0
Mumme from Braunschweig	61.4	0.4*
March- = Kufen-beer	49.6	0.8
Fredersdorf beer	45.5	1.7
Mannheim beer	29.8	1.7
Merseburg beer	43.4	2.1

\*: There were additional other ingredients: spruce, ginger, molasses, and so on.

**Table 3.** Alcohol and dry matter of Berlin beers (Neumann 1735).

Beer	Alcohol w/w %	‘Extract’ w/w %
Quedlinburg ‘Gose’	3.1	6.3
Bernau beer	2.5	7.2
Crossen beer	2.5	5.3
Rupin beer	2.5	6.1
>Halberstadt ‘Breyhan’	2.5	4.7
Carthäuser Bier (Frankfurt/Oder)	2.2	4.0
Cottbus beer	1.9	3.4
Berlin brown beer	1.9	12.2
Berlin white beer	1.9	5.3
Berlin home-brewed brown beer	1.9	11.9
Brandenburg beer	1.6	7.0
Lebus beer	1.6	7.8
Cöpenick ‘Moll’	1.3	6.9
Rodens table-beer	1.3	8.8
Local brown table-beer	0.9	8.8

between different types (Table 1 also includes ‘white beers’). Indeed, the data in Table 1 only provides generalized data as each city used different grain ratios, volumes, malt types, and so on. The red/brown/black beers were even more diverse with the only common factor being that barley malt and hops were the major ingredients (Table 2). Some of them contained wheat malt or even oats, albeit barley malt always dominated. It is possible to

roughly differentiate between three type of beers: namely those for long-term storage, immediate consumption, or light beers. Long-storage products (1) (e.g. lager beer, Märzen beer, and bitter beer) usually contained more hops than other beers. According to some ordinances (e.g. Einbeck, Munich) these beers had to remain in the cellar for second fermentation for at least 8 days. Beers for immediate consumption (2) included winter beer or middle beer. Light beers (3) such as Kofent, Dünnbier, and Nachbier, were usually made from the second washing of the spent grains. Not surprisingly, ‘extract’ values (probably soluble matter only) and alcohol content (as a measure for unfermented/unfermentable matter and attenuation) also varied greatly. Compared to modern malt dosages, historic dosages were extremely high because raw materials were not at such an adapted state as they are today (because of cereal breeding and selection). Such high ratios support the survival of microbes as a lot of particles and ‘colder grain agglomerate spots’ (also partially containing air pillows) during mashing and boiling can hide microbes from full heat input. Caspar Neumann was one of the first chemists to measure these attributes and described an interesting range of values for beers that he found in Berlin (Table 3) (Neumann 1735). Alcohol yields were low and it is clear that mashing efficiency varied greatly. This comes as no surprise as there were air dried malts, which are relatively wet and enzymatically active when used quickly, and mouldy later; and kilned malts, which were likely to be less enzymatically active, but stable for longer periods.

## Historical patterns of bottom and top-fermentation

Much has been written and inferred about so-called top-fermenting and bottom-fermenting yeast. The current meaning of the terms is that top-fermenting refers to the ale yeast *S. cerevisiae* and bottom-fermenting to the lager yeast, *S. pastorianus*. Modern lager beer is made via a cold fermentation with *S. pastorianus* whereas ale is fermented at warmer temperatures using *S. cerevisiae*. This difference between top- and bottom-fermenting yeasts has been known at least since the Middle Ages and the first book on brewing even differentiates between top-fermenting (*flos cerevisiae*) and bottom-fermenting (*feces, cerevisiae sedimentum*) yeasts (Placotomus 1551). The simultaneous presence of both

types of yeast in the 15th and 16th centuries can also be inferred from various German brewing ordinances. For example, those of Wismar (1535), Cologne (1608), and others explicitly ban the use of bottom-fermenting yeasts. Another brewing ordinance, from Quedlinburg (1600 AD), concerning a 'Gose' wheat beer states that both 'barmen' (bottom) and 'ghare' (top) must remain with the brewer (Lorenz 1916). A dispute between Munich's bakers and brewers (1483) provides evidence that the Bavarian 'Bohemian-type' beer (see below) was produced with a bottom-fermenting yeast. This dispute centred on the bakers' refusal to accept brewer's yeast (to which they were bound) because of its altered properties. It is well-known that bottom-fermenting yeasts are unsuited to baking, thus the rise of yeast fabrication following the introduction of lager beer. One must be careful, however, when extrapolating the modern meaning of species and processes to historical practices and descriptions. *Saccharomyces cerevisiae* is a pure species whereas *S. pastorianus* is a hybrid of *S. cerevisiae* and *Saccharomyces eubayanus*. Since the Middle Ages, it has been known that viable yeasts accumulated at the bottom of fermentation vessels but these could also have been *S. cerevisiae*, as, under certain conditions, top-fermenting yeasts also sink to the bottom. Truly cold (i.e. bottom) fermentation technology for primary and secondary fermentations, has only been regularly used since the 15th century and then only in regions south of the German low mountain ranges framing northern Bavaria (Fig. 1). In these regions, all brewing yeasts (fermentation slurries) were mixtures of top- and bottom-fermenting yeasts so the temperature of the fermentation environment played a decisive role in dictating the fermentation style. Interestingly, while bottom (cold) fermentation prevailed in Bavaria, top fermentation dominated in Bohemia despite the brewing methods and conditions being similar. Thus, whether the brewer used yeast from the top or the bottom of the fermentation vat or from the storage barrel to inoculate a new batch also played a critical role. From history, no single place or date for the beginning of bottom fermentation can be identified. As the brewing ordinances in Bavaria from the middle of the 16th century, most notably the purity law (German: Reinheitsgebot) from 1516, stipulated that brown beer had to be produced by bottom-fermentation, it must have been an established, well-functioning technology by then.

In fact, writers from the Middle Ages distinguished four different types of fermentation, three of which were in use all over Europe, and a fourth, that developed in Central Europe south of the German low mountain range (roughly comprising today's Bavaria and Czech Republic). It was this cold fermentation that underpins the emergence of the modern lager yeast, *S. pastorianus*. We, hereby describe each of the fermentation methods.

1. Wild yeasts/spontaneous fermentation. This was not only the case with Lambic-type beers, but also with some wheat beers of the Gose type. The LAB always present in these fermentations gave the beer a sour taste.
2. Barrel fermentation. this was widespread in Bohemia and the usual procedure for Bavarian wheat beers. The wort was inoculated with 'top-fermenting' yeast and filled into barrels, where it was fermented for several days. Yeast emerged from the bung hole and was collected. Once fermentation ceased, barrels were filled to the bung with water or wort and sealed.
3. Mixed top/bottom fermentation. This was the most widespread technology used until the 19th century. Typically, wort was inoculated with 'top-fermenting yeast' and fermented in vats for 3–6 days (primary fermentation). Dur-

ing this time, yeast rose to the surface, but some also settled at the bottom. The resulting 'green' beer was then filled into barrels, still open at the bung hole, and continued to ferment and eject top-fermenting yeast. The ejected yeast was used for new inoculations. Once this process stopped, the barrels were plugged, but fermentation continued at a slower pace. It is noteworthy that Christian Kobrer differentiates further between 'warm' and 'top' fermentation at this point. While 'top' fermentation is essentially the process described above, 'warm' fermentation resembles white (wheat) beer fermentation but is performed at warmer temperatures (~16–25°C) (Kobrer 1581). It is suited to cheap, weak beers only, which are destined for immediate consumption. The yeast for 'top' fermentation comes from previous top yeast recovered from the vat, while the inoculum for 'warm' fermentation comes from a barrel.

4. Bottom fermentation. As well as the barrel and top fermentation described above, Kobrer adds one more type practiced in the 16th century, namely, cold bottom-fermentation for the beers in the Upper Palatinate (Fig. 1) (Kobrer 1581). Dried bottom yeast from a previous fermentation was used to inoculate these beers. The capacity of yeast to survive drying is variable and one could speculate that sporulation played a role in this process, perhaps even in contributing a selective pressure. A preculture of these yeasts was added to cold wort and a slow fermentation was performed at cold temperatures (impeding not only top-fermenting yeasts but also bacterial growth). Afterwards, the green beer was transferred into storage barrels to mature for an extended period of time. Contemporary authors linked the type of fermentation to the prevailing temperature of the wort and the fermentation environment. Thus, 'cold fermentation' presumably referred to a comparatively slow fermentation at low temperatures (below 10°C), leaving more unfermented carbohydrate for a secondary fermentation (see below). From the 15th century onwards, Franconian and Bavarian brewing ordinances frequently mention 'cold fermentation', which in most cases (not always accurately) is equated with 'bottom fermentation'. By 1447, use of the German word 'unter' (under) when describing fermented beer gives a strong indication of the use of bottom-fermenting yeast. Bottom fermentation might have spread properly from Franconia and the Upper Palatinate south into Bavaria. It is noteworthy, that a strong beer at the end of the 15th and the beginning of the 16th century was called a 'Bohemian-type beer' in Bavaria. This was likely a bottom-fermented beer. Heiß and Balling both agree that in Bohemia, as in Bavaria, wort was prepared by similar decoction mashing and, compared to most other countries, cooled to very low temperatures prior to yeast inoculation. However, while only 'top'-fermentation was practiced in Bohemia until the 19th century, bottom fermentation prevailed in Bavaria for the most part, at least from the 17th century. Thus, the description of 'Bohemian-type beer' in this context refers to the brewing technology rather than the fermentation style.

In the mid-19th century, it was also acknowledged, that even in the usual top-fermenting brewing process, top fermentation develops into a bottom fermentation, as soon as the green beer was transferred from the fermentation vat into barrels, yeast had been ejected through the bung, and the barrels were locked. However, 'top fermentation' prevailed north of the low mountain ranges until the 19th century. Why then did bottom fermentation become



established in Franconia and Bavaria from the 15th century onwards? One reason could have been the paucity of grain in Upper Franconia, the Upper Palatinate, and Upper Bavaria. To produce a stable lager beer, it might have been advantageous to ferment the thinner worts more slowly (by restricting yeast metabolism using cold temperatures) (Noback 1874). Another important factor could have been the climate. There were a series of cold periods from the 13th to 17th century (Kromer and Friedrich 2007, Camenisch et al. 2016). Average temperatures, particularly in winter, were distinctly lower in southern regions than north and west of the low mountain ranges (Hoy 2006). Furthermore, at least from the 14th century, brewers in Franconia and the Upper Palatinate kept their barrels in rock cellars for long-term storage. Consequently, cold selection might have played an important role in domesticating cold-tolerant yeasts (Gunzelmann 2010).

