LALLEMAND LAGER STRAIN SELECTION

Lager is the most popular beer in the world. Clean and refreshing, this style has won over 90% of the international beer market.

The production of lagers is a recent innovation in the history of brewing, which is measured by thousands of years. The appearance of lagers is attributed to the XV-XVI centuries, and Bavaria is considered the birthplace of the style.

The key feature of the lager style is using the authentic type of yeast called *Saccharomyces pastorianus*. In the 1980s, while studying the DNA of the lager yeast, researchers discovered that it was a hybrid of the ale yeast *Saccharomyces cerevisiae* and a hitherto unknown microorganism. But in 2007 microbiologists found that the genes of that microorganism are 99.5% identical to a yeast found in Patagonia, which the local population used to produce alcohol at low temperatures. Yeast was contained in "galls", spherical growths on southern beech trees, inside of which juice is fermented (Figure 1). This strain was named *Saccharomyces eubayanus*.

FIG. 1: Galls growing on a southern beech tree in Patagonia.

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Lager yeast strains are classified into different lineages based on their genomic structure. Each *S. pastorianus* strain has a subgenome derived from both the *S. cerevisiae* and *S. eubayanus* parental strains (Figure 2). The two traditional lager groups arose by natural hybridation events. **Group I (Saaz)** lager strains are allotriploid with three sets of chromosomes, one from *S. cerevisiae* and two from *S. eubayanus*. Due to the greater contribution from the *S. eubayanus* subgenome these strains are more cryotolerant. **Group II (Frohberg)** lager strains are allotetraploid with four sets of chromosomes, two from *S. cerevisiae* and two from *S. eubayanus*. Due to the greater contribution from *S. cerevisiae* these strains exhibit more robust fermentation characteristics including broader temperature and alcohol tolerance. The most well-known lager yeast strain Weihenstephan 34/70 as well as **LalBrew Diamond™** belong to the Group II lineage. Both Group I and Group II strains are genetically very similar and have changed very little over the centuries since their domestication in 15th century Bavaria. Some brewers have used neutral ale strains to ferment at colder temperatures in order to produce neutral beers that are "lager-like", but not considered true lagers since they are not fermented with *S. pastorianus*. **LalBrew Nottingham™** is an excellent option for brewing pseudo-lagers due to its neutral profile and broad fermentation temperatures range.

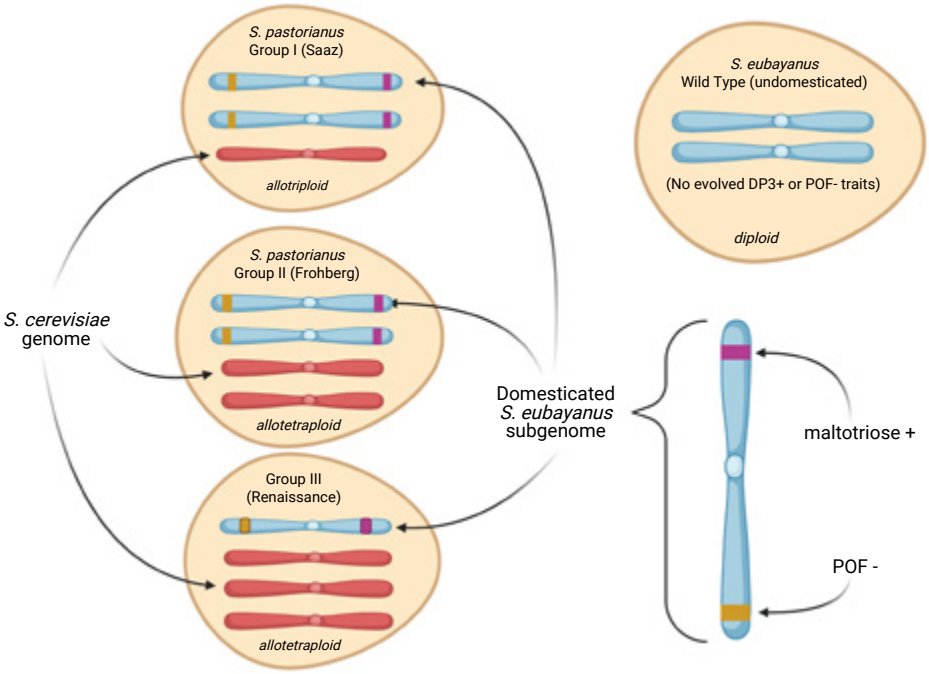

FIG. 2: Comparative genomic structure of Group I (Saaz), Group II (Frohberg) and Group III (Renaissance) lager strain lineages.