### Bottom-fermenting lager strains born from mixed cultures ‘Stellhefen’

When discussing fermentation types, it must also be stressed, that brewing yeasts were always a mixture of wild, top- and bottom-fermenting species and in most cases probably also LAB until the end of the 19th century (and in smaller breweries much longer). This has been acknowledged repeatedly in the 18th and 19th centuries. These mixtures were called ‘Stellhefen’ and contained fast- and slow-fermenting brewing yeasts, as well as other wild yeasts. Among many others (e.g. Paupie, Munz), the preeminent authors of brewing manuals at the middle of the 19th century, Carl Balling and Philipp Hei, describe that top-fermenting yeasts can be transformed into bottom-fermenting ones by repeatedly cultivating them at low temperatures and picking the sludge at the bottom for further inoculation (Hei 1853, Balling 1854). This is further supported by a brewer from Bamberg in 1809, who states that the bottom fermentation of cold worts and storage in rock cellars was the customary procedure in Bamberg. Sometimes, however, there was a lack of bottom yeast when brewing resumed in late autumn after the summer break. Brewers, therefore, started with top-fermenting yeast, which was then successively replaced by bottom isolates (Seifert 1818). The icon of Munich brewers, Gabriel Sedlmayr, made a concurring statement when providing expertise for the Bavarian ministry of trade in 1849. He explained that bottom-fermenting yeasts were used in almost all regions of Bavaria to produce brown beer, except in autumn, when brewing had to start without any bottom-fermenting yeast being available. Only then would top-fermenting yeast be used to successively obtain bottom-fermenting yeast from it (Sedlmayr 1951). Therefore, it seems likely that obtaining bottom from ‘top’-fermenting yeast was a widespread technique. The modern interpretation of these data would be that cultures of top-fermenting yeasts were never pure and always contained a bottom-fermenting yeast inoculum that could be selected by enrichment cultivation (i.e. cold fermentation).

In times past, brewers lacked filtration and tried to remove the beer between the cake of top-fermenting yeasts and the sediment from vats, but this was far from quantitative. Mixtures of top- and bottom-fermenting yeasts remained in the beer transferred to the barrels. Recovering the yeast ejected from the bung hole of fermenting barrels removed relatively more top-fermenting yeast and left bottom yeasts for a second (barrel-) fermentation. Taking yeast from barrels that had been stored for a while was frowned upon (rightly, as it might contain multitudes of spores). It has been noted (Powell et al. 2004) and previously described in de-

tail (Schnfeld 1938) that early brewers controlled their fermentations by carefully selecting the inoculum from earlier batches. Taking yeast from the middle layer of a (top-) fermentation vat (still fermenting) selected purer top-fermenting yeasts (the same was also true for the middle layer of bottom yeast). Taking it from a fermenting barrel, brewers always obtained a mixture of top, bottom and wild yeasts, and spores (and probably bacteria as well). It should be noted that this was by no means an inferior technique according to the standards of the time. Different yeasts in a mixture, adapted to different niches (vat fermentation/open barrel fermentation/storage fermentation) suited the needs of brewers and resulted in the distinctly flavoured beers that consumers appreciated.

In summary, it is important to note that (i) bottom fermentation was well-established in Bavaria by 1600 and (ii) bottom fermentation was essentially performed by mixtures of top- and bottom-fermenting yeasts. Even after centuries of bottom fermentation, mixtures of top- and bottom-fermenting yeasts were clearly prevalent. However, as judged by Hansen’s work, by the end of the 19th century, lager yeast (*S. pastorianus*) was the dominant constituent of these mixtures. Possibly, it was selected for each year anew over the cold brewing season. It seems that this yeast was the most adapted and stable with regard to Bavarian lager brewing technology (its decoction mashing, the fermentation, and storage condition and facilities) and climate. However, it should be noted that almost all of what we know applies only to Munich and to Mrzen or ‘summer’ (lager) beers, which were brewed in March and no later than 23rd April (Saint George’s day). They were stronger and stored with residual extract in cooled cellars. Fermentation could, therefore, be extended and the beers were stable for a long time throughout the summer. Often beer gardens with chestnut trees that cast a shadow were above the cellars with ice that cooled the beer barrels. Even Munich winter beers (cheaper and destined for immediate consumption) were different, and the brewing technology used in Franconia and Swabia was distinctively different. However, little remained from these technologies. It was the Munich Lager beer that was to be replicated all over the world.

### Hypothetical scenarios for the historical lager yeast hybridization

It is worth considering how the historical facts above relate to proposals regarding the origins of the lager yeast, *S. pastorianus*. Current opinion (quite simplified) is that a rare *S. eubayanus* was accidentally introduced to an existing *S. cerevisiae* habitat, the two interacted and formed a new species, *S. pastorianus* (Gallone et al. 2017a, Sampaio et al. 2017). Libkind et al. (2011) reported the isolation of *S. eubayanus* in South America in 2011, and the yeast was subsequently found in Argentina, Chile, Tibet, China, New Zealand, USA, and most recently in Ireland (Libkind et al. 2011, Bing et al. 2014, Peris et al. 2014, Gayevskiy and Goddard 2016, Eizaguirre et al. 2018, Nespolo et al. 2020; Bergin et al. 2022). Relatively few isolates of *S. eubayanus* that are not associated to *Nothofagus* sp. and *Cyttaria* sp., have been found so far in nature, indicating it is quite rare and cannot thrive broadly outside a very special habitat. The latter might be defined by uninhabited wilderness, a moderate climate, and the presence of sugars. Two main scenarios have previously been presented to explain the origins of the *S. eubayanus* parent of *S. pastorianus*. *S. eubayanus* may have existed in wild European populations when bottom-fermenting brewing was developed, or *S. eubayanus* may have been intro-

duced from China/Tibet (e.g. via the Silk Road), prior to which time brewers had used a different non-*S. pastorianus* yeast to produce bottom-fermented beer (Boynton and Greig 2014). Further archaeological and historical studies are necessary, which need to be combined with microbiological and DNA-based analyses, to unravel the mystery of which yeasts were used to produce the first bottom-fermented beers and how to explain the *S. pastorianus* hybridization (Boynton and Greig 2014). So far, *S. eubayanus* has not been reported in Central Europe, although a broad set of samples and habitats were analysed, and tailored methods were applied to cultivate cryotolerant yeasts. (Hutzler 2021). If the first, *S. eubayanus* had come from far away (and survived for a long time under adverse conditions, maybe as spores), it most likely would have entered a brewing habitat in a major city. There, exotic and expensive products were traded and at times used to fortify beers. On the other hand, the recent discovery of wild *S. eubayanus* in Ireland (suggests that there were, and still are, European populations of this yeast and supports the idea of European ancestry for *S. pastorianus* (Sampaio 2022). The Irish isolates belong to the same Holarctic clade as those from Tibet and genome analysis shows contributions from both populations to the *S. eubayanus* component of *S. pastorianus* (Bergin et al. 2022).

The generally accepted view is that bottom fermentation was responsible for the formation of *S. pastorianus* and the selective pressure that cold fermentation imposes could be seen to support this hypothesis. In this scenario, the emergence of *S. pastorianus* and the spread of bottom fermentation are inextricably linked. This is not consistent, however, with historical records of the new (bottom) fermentation technology in many places between 1400 and 1600. Bottom fermentation was certainly practiced at that time in Bavaria, likely originated from the rural landscapes in Franconia and the Upper Palatinate along the Bohemian border. It is important to note that brewing with mixtures of top and bottom-fermenting yeasts from the 14th century does not prove that the fusion between *S. cerevisiae* and *S. eubayanus* had occurred in the 14th century. Other yeasts in the Stellsbrenn mixture could also contribute to an overall bottom-fermentation-like process. Bottom-fermentation could have been accomplished by other flocculating, cold-adapted yeasts, and top-fermenting yeasts sediment like bottom-fermenting ones under appropriate conditions at cold temperatures (Schönfeld 1938). In fact, the information currently available rather points to a slow adaption of brewing yeasts to colder temperatures in multiple locations. Indeed, *S. eubayanus* itself is one such candidate. Originating from the sparsely populated regions of Franconia and the Upper Palatinate, it could have settled in a brewing habitat facilitated by cold temperatures. Under such conditions, the brewing performance of this yeast resembles contemporary *S. pastorianus* Saaz and Froberg (see later) types of lager strains (Table 4). With respect to the levels of 3-methyl-butyl-acetate (isoamyl acetate; banana flavour; threshold: 1.6 mg/l) and ethyl acetate (fruity flavour; threshold: 33 mg/l.), the *S. eubayanus* isolate resembles the Froberg type, while it is more similar to the Saaz type with respect to fermentation attributes. Possibly there have been other *S. eubayanus* variants in the past.

Phylogenetic analysis places the critical step of generating our present bottom-fermenting yeasts between 1550 and 1800 (Gallone et al. 2019) and it is believed that the parents of *S. pastorianus* were a top-fermenting strain of *S. cerevisiae* and a bottom-fermenting strain of *S. eubayanus*. It has been proposed by various groups (Dunn and Sherlock 2008, Gibson and Liti 2015, Okuno et al. 2016, Gallone et al. 2017a, 2019, Sampaio et al. 2017, Salazar et al. 2019) that there was a unique event, followed by subsequent evo-

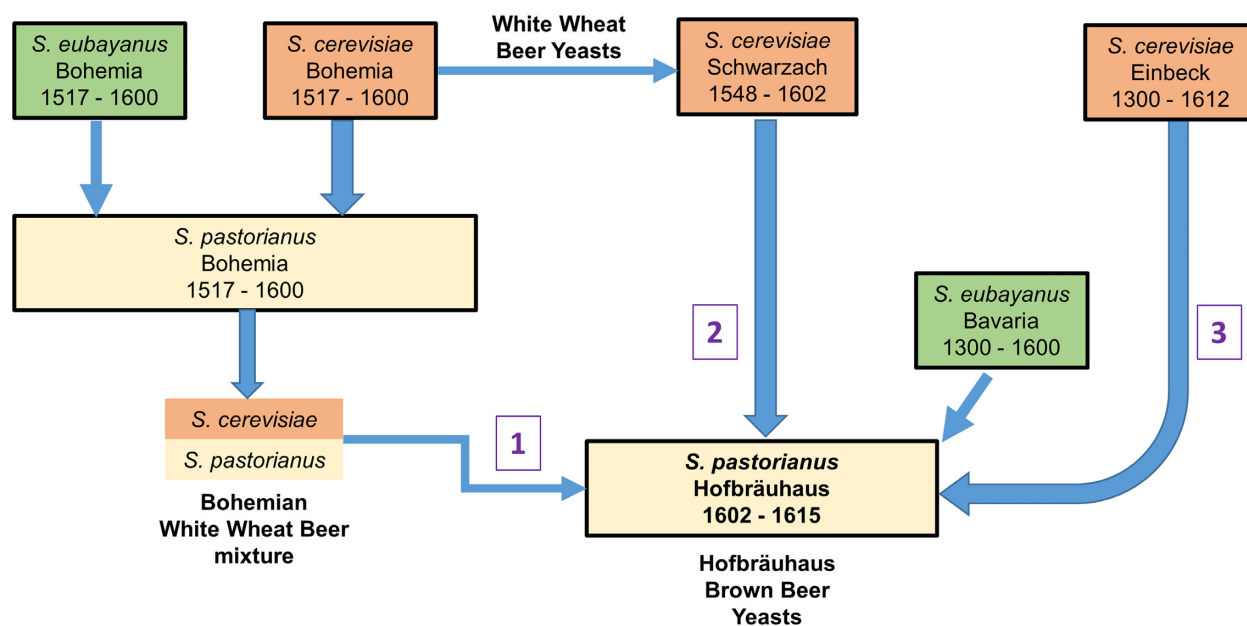
lutionary steps, to generate the prototype *S. pastorianus* lineage(s). Considering that bottom-fermentation was widespread long before the proposed founding hybridization event around the end of the 16th century, it is worth considering other explanations for how *S. pastorianus* arose. While it has been assumed that the critical step was the encounter of a rare *S. eubayanus* with an abundant *S. cerevisiae*, an alternative hypothesis is that the key event was the bumping of a particularly suitable *S. cerevisiae* into an *S. eubayanus* population. The strains involved could be domesticated top-fermenting *S. cerevisiae* and cold-resistant *S. eubayanus*, which was part of the brown beer yeast mixture being used in Bavaria at that time. Although we do not know much about the parental *S. eubayanus* population, there are very good data regarding the *S. cerevisiae* lineage that formed the second parent of *S. pastorianus*. Phylogenetic analysis of *S. cerevisiae*, identified several splits among beer strains, including the emergence of a Belgium/Germany ale clade, including a subclade of wheat beer strains, from which the *S. cerevisiae* parent of *S. pastorianus* came (Gallone et al. 2017b). Thus, formation of the Belgium/Germany ale lineage could be seen as the prerequisite for a subsequent formation of *S. pastorianus*. If the development of domesticated lineages of *S. cerevisiae* and *S. eubayanus* were independent events, they must have been connected somehow to enable the interaction that formed *S. pastorianus*. Yeasts in concentrations necessary to allow the selection of a new variant would likely travel via raw materials, products (yeast, beer, and wine), or humans. However, yeasts were typically exchanged only within a neighbourhood, a city, or a nearby location, and only in exceptional emergencies was yeast brought from further away. In such a desperate situation, the city of Einbeck brought yeast in Hildesheim (roughly 45 km) and Göttingen (37 km). Heinrich Knaust (Knaust 1575) mentions that the yeast from Duderstadt was renowned for its stable performance and regionally sought after to remedy faulty fermentations. But these are certainly exceptions. The same holds true for beer, which was rarely transported further than a couple of kilometres. The distance a wagon could cover to deliver beer and return within a day was 20 km. Only exceptional beers were traded over long distances, mostly by sea or river transport. Beers that travelled overland were a sensation and even then, and only with a few exceptions, not more than ~200 km. Thus, for an encounter of two exceptional yeasts domesticated under very different conditions, special conditions must be met. We suggest that suitable conditions for the encounter described above could have been provided by the Hofbräuhaus in Munich shortly after 1600 and propose two different scenarios that are consistent with the phylogenetic time calculations for the hybridization event by the Leuven yeast group (Gallone et al. 2019) (Fig. 2).

In the first case, the *S. cerevisiae* came to Munich from Bohemia via Schwarzach. The period from 1500 onwards saw a surge in white (wheat) beer brewing, with Bohemia becoming a well-documented stronghold. White beer is first mentioned in 1496 in the records of Budweis/České Budejovice (Peterka 1917). After the Bohemian gentry gained the right to brew beer in 1517 (Woltmann 1827), the sector greatly expanded and vast quantities of wheat beer were exported to Bavaria. To limit the economic damage and to confer a distinction, in 1548 the Bavarian duke Wilhelm IV awarded Hans VI von Degenberg a special privilege to brew and sell wheat beer in the regions bordering Bohemia—in contravention of the 1516 Bavarian Rheinheitsgebot purity law. The Degenbergs had already built a magnificent white beer brewery in Schwarzach, which was to become famous for its exceptionally high-quality beers, most likely with a yeast that came from Bohemia. When the Degenbergs finally died out in

**Table 4.** Comparison of fermentation attributes from *S. eubayanus* and other yeasts (Gibson et al. 2013).

Type	Strain		Maltose (g/l)	Maltotriose (g/l)	Ethanol (original)	3-Methyl-butyl acetate	Ethyl acetate
15° P wort			68.5–	18.9			
Ale	A56	22°C	0.2	14.2	6.1	1.8	25.2
Ale	A60	10°C	58.0	18.0			
Ale	A60	22°C	0.3	6.3	6.4	0.7	20.8
Frohberg	A03	10°C	0.4	8.6	6.4	1.0	17.6
Frohberg	A03	22°C	0.3	5.2	6.5	2.2	26.6
Frohberg	A15	10°C	0.2	6.6	6.5	2.2	22.8
Frohberg	A15	22°C	0.3	3.1	6.5	2.1	24.0
<i>S. eubayanus</i>	C902	10°C	6.8	18.8	5.1	1.7	23.4
<i>S. eubayanus</i>	C902	22°C	46.9	18.5			
Saaz	A11	10°C	4.0	18.7	5.3	0.3	9.5
Saaz	A11	22°C	0.4	18.7	5.6	0.4	16.2
Saaz	>A12	10°C	18.1	18.7	4.4	0.3	4.3
Saaz	A12	22°C	13.4	18.6	4.8	0.3	13.2

## Hypothetical origin of *S. pastorianus*



**Figure 2.** Hypothetical routes to the origin of *S. pastorianus*. From genetics, it is known that *S. pastorianus* arose by the hybridization of *S. cerevisiae* and *S. eubayanus* but the question is where and when this occurred. One theory is that *S. pastorianus* emerged in Bohemia and became part of the Bavarian *Stellhefen* mixture (Bavarian pitching yeast mixture) that came to be used in the Hofbräuhaus in Munich (1). The weakness with this argument is that it posits a Bohemian origin to both *S. eubayanus* and *S. pastorianus*, which are bottom-fermenting yeasts, whereas top-fermentation was the norm in Bohemia. The alternative hypothesis that emerges from our historical research is that *S. eubayanus* was part of the normal community of bottom-fermenting yeasts used in Bavaria from the 14th century and the critical event took place in the Hofbräuhaus in Munich early in the 17th century when particular top-fermenting *S. cerevisiae* strains entered the brewery. The attractive feature of this hypothesis is that historical records document two such introductions at the time that phylogenetic calculations say that the hybridization happened. These introductions, from the wheat beer brewery in Schwarzach (1602) (2) and the Einbeck brewery (1612) (3) led to the unique situation of a brewery producing both top- and bottom-fermented beers at the same time. The relationship between the *S. cerevisiae* subgenome of *S. pastorianus* and the genome of modern wheat strains of *S. cerevisiae* may favour the Schwarzach origin, but both are plausible. Perhaps future work that will uncover more genomic information about the ancestral populations of *S. eubayanus* and *S. cerevisiae* in the different regions will ultimately resolve the issue.

1602, Bavarian duke Maximilian conferred the privilege from 1548 on himself and seized the Degenberger's white beer breweries. Immediately, an expert brewer and the appropriate yeast were brought to the ducal Hofbräuhaus in Munich—where only brown (bottom-fermented) beer had been brewed since its foundation in 1591—and started brewing (top-fermented) wheat beer there.

This was a tremendous success and the white beer from the Elector's court attained some fame in the 18th century. Krünitz noted that it resembled English ale to some extent. A Bavarian princess, married to the king of Saxony, built a brewhouse to replicate this in Dresden. And elector Max Emanuel probably brewed it in Brussels, where he governed as vice-regent. The stunning success of

white beers (not only in Bavaria) might be attributed in part to rising average temperatures between ~1680 and 1750 (Luterbacher et al. 2004). Returning to the early days of brewing white beer in Munich, until a separate ‘weißes’ (white) Hofbräuhaus was established in 1607, bottom-fermented ‘brown’ and top-fermented ‘white’ beers were brewed in rotation, day and night, at the same brewery (Letzing 1990, Gattinger 2007). Thus, according to this proposal, the most likely *S. cerevisiae* ancestor of *S. pastorianus* is the top-fermenting wheat-beer yeast from the Schwarzach brewery that hybridized with *S. eubayanus* within a ‘Stellhefen’-yeast mixture at the Munich Hofbräuhaus. This hypothesis is supported by the data showing that the *S. cerevisiae* component of the *S. pastorianus* genome is phylogenetically related to wheat beer strains (Gallone et al. 2019). As Bohemian and Northern Bavaria were very much alike with respect to climate, raw materials, and brewing technology, and yeast and technology transfer within those connected regions was usual, it is very likely that these *S. cerevisiae* strains originated in Bohemia. When average temperatures dropped again in the 18th century, white beers quickly lost their appeal and in 1798, the Bavarian elector Karl Theodor revoked his monopoly on top-fermented beers. Wheat beer brewing decreased and stopped; wheat beers had become unattractive in Bavaria. The epoch of lager brewing had come.

There is a second plausible scenario in which the ale-yeast would have come from Einbeck. The extremely stable top-fermented ale (wheat-barley export-lager beer) from this little city on the southern rim of the Harz Mountains was the most famous beer in Germany between the 14th and 17th centuries. Although terribly expensive, the Bavarian dukes bought some casks for their table every year. To save this expenditure, in 1612, Duke Maximilian hired a brewer (presumably including his yeast) from Einbeck to prepare this beer in Munich. Some years later (1614 or 1617) the ‘Ainbock’ (later ‘Bockbier’) was served at the duke’s table. Ainbock was initially—like the original—a top-fermented beer made from wheat and barley. However, the brewing technology in Einbeck was special and differed significantly from the one in Munich. The yeast used in Einbeck also differed from other wheat beer yeasts in northern Germany. When the Hannover type of wheat beer (Broyhan) became fashionable in Einbeck (like elsewhere), a brewing ordinance prohibited using the same equipment for making Broyhan and Einbeck beer. Moreover, Broyhan yeast was definitely not to be used to make Einbeck beer. This indicates a distinct difference in the fermenting pattern/profile of the styles. Special beer was brewed in Einbeck since at least the 14th century (Feise 1928) and its special characteristics could have been associated with particular yeast strains. Thus, a potential split or differentiation of ale yeasts could have happened in northern Germany after the introduction of Broyhan (allegedly in 1528, possibly a modification of the famous white beer from Hamburg) or its introduction in Einbeck around 1600. One variety would then have been transferred to Munich. For some time, the ‘Einbock/Ainbock’ brewed in the Duke’s court was a strong ‘Weizenbock’ (Ester 1757) but it later became a very special Märzen type beer (Kreittmayr 1761). Thus a transformation from an originally top-fermented wheat beer to an strong bottom-fermented Märzen could be observed, which may indicate the presence of *S. pastorianus*. Kreittmayr attests that it was a special, stronger beer, sold every year for a few weeks seasonally only by the elector’s brown-beer Hofbräuhaus from Corpus Christi. Everybody was free to produce it as it was at that time (and as far back as Kreittmayr knew) a brown bottom-fermented beer. However, despite being the most coveted beer in Munich, due to both its expensive manufacture and the statutory cap on beer

prices due to the purity law, it was not profitable to produce. Accordingly, this beer was only brewed at the Hofbräuhaus.

The common feature of both these scenarios is that a top-fermenting beer yeast was introduced to the Hofbräuhaus and subsequently there was a switch of production from top- to bottom-fermented strong beer. There is no reason to think that this encounter between a particular ale (white beer) lineage and an *S. eubayanus* population in the Hofbräuhaus was unique and many more such encounters might have taken place (although bottom fermentation at that time was only widespread in the South), although no molecular traces of the resulting yeasts have been detected to date. Indeed, it is reasonable to believe that multiple different encounters and hybridizations took place but, for various reasons that are described below, the lineages that emerged from the Munich Hofbräuhaus became dominant in European brewing.