Recently, classical and non-GMO methods have been used to breed novel lager hybrid strains that are distinct from the Group I and II traditional lineages[1]. These novel **Group III (Renaissance)** strains are allotetraploid with four sets of chromosomes, three from *S. cerevisiae* and one from *S. eubayanus*. The first commercial example of the Group III lager strains is **LalBrew NovaLager™**, which represents the first major innovation in lager yeast strains in centuries. Due to a greater contribution from the *S. cerevisiae* subgenome the **LalBrew NovaLager™** strain demonstrates tolerance to warmer temperatures, more robust and rapid fermentation, a unique flavor profile and low levels of diacetyl and H_2S while maintaining cryotolerance imparted by the *S. eubayanus* subgenome (Figure 3).

FERMENTATION KINETICS

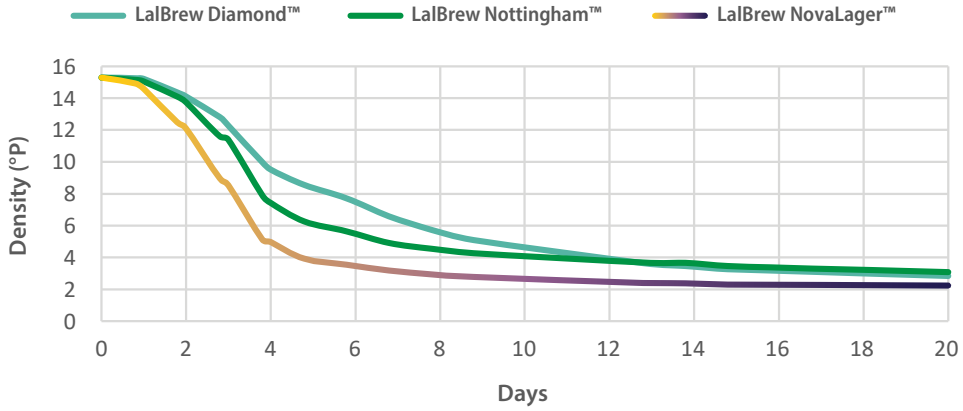

FIG. 3: Fermentation kinetics of different LalBrew® Premium Series yeast strains for lager styles. Standard all-malt 15°P pale wort pitched with 1.5 million cells/ml/°P and fermented at 12°C.

[1] Turgeon, Z., Sierocinski, T., Brimacombe, C. A., Jin, Y., Goldhawke, B., Swanson, J. M., Husnik, J. I., & Dahabieh, M. S. (2021). Industrially Applicable De Novo Lager Yeast Hybrids with a Unique Genomic Architecture: Creation and Characterization. *Applied and environmental microbiology*, 87(3)

[2] https://www.lallemandbrewing.com/en/technical-paper/hydrogen-sulfide-h2s-beer/

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QUICK FACTS

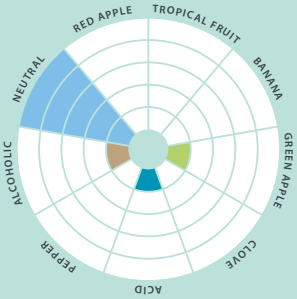
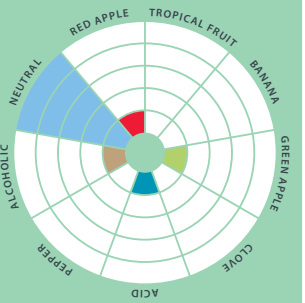
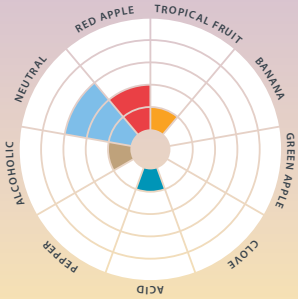
SPECIES	*Saccharomyces pastorianus*	*Saccharomyces cerevisiae*	*Saccharomyces pastorianus*
LAGER CLASSIFICATION	Group II (Frohberg)	Pseudo-lager	Group III (Renaissance)
HYBRID GENOMIC COMPOSITION	50% *S. cerevisiae* 50% *S. eubayanus*	100% *S. cerevisiae*	75% *S. cerevisiae* 25% *S. eubayanus*
MELIBIOSE UTILIZATION	+	-	+
ATTENUATION RANGE	77-83%	78-84%	78-84%
FLOCCULATION	High	High	Medium
TEMPERATURE RANGE	10-15°C (50-59°F)	10-25°C (50-77°F)	10-20°C (50-68°F)
ALCOHOL TOLERANCE (ABV)	13%	14%	13%
PITCHING RATE	100-200 g/hl	50-100 g/hl	50-100 g/hl
FLAVOR & AROMA			
	Neutral	Slightly fruity, neutral	Clean, low to medium ester, no sulfur

TABLE 1: Comparison of LalBrew® Premium Series strains for lager styles.