## Spread and dominance of Hofbräuhaus *S. pastorianus*

Regardless of the events that led to the creation of *S. pastorianus*, it is clear that strains from Bavaria spread all over Europe and are the source of all modern lager yeast strains. We explore here how this came to pass. Hofbräuhaus yeasts would have spread among Munich brewers and were widely used throughout the subsequent centuries, long before the development of pure brewing starter cultures (1883 onwards). The Hofbräuhaus was the most respected brewhouse in Munich both for its quality beers and for innovation and, by 1800, it was the largest brewery in Bavaria. However, the main argument for the dominance of Hofbräuhaus *S. pastorianus* might be due to a particular head brewer (brewmaster), Gabriel Sedlmayr the Elder, being appointed at the Hofbräuhaus in 1806. Even though Sedlmayr left just 1 year later (1807) and acquired the ‘Oberspatenbräu’ in Munich, he certainly had access to Hofbräuhaus yeasts. Together, he and his son, Gabriel Sedlmayr the Younger (along with other Munich brewers and scientists such as J. v. Liebig, B. Herrmann, and C. v. Kaiser) made the Spatenbräu a model brewery of modern industrialized lager beer fabrication. The success of the Munich brewing community rested on the close collaboration between the still craft-like brewers and brewing academia. Brewers from all over the world flocked to the Munich brewer school to learn the new technology of lager brewing while practicing in one of the breweries, preferentially at Sedlmayr’s Spaten. Thus, from 1837 till 1867 (when Weihenstephan took over), a total of 844 scholars attended the polytechnic academy. Among the brewers visiting Munich were those who introduced lager brewing to Berlin, Dortmund, and Switzerland (Sedlmayr 1951). The graduates probably took yeast as well as know-how with them, because there was no alternative if one wanted to produce lager beers. Selection/domestication of bottom-fermenting yeasts had been prevented by climate and tradition outside old (electorate) Bavaria. Even by this point, in the mid-19th century, ‘little glacial’, ice houses and elaborate artificial cooling were indispensable north of the low mountain ranges for lager brewing. Moreover, brewing even in most of the small brewhouses in Bavarian Swabia and in Franconia was less suitable than the integrated approach in Munich for the increasingly industrial-scale beer production (Anonymus 1845). Thus, everyone who intended to produce lager beer on a large scale had to use the fairly standardized Munich/Bavarian approach, including its yeast. Sedlmayr, in the old craft tradition, generously shared his knowledge and his yeasts with



friends and colleagues. His friend J. C. Jacobsen, owner of the Carlsberg Brewery, carried a tin can with the valuable yeast from Munich to Copenhagen in 1845, travelling in a stage coach and eagerly cooling his can at every stop with cold water (Sedlmayr 1951). In the 19th century, such long-distance shipments of yeast were no longer exceptional. When the brewer Georg Lederer (another of Sedlmayr's friends) started brewing 'Bayrisch beer' in Paris in 1850 while exiled from his native Nuremberg, he had yeast sent from home (Sprotte 2016). Similarly, German brewers emigrating to America to become founders of the lager beer industry there brought yeast with them.

## Development of methods for the isolation of pure yeasts by Hansen and Lindner

It is important to realize that the yeasts being distributed by the Spaten brewery in the 19th century were not pure cultures—these were "Stellhefen" mixtures that were dominated by strains of *S. pastorianus*. The capacity to consistently brew high-quality lager beer with them relied on the close adoption of the integrated Munich brewing process. In Copenhagen, Jacobsen noted that his brewery had done perfectly well using the same yeast for 36 years until the brewery skipped the traditional summer break and brewed all year around (Anonymus 1886, Lüers and Weinfurter 1931). Brewing all year round was an economic necessity for industrial breweries but it came with an elevated risk of spoilage. This risk, along with the increased frequency of poor-quality beer, motivated the chief scientist at Carlsberg, Emil Christian Hansen, to research the yeasts involved in the beer fermentation, a path that culminated in the development of the first pure yeast cultures. Using pure yeast for each brew anew would remedy the problem to some extent, because it prevented the increase of 'wild yeasts' in the population over repeated cycles. Hansen developed a cultivation method based on a fractionated streaking technique to obtain colonies from single cells (optimally if cells do not stick together).

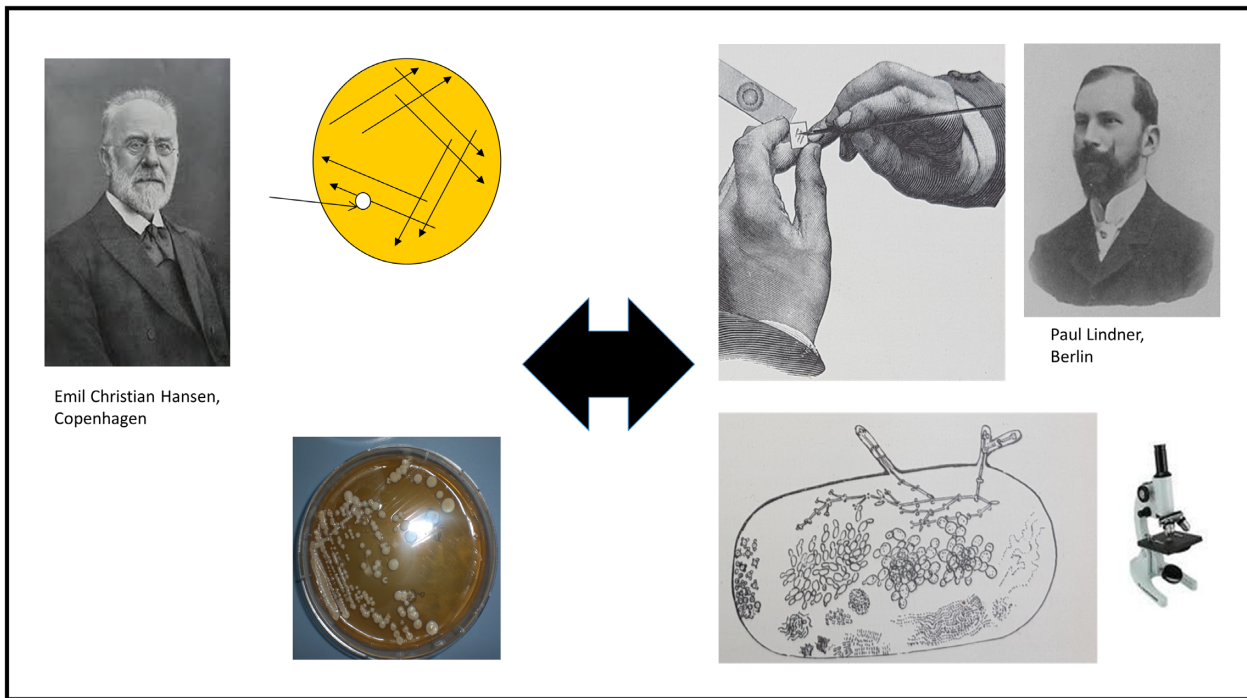
In 1883, Hansen recovered four isolates from his Carlsberg yeast, only one of which was a domesticated yeast ('Kulturhefe', i.e. a yeast with distinct fermentation profile, which performed reproducibly in practice) and three so-called 'wild yeasts', categorized according to the then accepted nomenclature as '*Saccharomyces pastorianus*' (Hansen 1895). Around this time, Paul Lindner, working at Delbrück's research institute at Berlin, developed an alternative approach to obtain pure yeasts ("Tröpfchenkultur"). Those two single cell lineage isolation methods were described in different references and are illustrated in Fig. 3 (Lindner 1891, 1901, 1909, 1910, Hansen 1895, 1905, Hansen and Klöcker 1911). This was the beginning of pure monofermentations and the observation and research of pure yeast lineages deriving from single clones. It was no wonder that many breweries soon established a laboratory with a pure yeast culture system. By 1895, a total of 115 lager breweries had adopted Hansen's system while another 18 lager breweries used the alternative system developed by Lindner (Hansen 1895). Not everybody agreed with the doctrine of pure cultures. For example, Max Delbrück objected to Hansen's methods as late as 1887 based on the reasoning that mixtures of yeasts with different properties were more suited to imparting distinct flavours. The English model (main top fermentation/*Brettanomyces* storage fermentation) still prejudiced German thinking. And even Hansen himself discussed the pros and cons of yeast mixtures in his book on fermentation (Hansen 1895). Moreover, it should be remembered that 'pure' strains only became available once

Hansen had introduced clonal selection to brewing. The idea of pure strains fitted perfectly with industrial fermentation concepts as it introduced criteria to standardize fermentations and allowed specific strain improvement. It also made collecting cultures attractive, as strains that performed consistently under given conditions became a commercial asset.

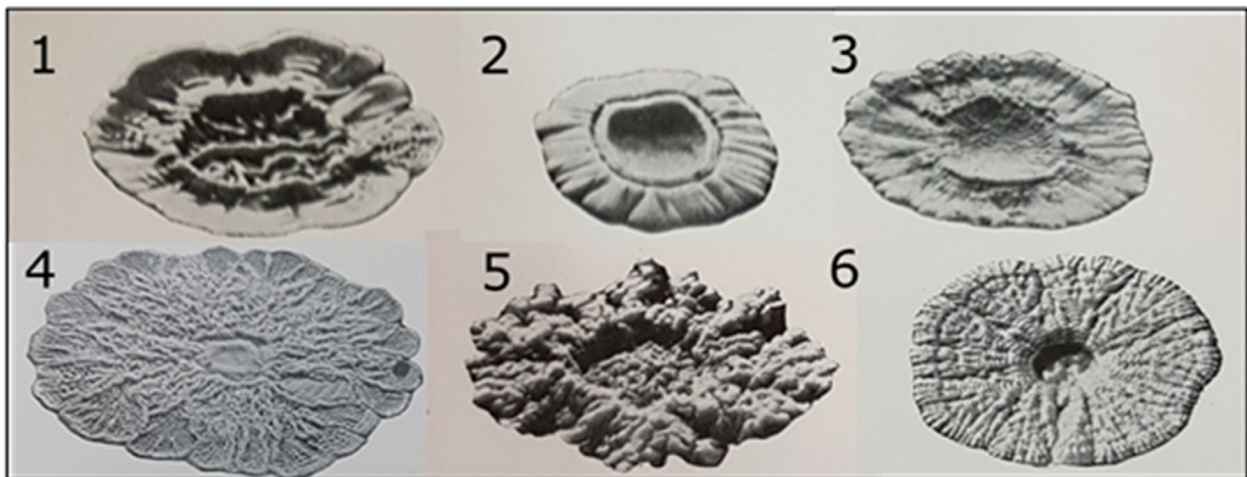
It has been testified by J. C. Jacobsen, as well as by Hansen, that the yeast samples from which Hansen isolated the pure strains, descended directly from the sample derived from the Spatenbräu in 1845 and it is now evident that Lindner's cultures were also of Bavarian origin. In addition to pure cultures, Lindner developed a method on thick wort-agar plates or wort-agar bottles to cultivate giant colonies deriving from pure cultures. The resulting colony morphology was also a differentiation criterion of the pure yeast culture (Fig. 4). Two distinct types of bottom-fermenting yeasts—slow and fast fermenting—were subsequently characterized. While there is little doubt that the fast-fermenting variety originated in Bavaria, theoretically, the slow-fermenting variety could have emerged by an independent event in Bohemia. However, as Hansen isolated a slow-fermenting strain (*Saccharomyces monacensis*) from a German sample, it is equally as likely that both scientists were purifying yeasts that were of Bavarian origin. The loss of culture collections during World War II, the arbitrary naming of isolates, and the periodical changes in yeast systematics, hampers attempts to trace today's specimens back to their original source. We discuss below the most likely interpretation of what the different isolates actually were in terms of modern yeast species.

## Differentiation of the two modern *S. pastorianus* lineages

Despite the confused taxonomy, it is now accepted that there are two extant modern lineages of *S. pastorianus*, usually referred to as referred to as Group I ('Saaz') and Group II ('Frohberg'). The identification of these two groups dates right back to the time of the initial isolations by Hansen and Lindner. Hansen noted that one of his most widely circulated isolates, 'Unterhefe Nr. 1' formed spores only after 5–6 days at 25°C, whereas another bottom-fermenting isolate, 'Unterhefe Nr. 2' sporulated rather readily (Hansen 1895). 'Unterhefe Nr. 1' is the original Spaten–Carlsberg yeast and was named *Saccharomyces carlsbergensis*. This strain was later recognized as *S. pastorianus* but the synonym *S. carlsbergensis* has been widely used in the literature. The second isolate, 'Unterhefe Nr. 2', was recovered from a sample sent from Germany shortly after the announcement of Hansen's results. Hansen put this isolate to work at Carlsberg and immediately realized that it behaved completely differently to Unterhefe Nr. 1 (Aubry 1885). While Unterhefe Nr. 1 fermented vigorously, Unterhefe Nr. 2 worked slowly. Despite these differences and Hansen's classification as *S. monacensis*, Unterhefe Nr. 2 is, in fact, a variant of *S. pastorianus*. In Berlin, Lindner also isolated yeasts with different fermentation properties. One, named 'Saaz', originated from the Common Brewhouse (Bürgerliches Brauhaus) at Saaz in Bohemia, where Dr Reinke from the Berlin Institute had sampled it. The other isolate, 'Frohberg', had been isolated from a sample sent to the Berlin institute by the owner of Frohberg's brewery at Grimma in Saxony (Reinke 1891). In the Berlin Institute, Saaz-type strains were classified as 'S' (German 'schwer' (slow)-fermenting) and Froberg-type as 'F' (German 'flott' (fast)-fermenting) (Reinke 1891), but the most common terminology for lager yeasts now is Group I (S; Saaz) and Group II (F; Frohberg). Despite the differences mentioned above, both Unterhefe Nr 1 and Unterhefe Nr 2 belong to Group 1 (Saaz).



**Figure 3.** Pure culture techniques developed by Emil Christian Hansen and Paul Lindner. Two different methods for the generation of pure monocultures of yeast were developed by Hansen and Lindner. Hansen's method was a fractionated single-colony streak on solid medium whereas Lindner's involved single droplet isolation under the microscope. In Lindner's method, mini droplets of a liquid sample (e.g. wort, green beer, and beer) were placed onto scratches of a glass slide and viewed using a microscope. If there were single cells in the field, they could be transferred into sterile wort by using sterile blotting paper. Both methods were adopted by breweries and used to develop pure cultures of *S. pastorianus* from St. Pauli Beer Co. mixtures of brewing yeasts.



**Figure 4.** Images of giant colonies of different yeasts visualized by Paul Lindner. Lindner developed a method to distinguish strains and species of brewing and wild yeasts by cultivating giant colonies on thick wort-agar. The different morphology is evident. Colonies 1 (Saaz) and 2 (Frohberg) show the two modern *S. pastorianus* groups; colony 3 is an *S. cerevisiae* Berlin wheat-beer yeast; colony 4 is *Saccharomyces farinosus* from Danziger Jopenbier (current name *Milleromyces farinosus*); colonies 5 and 6 are wild yeasts (Lindner 1909, 1910, Hutzler 2021).

The best-known strain of the Frohberg group is *S. pastorianus* TUM 34/70, which was sequenced in 2009 (Nakao et al. 2009), and whose history and path through time is described in detail in the publication by Gallone et al. (2019) in the supplemental note. The latter publication also describes the historical paths of another important strain (TUM 26) of the 'Frohberg' group, whose genome has also then been sequenced. TUM 26 was isolated between 1945 and 1956 from a yeast culture of the Sternburg brewery in Lützschen Leipzig, which is only a few kilometres from Fro-

hberg Brewery in Grimma (Meussdoerffer and Zarnkow 2016, Gallone et al. 2019). The Lützschen–Leipzig strain and the Frohberg–Grimma strain were probably the same. The Sternburg brewery Lützschen–Leipzig was founded in 1836–37 by Maximilian von Sternburg, who transformed it into a model lager brewery. The Bavarian Beer–Steam–Brewery Lützschen, as it was named originally, was built and run as a blueprint of the Munich original, and a master brewer of the Augustiner brewery (Munich) was hired as a consultant (Meussdoerffer and Zarnkow 2016, Gallone et al. 2019).

In all likelihood, the master brewer brought the yeast culture from the Munich Augustiner brewery to the Sternburg Brewery to start the first brewing batch. Together, this historical information also points towards a Bavarian, and more specifically Munich, origin of the Frohberg lineage (Meusdoerffer and Zarnkow 2016, Gallone et al. 2019). The genome sequence of the archetype of Group I strains, Hansen's 'Unterhefe Nr. 1' (*S. carlsbergensis*) was also determined, facilitating studies on the evolutionary relationship between the groups (Walther et al. 2014).

Almost immediately with the availability of pure yeasts, their fermentative properties were elucidated in detail. However, before discussing these results it might be useful, to look at the state of the brewing industries in Bavaria, Berlin, and Bohemia at that time. This was a period of rapid change from artisanal to industrial brewing. Although Munich was still home to the largest breweries at the time of Hansen's discovery, the brewing industry in Berlin grew rapidly and soon overtook Munich. However, the beer from Pilsen in Bohemia grew even faster from the 1860s onwards and would soon prevail. Thus, regional tradition as well as international customer preferences shaped the market. There were still distinct beer styles, differing in malting and mashing technologies. It was generally agreed that Munich style beers were less fermented and sparkling, but nourishing. This was achieved by using poorly dissolved malts (germination, high-temperature kilning), decoction-mashing, and cold, slow fermentation. In contrast, Viennese and particularly Pilsener beers were crystal clear, pale, and carbonized, with a slight resemblance to champagne. Certainly, different yeasts/fermentation technologies were essential to these beer styles. Moreover, the majority of breweries (in Bavaria 76%) still produced less than 1000 hl per year. In Saaz County, home to the original Saaz yeast, there were 56 breweries, of which only five were mechanized. Many of these small breweries obtained their yeasts from research stations or had their traditional yeast purified there. Only a few small breweries, like the one in Grimma where the Frohberg yeast originated, had a pure yeast apparatus (in this case a Lindner system). Thus, the isolated yeasts originated from small establishments that were still using mixtures of various yeasts as well as from larger brewing establishments that operated all year round with pure cultures. Studying the Saaz and Frohberg-type yeasts it soon became evident, that the different fermentation pattern was due to differences in their ability to metabolize different substrates (see Table 5). While the strong-fermenting Frohberg yeast suited the larger breweries well, the Saaz yeast seemed to be particularly adapted to the old Bohemian brewing process (Reinke 1891). The worts here were significantly weaker than elsewhere; in 1871 60% of the beers were produced from worts of 10°P (Noback 1874). The fermentation at the Bürgerliche Bräuhaus at Saaz—home of the yeast—was performed slowly at low temperatures yielding a degree of (real) fermentation of 33% (Reinke 1891). Moreover, differences were observed between the fermentation of Munich- and Berlin-type worts by S (Group 1) and F (Group 2) yeasts. Group 1 strains could only metabolize about 6/7 of the extract that was fermented by Group 2 strains. Later, this was correctly attributed to the inability of Group 1 yeasts to metabolize ( $\beta$ -) isomaltose (Koch 1894).

## Distribution and storage of brewing yeasts

Following the development of the technology for generating pure starter cultures, public and commercial scientific laboratories sprung up everywhere. In 1895, Hansen names a total of 22 such laboratories that used his technique, in Denmark (2), Sweden (1),

**Table 5.** Substrate usage of Saaz and Frohberg yeasts (Euler and Lindner 1915).

Yeast/sugar	Saaz	Frohberg
Inulin	–	–
Dextrin	–	–
Glucose	3	3
D-Mannose	3	3
D-Galactose	2	3
Fructose	3	3
Trehalose	?	2
Saccharose	3	3
Maltose	3	3
Lactose	–	–
Melibiose	2	3
Raffinose	3	3
$\alpha$ -Methyl-glucoside	3	3
$\beta$ -Methyl-glucoside	–	–
Arabinose, xylose, and rhamnose	–	–

– = negative, 2 = 2/3 usage, 3 = 3/3 usage

England (1), Germany (11), Bohemia (1), Austria (1), Russia (1), France (2), and USA (2). Soon, these institutes established culture collections of their own. In 1891, the collection of the Institute for fermentation in Berlin kept about 300 isolates, among them 200 yeasts (Lindner 1891). This institute purified yeasts from samples sent by breweries and provided expertise and appropriate yeasts from its own collection. In most of Bavaria, these services were rendered by the scientific station at Weihenstephan (Anonymus 1886). In 1895, Jörgensen (Copenhagen) provided yeasts to 66 smaller breweries as distant as South America or Australia. The institute's culture collection comprised more than 200 isolates. At the same time, the Bavarian experimental station in Nuremberg sent out some thousand litres of small pure yeast portions (Hansen 1895). Yeast specimens were stored in a dried state, preferentially mixed with sawdust. Asbestos, charcoal, or gypsum was also discussed, but probably less efficient (Lindner 1901). Another method was adsorption on sterilized filter paper. This method was also used to ship analytical samples. However, yeasts treated that way were stable for no more than 6–20 months and might even change before use. For longer preservation times, Hansen recommended storage in flasks with a sucrose (saccharose) solution. Yeasts remained remarkably stable under these conditions. Flask cultures sent in 1887 by Hansen to the British microbiologist A. Gordon Salomon could be fully reactivated after 34 years in 1921 (Ling and Nanji 1921). Of course, sporulation played a role in conservation and shipping. It was known that 'wild' yeasts sporulated much better than domesticated bottom yeasts ('Kulturhefen'). Hansen even recommended a quick check on spore formation as a rapid test for the purity of bottom-fermenting yeast cultures. Yeasts were also provided for free to Jacobsen's competitors Tuborg in Copenhagen and Heineken in Amsterdam. The correspondence between Heineken's director (1869–97) Wilhelm Feltmann and Jacobsen concerning yeasts from 1885 is revealing. Repeatedly, Feltman complained about the unsatisfying quality of the yeast he received and demanded better packaging. He proposed putting the yeast in a tin can, soldering the top and packing the can in a container filled with sawdust and ice. Meanwhile, Heineken brewed using pure cultures from Munich brewers and particularly the Hofbräuhaus, which is mentioned in several letters and even later judged the best yeast. These letters prove that pure cultures were readily available from many sources by 1885. In 1886, Feltman set up a laboratory for pure strain isolation at



**Table 6.** Timeline important steps, events, and inventions for beer brewing and fermentation (likely time period for *S. pastorianus* hybridization is marked with red bar).