LAGER FLAVOR AND AROMA

Hydrogen sulfide (H_2S)

All brewing yeasts produce some amount of H_2S during fermentation as a part of normal amino acid metabolism (See our Technical paper, Impact of Hydrogen Sulfide in Brewing[2]). With ale fermentations, H_2S is depleted efficiently by CO_2 scrubbing during active fermentation and reabsorption by the yeast after full attenuation. With lager fermentations, the cooler, slower fermentations result in less CO_2 scrubbing and the bottom fermenting, moderately flocculant lager yeast does not reabsorb H_2S as efficiently. Small amounts of H_2S at threshold detection levels produced by traditional lager strains such as **LalBrew Diamond™** are considered normal and desirable for many lager beers. However, poor wort nutrition or brewing techniques can result in elevated levels of H_2S and an undesirable aroma of rotten eggs. This can be avoided by adding nutrients to the wort (especially when using adjuncts) and leaving the beer in contact with the yeast before transfer or filtration to allow time for H_2S reabsorption. The **LalBrew NovaLager™** strain was selected for reduced H_2S production by disrupting specific sulfur metabolic genes (Figure 4). As a result, **LalBrew NovaLager™** does not require the long maturation times typical of traditional lager strains.

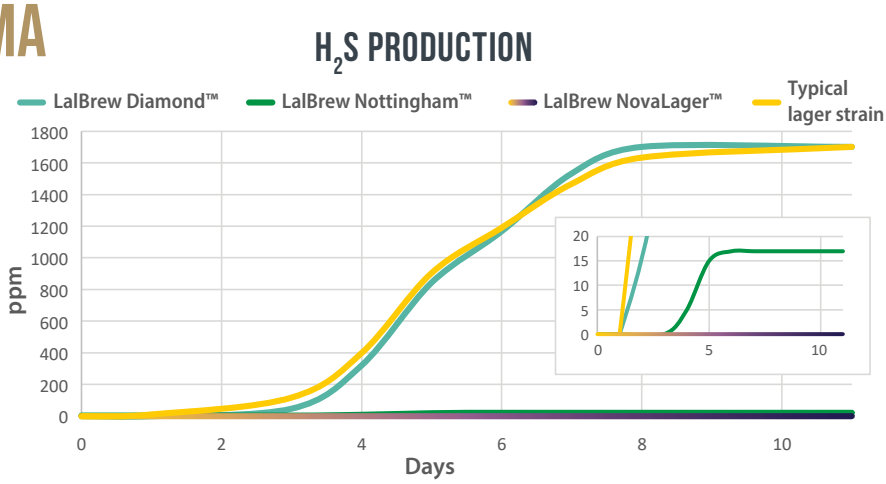


FIG. 4: H_2S production during fermentation with LalBrew® Premium yeast strains for lager beer styles. A typical lager strain is shown for comparison purposes. Traditional lager strains (**LalBrew Diamond™**, typical lager strain) produce more H_2S than ale strains (**LalBrew Nottingham™**). H_2S levels are undetectable for fermentations with **LalBrew NovaLager™**. Standard all-malt 15°P pale wort pitched with 1.5 million cells/ml/°P and fermented at 12°C.

Diacetyl

Diacetyl is a common fermentation byproduct that is perceived by most people as an off-flavor. It is produced from a side reaction by yeast metabolizing amino acids into valine. The yeast produces α-acetolactate, which is then excreted out of the cell. The α-acetolactate is then decarboxylated into diacetyl and reabsorbed back into the yeast at the end of fermentation where it is metabolized into acetoin, a flavorless compound. Diacetyl reabsorption by the yeast takes time and is faster at warmer ale temperatures compared to cooler lager temperatures. Diacetyl may be present in packaged beer when fermentations are incomplete, and the yeast is unable to completely reabsorb the diacetyl. For this reason, lager fermentations usually employ a diacetyl rest by raising the temperature at the end of fermentation to give the yeast time to reabsorb the diacetyl before transfer off the yeast (Figure 5). Diacetyl production can also be inhibited by using an α-acetolactate decarboxylase (ALDC) enzyme, which allows the direct breakdown of α-acetolactate into flavorless acetoin and prevents the formation and normal metabolism of diacetyl by the yeast cell.