1335	Brewing ordinance at Nuremberg (1323–35): 23. 'he who sells warm beer for cold beer is fined 5 pounds Heller
1350	Each year the counts of Schauenburg receive a donation of beer and <b>Gose</b> from Hamburg (1350–1400)
1374	Brewers in Hamburg create a new beer type from wheat and barley
1378	Expenses for the castle at Celle include two tuns of Einbeck beer
1380	Brewers and innkeepers at Nuremberg must have a deep cellar
1409	Brewing ordinance at <b>Landshut</b> : the cold beer shall remain in the brewhouse until fermentation has ended; the warm beer may be carried when fuzzy foam has emerged
1421–1550	Spöer Minimum (SPM) in solar activity
1435	Koelhoff's Chronicle in Cologne reports that Dutch beer spreads in the Rhineland's after a freeze in May has destroyed the vines and wine has become very expensive
1447	The word 'biere' is used officially for the first time in Paris
1473	Brewing ordinance of the <b>Munich</b> council: but for top fermenting beers, beers (bottom fermented) should be stored for at least 8 days before giving out
1474	Quarrel between <b>bakers and brewers at Munich</b> begin. It will continue sporadically until 1517 indicating that it took a long time until bottom fermentation was established, and all of its implications were manageable
1480–90	The chronicles of <b>Nabburg</b> (Palatinate) states that for the first time beer was brewed for the summer by bottom fermentation. In <b>1487</b> , a council ordinance established that henceforward only bottom fermentation should be used in the city's brewhouses
1480	At <b>Auerbach (Palatinate)</b> the 'cold' fermentation is successively introduced between 1480 and 1490
1483	The count Palatine decides in a quarrel at <b>Cham</b> (Palatinate) that new beer storage cellars might be built within the towns limits, but that ' <b>Bohemian' beer</b> might be served only with a permit of city authorities
1490	According to the records of the bakers–brewers quarrel in <b>Munich</b> , in 1483 the brewer Jacob Regenecker from Eger (originally a free city at the border of Franconia/Bohemia/Palatinate, then in the possession of the Bohemian crown) comes to Munich, gets the citizenship and starts to brew ' <b>Bohemian' beer</b>
1492	An ordinance from <b>Hamburg</b> decrees that beer has to rest for 8 days (export-lager beer) or 3 days in summer (ordinary beers), respectively before sent out
1513	The city council at <b>Nuremberg</b> orders the city's physician to examine the beer dregs (probably bottom yeast) for its possible health hazards
1516	Christian <b>Mumme at Brunswick</b> allegedly creates a new brown beer. In reality, this was a brown beer already brewed for some time, which resembled that at Einbeck
1517	A city ordinance from <b>Munich</b> limits the brewing season to the time between Michaeli (29th September) until Georgi (23rd April), beer might only sent out 8 days after brewing
1526	Announcement of the Bavarian purity law (Bayerisches Reinheitsgebot) in Ingolstadt by the dukes Dukes Wilhelm IV and Ludwig X. Only water, barley, and hops were allowed as beer raw materials
1535	In Bohemia, the contract of St. Wenceslaus day restricts the brewing rights of the Bohemian cities and cedes to the nobility the right to brew on their properties at will. This results in a surge in breweries and white beer production
	Kurt Broihan (Broyhan) allegedly creates a new wheat/barley white beer at <b>Hannover</b> resembling the white beer from Hamburg. This beer quickly captures the markets and is replicated at many places. Even the first white beers at Berlin at the end of the 16th century were modelled according to this beer
	A brewing ordinance at Wismar bans the use of bottom yeast 'underbarne' for home brewing (in contrast to top yeast, 'gest', which might be used by bakers. Repeated in 1635



Table 6. Continued

1547	In 1546/47, Hans VI von Degenberg builds a magnificent white beer brewhouse at Schwarzach. Die extraordinary quality of the white beer from there is often confirmed by contemporaries. Bavarian Duke Wilhelm V. at his residence at Landshut draws his white beer from Schwarzach
1548	Duke Wilhelm IV bestows on his chamberlain in lower Bavaria, Hans VI. von Degenberg the privilege, to brew and sell white beer in the territory north of the Danube
1551	Johannes <b>Placotomus</b> (Brettschneider, 1514–77) publishes 'de natum et viribns cerevisiarum et de mulso opusculum', from which H. Knaust later copied most of his book
1567	<b>A Ducal ordinance interdicts white beer brewing in all of Bavaria.</b> Thus, Degenberg has a monopoly
1573	<b>Heinrich Knaust</b> publishes in 1573 'Fünff Bücher, Von der Göttlichen vnd Edlenn Gabe, der Philosophischen, hochthwren vnd wunderbaren Kunst, Bier zu brawen'
1581	<b>Christoph Kobrer</b> publishes in 1581 'Gründliche und Nütze Beschreibung der Weinhawer und Bierbreyer-Practick und der ganzen Kellnermeister-Kunst'. It contains the first description of bottom fermentation
1585	Dr <b>Thaddaeus Hagecius von Hajek</b> publishes the first Bohemian brewing manual: 'De cerevisia eiusque conficiendi ratione, natura, viribus et facultatibus opusculum'. He reports about white beer brewing and states that the beer from Saaz is thought to be the best in Bohemia
1589	Chamberlain <b>Heinrich von Schwarzenberg</b> receives a Ducal patent for brewing wheat beer north of the Danube. This privilege remained only of local importance and never fully exploited. However, the Schwarzenbergs had already large estates in Bohemia
1602	Founding year of the <b>Munich</b> Hofbräuhaus. Its first high quality brown beer might have been served at the ducal table in 1591/92
1602	At 10th June 1602, Hans Sigmund von <b>Degenberg</b> , the last of his name <b>dies</b> . Duke Maximilian immediately takes all property, particularly the wheat beer brewhouses. The head brewer from Schwarzach is ordered to Munich to brew wheat beer at the Hofbräuhaus. On 24th October yeast is fetched from Schwarzach
1603	An ordinance at <b>Cologne</b> interdicts the use of bottom yeast ('Knupp') repeated in 1698
1607	From 1602 to 1607, the ducal wheat beer is brewed in the old, 'brown' Hofbräuhaus at <b>Munich</b> . Then a new 'white' Hofbräuhaus is ready and quickly expanded
1607	The first settlers in <b>Virginia</b> receive beer from England
1609	The Governor and Council of Virginia advertised in 1609 for two brewers to be sent to the colony. But the situation was still fairly desperate in 1613
1612	Brewer Elias Pichler from Einbeck is recruited by Duke Maximilian to produce Einbeck-style beer in Munich
1614	The first 'ainpöckisch' beer is served in Munich
1680	1680 Antony Leeuwenhoek studies yeast under the microscope
1798	Electo Karl Theodor revokes the monopoly on wheat beer. Wheat beers have become unattractive
1806	Gabriel Sedlmayr d. Ä. becomes head brewer at the Munich Hofbräuhaus, the largest brewery at Munich
1807	Sedlmayr resigns at the Hofbräuhaus and buys the Oberspatenbräu
1835	Sedlmayr sends air dried yeast, mixed with hops, to Scotland
1837	Sedlmayr sends yeast packed in a butter tube ('the Dutch way') to London
1841	Sedlmayrs son in law Deiglmayr buys the Franziskanerbräu
1842	Joseph Sedlmayr (brother) buys the Leistbräu
1845	Karl (son of J. C.) Jacobsen carries some pounds of Spaten-yeast in a tin can to Copenhagen
1848	Gabriel Sedlmayr buys the Kalteneckerbräu
1855	Josef Sedlmayr buys the Menterbräu
1863	In Bavaria, the brewing season is extended
	Sedlmayr buys the Dürnbräu

Likely event for *S. pastorianus* hybridization

Table 6. Continued

1871	Restrictions on brewing seasons are revoked, brewers may produce all year. However, Spaten only brews all year round in 1888
1876	Linde patent on the ammonia-refrigerator. 1877 Dreher in Vienna buys a Linde system for his fermentation cellar at Triest
1878	Lister publishes a dilution method to isolate clonal LAB
1879	Doctorate E. Ch. Hansen
1882	In Robert Koch's Lab agar plates are used, following a suggestion of Fanny Angelina Hesse. 1883 published. First storage cellar cooling with a Linde system at Dortmund
1883	Hansen isolates Carlsberg yeast # 1. (Unterhefe Nr 1)
1884	1.1.1883 The VLB at Berlin is founded by Richard Roesicke, Hugo Thiel, and Max Delbrück Hansen publishes a short note on his results in Zeitschr. f. d. ges. Brauwesen. 1884. A more extensive article appears only in 1888
1885	Jacobsen sends the pure Carlsberg yeast to Munich where it is used for the first time 11th May 1884 Aubry's Munich research station produces pure yeast for member breweries and interested customers. He offers two basic varieties: fast and slow fermenting
1886	Pure yeasts are distributed to 107 members of the Munich station. These were in part purified from samples sent in from the respective brewery and in part from the station's culture collection of Bavarian yeasts
1888	Paul Lindner's thesis 'Die Sarcina-Organismen der Gärungsgewerbe' is accepted by the faculty. He publishes 'ueber einige Gärversuche mit verschiedenen Hefen'. Wochenschrift f. Brauerei. 1888. No. 14., mentioning Saaz and Frohberg yeasts
1889	Aubry's research station distributed 164 pure cultures
1890	After 2 years of strong rejection, the station in Berlin accepts Hansen's methods and conclusions and bestows on him a honorary membership In 1890 and 1891, attention was directed by Otto Reinke and by M. Delbrück to two yeasts, Saaz and Frohberg, the first of which does not ferment wort to the same extent as the second, except in the presence of diastase or malt extract
1891	M. Irmisch studied 37 different yeast varieties and differentiates between two basic types: Frohberg und Saaz: 1891 Bd. 8: 1135 Der Vergährungsgrad, zugleich Studien über zwei Hefecharaktere'. Wochenschrift für Brauerei, 1891 Bd. 8: 1135
1893	Lindner publishes the droplet-purification method
1895	Jørgensens Lab at Copenhagen has a collection of about 200 pure yeasts 22 public laboratories (DK, S, D, F, A, USA) use Hansen's apparatus Lindners 'Mikroskopische Betriebskontrolle in den Gärungsgewerben'
	E. Prior at Nuremberg doubts that Saaz and Frohberg are distinct varieties. Bayr. Brauer-Journ., Jahrg. v., 1895 Arminius Bau proposes a new systematic for yeasts: 'Der Sammelbegriff Saccharomyces cerevisiae'; Wochenschrift für Brauerei, 1894 Nr. 43 S. 1366
1901	Carl Theodor Robert Frohberg (1850 Böhlen–1905 Grimma), Brauereibesizer in Grimma von 1872–1901. Probably closed due to bankruptcy!

Heineken and isolated their own strains. Soon Heineken sold surplus yeasts to breweries in Austria, Belgium, France, and Germany. In a letter to Jacobsen, Feltman had asked him to charge a fee for the yeast 'as it is usual among colleagues'. Thus, while Jacobsen rejected Hansen's repeated urgings to make yeast culturing a business for a long time, Feltman did so and had no scruples charging Carlsberg when he eventually delivered yeast to Copenhagen (Unger 1998). After 1900, the time when yeasts were given away freely had passed.