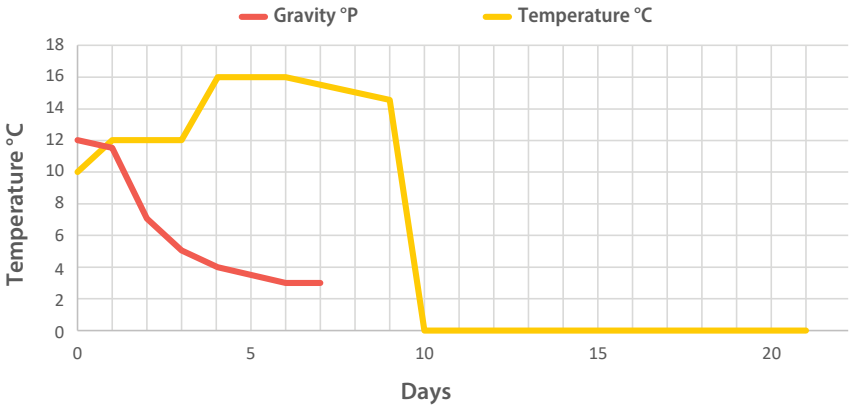


FIG. 5: A typical diacetyl rest is performed by increasing the temperature for several days at the end of active fermentation

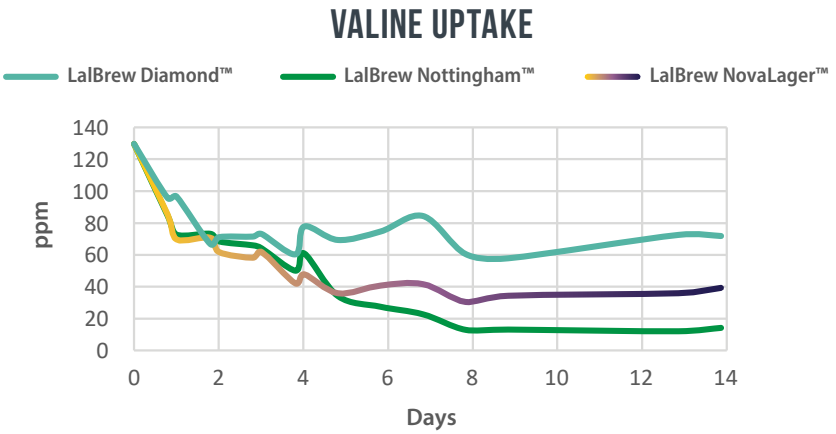


FIG. 6: LalBrew NovaLager™ exhibits higher "ale-like" uptake of valine similar to LalBrew Nottingham compared to traditional lager strains.

Strain selection will also impact diacetyl production. Ale strains such as **LalBrew Nottingham™** will tend to produce less diacetyl as a result of more efficient valine uptake. **LalBrew NovaLager™** demonstrates valine uptake and diacetyl levels that are similar to ale strains (Figures 6-7), which contributes to shorter maturation times required for this strain compared to traditional lager strains.

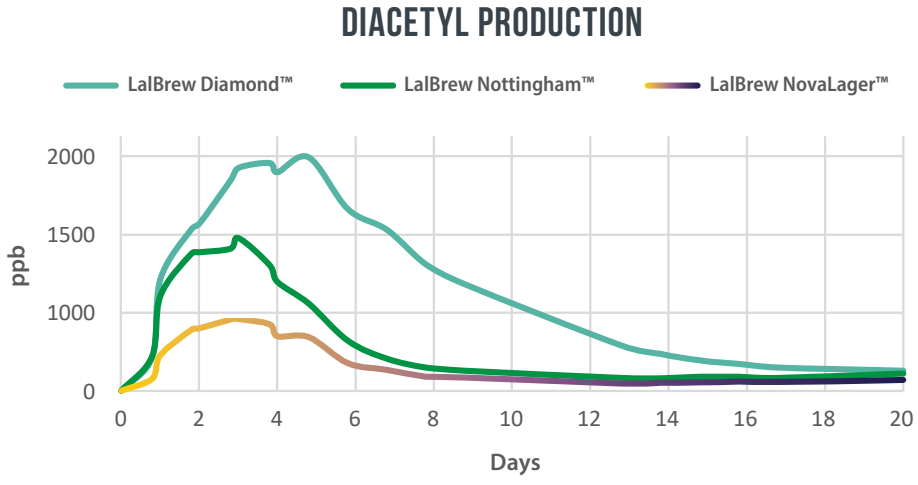


FIG. 7: The level of diacetyl produced by different yeast strains for lager styles.

Esters and Hop Biotransformation

Traditional lager strains such as **LalBrew Diamond™** produce few esters and are very neutral leading to clean, dry and refreshing beers. Modern interpretations of lager styles tend to be more flavorful, often with higher hop rates than traditional lager beers. The **LalBrew NovaLager™** strain produces low to medium esters for a more aromatic lager beer, and expression of a β-glucosidase enzymes promotes biotransformation and complexity of hop aromas.