In the wake of Hansen's work, new strains were regularly isolated, characterized, and categorized. As unambiguous special properties for classifying yeasts and no systematics had yet agreed upon, much relied on observation under undefined conditions. However, these efforts led to the discovery of such important species/strains as *S. bayanus* (H. Will, 1891, from turbid beer), *Saccharomyces ludwigii* (later *Saccharomyces ludwigii*) (C. Hansen 1889, from the slime of oak trees) and *Schizosaccharomyces pombe* (P. Lindner 1893, from an African beer) (Barnett and Lichtenhaler 2001). Although yeast nomenclature has been changed multiple times, and some familiar species names like Hansen's *S. carlsbergensis* are considered synonyms, some others were discontinued and later reinstated (*S. uvarum*), many originally named strains are still included in culture collections. As the origin and history of strains is often somewhat blurred, it might be difficult to trace a given isolate back in time. Thus, one *S. bayanus* isolated by Beijerinck in 1894 and described in 1898 is typified today by strain CBS 395<sup>T</sup>. On the other hand, a *S. bayanus* described by Will (1891) and listed in 1895 by Saccardo (Sylloge Fungorum XI, p. 457) is typified with strain CBS 380T (Nguyen and Boekhout 2017). The frequent change of nomenclature leads to problems as summarized by Ernst Mayr in 1989: 'A brewer, for instance, is disconcerted when, ordering a culture of *S. carlsbergensis* from a culture collection, he receives one labelled *Saccharomyces cerevisiae*, *Saccharomyces uvarum* or *Saccharomyces bayanus*' (Barnett 2004). Accordingly, Will's *S. bayanus* (1891) is listed in ~191 accession numbers, while Beijerinck's yeast is registered today in ~49 and Hansen's Unterhefe Nr. 2, *S. monacensis* (1908) in 60 accession numbers.

## Summary and perspective

Our results of the historical research and their combination with published phylogenomic data allow us to propose the emergence and distribution of the *S. pastorianus* lager yeast in three main stages of evolution and transfer (Fig. 1, Table 6). First, yeast mixtures came to Munich from the heartlands of bottom-fermented beer (Franconia, Upper-Palatinate) where bottom fermentation has been practiced since at least the 14th century. After the Purity Law (Reinheitsgebot) of 1516, Bavaria was, with a few exceptions, a land with a purely bottom-fermented brewing method. Second, in 1602, the Hofbräuhaus Munich commenced brewing top-fermented beer (transfer of the right of the Degenberg aristocrats from Schwarzach to brew wheat beer to the Hofbräuhaus Munich). From 1602 until at least 1607 (1607 saw the establishment of a separate wheat beer brewery for the Hofbräuhaus), bottom- and top-fermented beer was produced side by side under one roof in very large volumes. In the period 1602–15, brewmasters from Schwarzach and Einbeck, along with their yeast, were active in the Hofbräuhaus Munich. This period and location are the most likely scenario for the hybridization to produce the bottom-fermenting *S. pastorianus* lager yeasts. Unlike its parents, *S. cerevisiae* and *S. eubayanus* (Rodríguez et al. 2014) (Goddard and Greig 2015), *S. pastorianus* does not sporulate well, if at all. This might indicate, i.e. unfit to thrive in natural environments and thus relied

exclusively on human propagation for longer-term survival. It is, therefore, quite likely that the bottom-fermenting yeasts, if they originated from a single event, remained confined to the regions south of the low mountain ranges. Third, and much later (1807), Sedlmeyer the Elder brought the yeasts of the Hofbräuhaus Munich to the Spatenbrauerei, which he and later his son developed into the flagship bottom-fermenting brewery. The Spaten brewing technology and associated *Stellhefen* became widely distributed, including to the Munich breweries Spatenbräu and Leistbräu, the experimental governmental brewery at Weihenstephan, and significantly in 1845 to the Carlsberg Brewery in Copenhagen. In 1883, at the Carlsberg brewery, Hansen developed pure cultures of the Spaten *S. pastorianus* and in turn these were widely distributed to many European breweries, among them the Franziskaner brewery in Munich, the Liesinger brewery in Vienna, the Cracauer brewery at Magdeburg (Aubry 1885), and even the Heineken brewery in Amsterdam. In Berlin, Lindner also developed techniques for purifying and studying yeasts and, using either Hansen's or Lindner's techniques, other breweries proceeded to isolate pure yeast strains of *S. pastorianus*. All of these derive from bottom-fermenting *Stellhefen* that were used in Bavaria, very often directly sourced from the Spaten Brewery, in some cases, many years before.

Early on, analysis of fermentation parameters defined two main groups of *S. pastorianus* strains and genomic data later confirmed this. The underlying ploidy of Group 1 (Saaz) strains is triploid, comprising mainly one *S. cerevisiae* subgenome and a diploid *S. eubayanus* subgenome, whereas that of Group 2 (Frohberg) strains is tetraploid, with diploid *S. cerevisiae* and *S. eubayanus* subgenomes. Subsequent genome evolution, changes in ploidy, gene duplication, loss of heterozygosity, and so on further complicates the landscape but the underlying structure is clear. The relationship between the two groups has been debated but it is now accepted that the two groups share an ancestry and do not represent two completely independent hybridization events (Salazar et al. 2019). This does not exclude the possibility that other hybrids may have formed over the course of brewing history. In fact, if one considers that large-scale brewing took place over hundreds of years, using mixtures of related yeast species, with variable environmental conditions and strong selective pressure, it seems very likely that this was the case. While, without doubt, *S. pastorianus* is an excellent bottom-fermenting brewing yeast, perhaps its occurrence in a technologically advanced brewery that was willing to share know-how and yeasts also contributed to its achieving dominance. It is also interesting to note that both Groups 1 and 2 *S. pastorianus* were maintained in the yeast mixtures that were used in Bavaria for hundreds of years, suggesting that their combined activity produced the desirable lager beers. The development of pure starter cultures facilitated the development of very large, industrial breweries and very consistent, high-quality beer—but perhaps as suggested by Delbruck, with the loss of some characteristics of those beers produced in the winter months in Bavaria from the 16th to the 20th century. Indeed, there is a certain circularity in the current efforts to create new beers by using consortia of strains, developing new hybrids, and tapping into yeast biodiversity.

## Acknowledgements

Roswitha Meussdoerfer for the posthumous release of text written by her husband Franz.

**Conflicts of interest.** None declared.

## References

- Anonymus. Über Gärung und Hefe. *Dinglers Polytech J* 1886;**6**:419–24.
- Anonymus. Ueber das bayerische bierbrauereiverfahren. *Dinglers Polytech J* 1845;**96**:478–82.
- Aubry L. Ein Beitrag zur Klärung und Richtigstellung der Ansichten über reine Hefe. *Zeitschrift für das gesamte Brauwesen* 1885;**7**:133–9.
- Balling CJN. *Die Gährungschemie Wissenschaftlich Begründet und in Ihrer Anwendung auf Die Weinberei-Tung, Bierbrauerei, Branntweinbrennerei und Hefenerzeugung practisch dargestellt*. Verlag der J. G. Calve'schen Buchhandlung. Charleston: Nabu Press, 1854.
- Barnett JA, Lichtenthaler FW. A history of research on yeasts 3: emil Fischer, Eduard Buchner and their contemporaries, 1880-1900. *Yeast* 2001;**18**:363–88.
- Barnett JA. A history of research on yeasts 8: taxonomy. *Yeast* 2004;**21**:1141–93.
- Bergin SA, Allen S, Hession C et al. Identification of European isolates of the lager yeast parent *Saccharomyces eubayanus*. *FEMS Yeast Res* 2022;**22**:foac053.
- Bing J, Han PJ, Liu WQ et al. Evidence for a Far East Asian origin of lager beer yeast. *Curr Biol* 2014;**24**:R380–381.
- Boynnton PJ, Greig D. The ecology and evolution of non-domesticated *Saccharomyces* species. *Yeast* 2014;**31**:449–62.
- Camenisch C, Keller KM, Salvisberg M et al. The 1430s: a cold period of extraordinary internal climate variability during the early Spörer Minimum with social and economic impacts in north-western and central Europe. *Clim Past* 2016;**12**:2107–26.
- Dunn B, Sherlock G. Reconstruction of the genome origins and evolution of the hybrid lager yeast *Saccharomyces pastorianus*. *Genome Res* 2008;**18**:1610–23.
- Eizaguirre JI, Peris D, Rodriguez ME et al. Phylogeography of the wild lager-brewing ancestor (*Saccharomyces eubayanus*) in Patagonia. *Environ Microbiol* 2018;**20**:3732–43.
- Estor JG. *Bürgerliche Rechtsgelehrsamkeit der Teutschen*. Bd. 1. Marburg: Weldige, 1757.
- Euler H, Lindner P. *Chemie der Hefe und der alkoholischen Gärung*. Mannheim: Akademische Verlagsgesellschaft, 1915.
- Feise W. *Das Brauwesen der Stadt Einbeck*. Berlin: Gesellschaft für die Geschichte und Bibliographie des Brauwesens, 1928.
- Gallone B, Mertens S, Crauwelse S et al. Genomics and evolution of beer yeasts. In: Bokulich NA, Bamforth CW (eds), *Brewing Microbiology: Current Research, Omics and Microbial Ecology*. Norfolk: Caister Academic Press, 2017a.
- Gallone B, Mertens S, Gordon JL et al. Origins, evolution, domestication and diversity of *Saccharomyces* beer yeasts. *Curr Opin Biotechnol* 2017b;**49**:148–55.
- Gallone B, Steensels J, Mertens S et al. Interspecific hybridization facilitates niche adaptation in beer yeast. *Nat Ecol Evol* 2019;**3**:1562–75.
- Gattinger K. *Bier und Landesherrschaft: Das Weißbiermonopol der Wittelsbacher unter Maximilian I. von Bayern*. München: Karl M. Lipp Verlag, 2007.
- Gayevskiy V, Goddard MR. *Saccharomyces eubayanus* and *Saccharomyces arboricola* reside in North Island native New Zealand forests. *Environ Microbiol* 2016;**18**:1137–47.
- Gibson B, Liti G. *Saccharomyces pastorianus*: genomic insights inspiring innovation for industry. *Yeast* 2015;**32**:17–27.
- Gibson BR, Storgårds E, Krogerus K et al. Comparative physiology and fermentation performance of Saaz and Froberg lager yeast strains and the parental species *Saccharomyces eubayanus*. *Yeast* 2013;**30**:255–66.
- Goddard MR, Greig D. *Saccharomyces cerevisiae*: a nomadic yeast with no niche?. *FEMS Yeast Res* 2015;**15**:fov009.
- Gunzelmann T. Bierlandschaft Bayern: keller als historische Orte des Konsums. *Archäologie – Geschichte – Geographie* 2010;**28**:7–53.
- Hansen EC, Klöcker A. *Gesammelte theoretische Abhandlungen über Gärungsorganismen*. Jena: G. Fischer, 1911.
- Hansen EC. Über die brutstätte der Alkoholgärungspilze oberhalb der Erde. *Centralblatt Für Bakteriologie, Parasitologie Und Infektionskrankheiten* 1905; **Abteilung II**:545 ff.
- Hansen EC. *Untersuchungen aus der Praxis der Gärungsindustrie: Beiträge zur Lebensgeschichte der Mikro-organismen*. München, Leipzig: R. Oldenbourg, 1895.
- Heiß P. *Die Bierbrauerei mit besonderer Berücksichtigung der Dickmaischbrauerei*. München: Selfpublished, 1853.
- Hermbstädt SF. *Chemische Grundsätze der Kunst Bier zu brauen*. Berlin: Carl Friedrich Amelang, 1814.
- Hoy A. Veränderung langjähriger Temperaturreihen in Mitteleuropa (und weltweit) seit dem 18 Jahrhundert. 2006. [https://www.regional-wetter.de/Klimaveraenderungen\\_seit\\_dem\\_18Jh.pdf](https://www.regional-wetter.de/Klimaveraenderungen_seit_dem_18Jh.pdf).
- Huntemann H. *Bierproduktion und Bierverbrauch in Deutschland vom 15. bis zum Beginn des 19 Jahrhunderts*. Dissertation Thesis, Georg-August-Universität zu Göttingen, 1970.
- Hutzler M. *Yeast biodiversity of traditional and modern hop beer fermentations and their targeted expansion via developed yeast hunting methods*. Habilitation Thesis, TU Berlin, 2021.
- Irsigler F. Ind machden alle lant beirs voll"- Zur Diffusion des Hopfenbierkonsums im westlichen Hanseraum. In: Wiegelmann G, Mohrmann RE, (eds), *Beiträge zur Volkskultur in Nordwestdeutschland*, Münster: Waxmann Verlag GmbH, 1996.
- Knaust H. *Fünff Bücher Von der Göttlichen und Edlen Gabe der Philosophischen, hochtheuren und wun-derbaren Kunst, Bier zu brawen*. Erfurdrt: Georgium Bawman, 1575.
- Kobrer. *Gründliche und Nütze Beschreibung der Weinhawer und Bierbriewer-Practick und der ganzen Kellermeister-Kunst, Regenspurg (today Regensburg)*, 1581.
- Koch A. *Jahresbericht über die Fortschritte in der Lehre von den Gährungsorganismen, 5. Jahrgang*, Braunschweig: Harald Bruhn, 1894.
- Kreitmayer WXA. *Anmerkungen über den Codicem Maximilianeum Bavaricum Civilem*. München: Zweyter Theil, 1761.
- Kromer B, Friedrich M. Jahresringchronologien und Radiokohlenstoff. *Geographische Rundschau* 2007;**59**:50–55.
- Landsteiner E. The crisis of wine production in late sixteenth-century Central Europe: climatic causes and economic consequences. *Clim Change* 1999;**43**:323–34.
- Letzing H. Das Weißbierprivileg Herzog Wilhelms IV von Bayern für Hans VI. von Degenberg. *Jahrbuch der Gesellschaft für die Geschichte und Bibliographie des Brauwesens* 1994/1995. Berlin: Gesellschaft für die Geschichte und Bibliographie, 1990.
- Libkind D, Hittinger CT, Valerio E et al. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. *Proc Natl Acad Sci USA* 2011;**108**:14539–44.
- Lindner P. *Atlas der mikroskopischen Grundlagen der Gärungskunde mit besonderer Berücksichtigung der biologischen Betriebskontrolle*. Berlin: Verlagsbuchhandlung Paul Parey, 1910.
- Lindner P. *Mikroskopische Betriebskontrolle in den Gärungsgewerben mit einer Einführung in die technische Biologie, Hefereinkultur und Infektionslehre*. Berlin: Verlagsbuchhandlung Paul Parey, 1909.
- Lindner P. *Mikroskopische Betriebskontrolle in den Gärungsgewerben*. Berlin: Verlagsbuchhandlung Paul Parey, 1901.
- Lindner P. Über die die Erkennung der Heferassen und ihre photographische Darstellung. *Wochenschrift Für Brauerei* 1891;**8**:815–6.
- Ling AR, Nanji DR. On the longevity of certain species of yeast. *Proc R Soc B* 1921;**92**:355–7.



- Liu L, Wang J, Rosenberg D et al. Fermented beverage and food storage in 13,000 y-old stone mortars at Raqefet Cave, Israel: investigating Natufian ritual feasting. *J Archaeol Sci Rep* 2018;**21**:783–93.
- Lorenz H. *Quellen zur städtischen Verwaltungs-, Rechts- und Wirtschaftsgeschichte von Quedlinburg*. London: Wentworth Press, 1916.
- Lüers H, Weinfurter F. Die Hefereinzucht in der Entwicklungsgeschichte der Brauerei. In: Schulze-Besse H (ed.), *Die Einführung der Hefereinzucht in den Brauereibetrieb*. Berlin: Gesellschaft für Geschichte und Bibliographie des Brauwesens e.V., 1931.
- Luterbacher J, Dietrich D, Xoplaki E et al. European seasonal and annual temperature variability, trends, and extremes since 1500. *Science* 2004;**303**:1499–503.
- Meussdoerffer F, Zarnkow M. *Brewing History Part III Cities - lecture manuscript*. Munich: TU München, 2018.
- Meussdoerffer F, Zarnkow M. *Das Bier, Eine Geschichte von Hopfen und Malz*. München: C. H. Beck, 2016.
- . In: Meussdoerffer FG, Eßlinger HM. *A comprehensive history of beer brewing*. (ed.), *Handbook of Brewing Processes, Technology, Markets*. Wiley-VCH, Weinheim, 2009, 1–42.
- Nakao Y, Kanamori T, Itoh T et al. Genome sequence of the lager brewing yeast, an interspecies hybrid. *DNA Res* 2009;**16**:115–29.
- Nespolo RF, Villarroel CA, Oporto CI et al. An out-of-Patagonia migration explains the worldwide diversity and distribution of *Saccharomyces eubayanus* lineages. *PLoS Genet* 2020;**16**:e1008777.
- Neumann C. *Lectioes publicae von vier Subjectis Diäteticis, Nemlich von den in hiesigen Gegenden gewöhnlichen und durch menschliche Hülffe zustande gebrachten Vierer Getränken*. Züllichau: Gottlob Benjamin Fromman, Buchhändler des Waisenhauses, 1735.
- Nguyen H-V, Boekhout T. Characterization of *Saccharomyces uvarum* (Beijerinck, 1898) and related hybrids: assessment of molecular markers that predict the parent and hybrid genomes and a proposal to name yeast hybrids. *FEMS Yeast Res* 2017;**17**: fox014.
- Noback G. Bier, Malz, so wie Maschinen und Apparate für Brauereien und Mälzereien. (Gruppe IV, Section 3.). In: Richter CT (ed.), *Officieller Ausstellungs-Bericht herausgegeben durch die General-Direction der Weltausstellung 1873*. Wien: K. K. Hof- und Staatsdruckerei, 1874.
- Okuno M, Kajitani R, Ryusui R et al. Next-generation sequencing analysis of lager brewing yeast strains reveals the evolutionary history of interspecies hybridization. *DNA Res* 2016;**23**:67–80.
- Peris D, Sylvester K, Libkind D et al. Population structure and reticulate evolution of *Saccharomyces eubayanus* and its lager-brewing hybrids. *Mol Ecol* 2014;**23**:2031–45.
- Peterka O. *Die bürgerlichen Braugerechtigkeiten in Böhmen: eine rechtsgeschichtliche untersuchung*. *Die bürgerlichen Braugerechtigkeiten in Böhmen: eine rechtsgeschichtliche untersuchung*. Prague: J G Calve, 1917.
- Placotomus J. *De natura et viribus cerevisiarum et mulsarum opusculum*. Wittenberg: Seitz, 1551.
- Powell CD, Quain DE, Smart KA. The impact of sedimentation on cove yeast heterogeneity. *J Am Soc Brew Chem* 2004;**62**:8–17.
- Reinke O. Über den mit verschiedenen Heferassen zu erzielenden Vergährungsgrad, seine bedeutung für die würzeuntersuchung und die bierbereitung. *Wochenschrift Für Brauerei* 8 1891;**39**:809–15.
- Rodríguez ME, Pérez-Través L, Sangorrín MP et al. *Saccharomyces eubayanus* and *Saccharomyces uvarum* associated with the fermentation of *Araucaria araucana* seeds in Patagonia. *FEMS Yeast Res* 2014;**14**:948–65.
- Salazar AN, Gorter de Vries AR, van den Broek M et al. Chromosome level assembly and comparative genome analysis confirm lager-brewing yeasts originated from a single hybridization. *BMC Genomics* 2019;**20**:916 1–18. <https://doi.org/10.1186/s12864-019-6263-3>
- Sampaio JP, Pontes A, Libkind D et al. Taxonomy, diversity, and typing of brewing yeasts. In: Bokulich NA, Bamforth CW (eds), *Brewing Microbiology: Current Research, Omics and Microbial Ecology*, Norfolk: Caister Academic Press, 2017, 85–118.
- Sampaio JP. *Saccharomyces eubayanus* – a tale of endless mysteries. *FEMS Yeast Res* 2022;**22**:foac058.
- Schönfeld F. *Obergärige Biere und ihre Herstellung*. Berlin: Paul Parey, 1938.
- Sedlmayr F. *Die Geschichte der Spatenbrauerei unter Gabriel Sedlmayr dem Älteren und dem Jüngeren 1807-1874*. Nürnberg: Hans Carl Verlag, 1951.
- Seifert JA. *Das Bamberger Bier, oder praktische, auf chemische Grundsätze gestützte Verfahrungsweise, Handgriffe und Gewerbs-Vortheile beim Brauen des Bamberger Bieres*. Bamberg: Selfpublished, 1818.
- Sieuwerts S, Bronac PA, Smid EJ. Mutually stimulating interactions between lactic acid bacteria and *Saccharomyces cerevisiae* in sourdough fermentation. *Food Sci Technol* 2018;**90**:201–6.
- Sprotte J. *Die Geschichte der Lederer-Bräu zu Nürnberg. Jahrbuch der Gesellschaft für die Geschichte des Brauwesens*. Berlin: Gesellschaft für die Geschichte des Brauwesens, 2016, 149–81.
- . In: Steinmeyer E, Steinmeyer E, Sievers E. *Sachlich Geordnete Glossare*(eds), *Die Althochdeutschen Glossen*. Berlin: Weidmannsche Buchhandlung, 1895.
- Unger R. Dutch brewing in the nineteenth century. In: Wilson RG, Gourvish TR (eds), *The dynamics of the international brewing industry since 1800 Dutch brewing in the nineteenth century*. New York: Routledge, 1998.
- Walther A, Hesselbart A, Wendland J. Genome sequence of *Saccharomyces carlsbergensis*, the world's first pure culture lager yeast. *G3: Genes, Genomes, Genetics* 2014;**4**:783–93.
- Wiegelmann G, Mohrmann R-E. *Nahrung und Tischkultur im Hanseraum*. In: Wiegelmann G, Mohrmann RE (eds), *Beiträge zur Volkskultur in Nordwestdeutschland*. Münster: Waxmann Verlag GmbH, 1996.
- Woltmann KL. *Geschichte von Böhmen*. Berlin: August Rücker, 1827.
- Zhao Z, Moghadasian MH. Chemistry, natural sources, dietary intake and pharmacokinetic properties of ferulic acid: a review. *Food Chem* 2008;**109**:691–702.