Characterization of Novel Hybrid *Saccharomyces pastorianus* for Application In Industrial Brewing

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Introduction

Saccharomyces pastorianus is comparatively new on the evolutionary landscape yet is responsible for 90% of modern global brewing output. The unique selective pressures associated with traditional lager production are believed to have created the right conditions for rare hybrids to thrive and eventually dominate a once niche brewing style. Evidence suggests the first instances of “true” German lager yeast likely diverged approximately 500 years ago, coinciding with the start of industrialized brewing practices (1,2).

Interestingly, and perhaps in part due to its more contemporary evolutionary status, strain among modern *S. pastorianus* is viewed as lower than that of domesticated "pure" *S. cerevisiae* (though this notion is being challenged as biocomputing technology improves (3)). The challenge in boosting this diversity arises from two expected extremes: either waiting out natural selection events to occur, or to make use of iterative bioengineering. A middle ground does exist, and due in part to the complex genomic structure of *S. pastorianus* and other domesticated yeast, significant artificial selection progress can be made in a relative short amount of time. The use of high throughput screening and *de novo* hybridization techniques combined with forced evolution have opened new avenues to rapid artificial diversity enrichment within the taxon of *S. pastorianus*. In these trials we document the first uses and exploration into a genetically distinct and stable lager yeast bred primarily for low-to-zero expression of hydrogen sulfide, temperature tolerance, and low excretion of alpha-acetolactic acid. The classical definition of lager yeast is preserved with the added tangible benefits phenotypic diversity.

Materials and Methods

Wort Substrate

Standardized fermentation conditions were used to maximize fermentation efficiency. Wort was prepared with 12.0 g Golden Light Dry Malt Extract (DME, Briess Malt & Ingredients Co. Chilton, WI, USA) per 100 g tap water (12.0°P) and 30 ppm of Isohop® kettle extract (John I. Haas, Yakima, WA USA). Final wort concentration was verified via Anton-Paar DMA 35 densitometer, coming out to 12.0°P. Fermentability is rated at approximately 83%.

Classical Strain Comparison

Single representative fermentations (n=1) were performed using standard EBC practices at 1.6 L at 12°C. Active dried samples of fresh hybrid lagers were used and pitched at a rate of approximately 1.0x10^7 viable cells/ml. Fermentations were compared to classic lager strains prepared in dried form (**Table 1**). Fermentations were allowed to proceed for 168 hours before termination and bottling (**Figure 1**). 10 ml samples were collected for VDK analysis (**Figure 2**).

Table 1. Strains selected for comparative benchmarking

Strain	Code	Approx. cells/ml
Hybrid 01	L-01	1.0x10^7
Hybrid 02	L-02	1.0x10^7
Lager 01	L-03	1.0x10^7
Lager 02	L-04	1.0x10^7
Lager 03	L-05	1.0x10^7

Table 2. Strains selected for H2S and VDK benchmarking

Strain	Code	Approx. cells/ml
LalBrew 1	H-01	1.0x10^7
Hybrid 01	H-02	1.0x10^7
Hybrid 02	H-03	1.0x10^7
Lager 01	H-04	1.0x10^7
Lager 02	H-05	1.0x10^7
Lager 03	H-06	1.0x10^7

Hydrogen Sulfide Quantitation and Mass-Loss Temperature Trials and

Hydrogen sulfide production was compared among six strains, including the same classical lager strains benchmarked in fermentation (**Table 2**). H2S was quantified using a modified version of the method outlined by De Guidi *et al.* (2021). Methodology and apparatuses prepared for analysis available upon request (**Figure 3**).

Tared 200 ml fermentation vessels were sterilized and prepared using 150 ml of 12.0°P wort. Strains selected for mass loss were performed in duplicate (n=2) for a total of 12 fermentations. Active dried yeast was was pitched at a rate of 1.0 g/L. Fermentation conditions were 15°C, 20°C, and 25°C at 120 RPM agitation in an Infors HT Ecotron Incu-shaker (Infors HT Canada) and mass was measured every 12 hours until the 168-hour cut off time was reached. Samples were collected for HPLC carbohydrate analysis. Supplemental and method information available upon request (**Figure 4**).

Quantitative Metabolite Analysis

Headspace analysis was performed using a Thermo TSQ with a SatbilWax DA column (60 m x 0.32 mm ID; 0.25 µm film thickness). When possible, requested analytes were quantified by comparison to two mixed standards typically used for congener analysis in distilled spirits or an organic acids standard. 1.0 ml of sample was added to 100.0 µL internal standard of 1-pentanol. Additinoal HPLC analysis was performed using a Biorad Ion Exclusion column (HPX-87H)

Sample chromatograms are not presented here and are available upon request along with chromatography conditions.

Results

Figure 1. Average Fermentation Kinetics: 12°C, Approx. 1.0x10^7 cells/ml

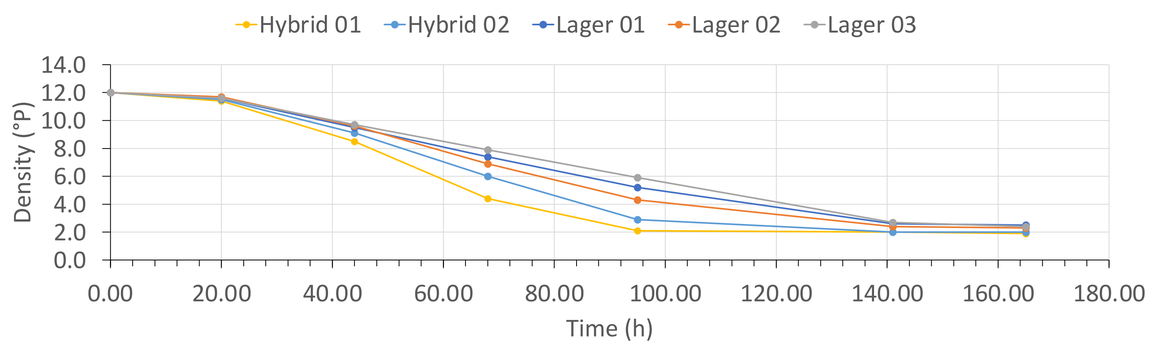


Figure 2. Average Post-Fermentation VDK samples (ppb), t=168 hours.

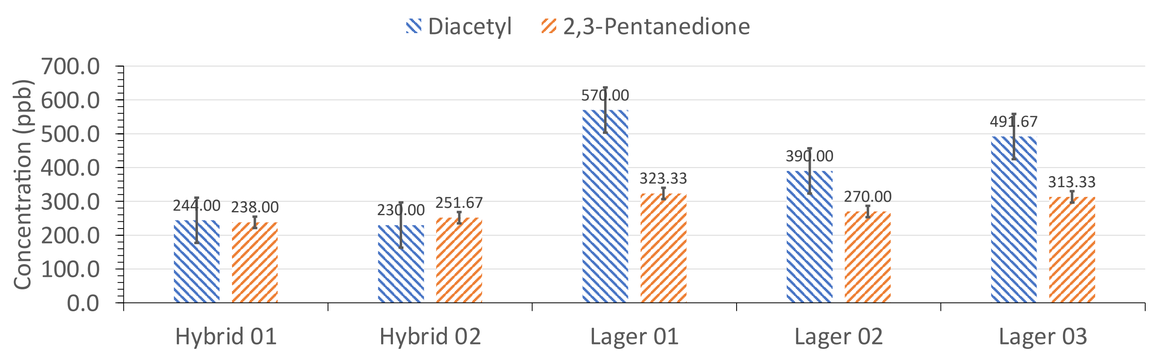


Figure 3. Total Hydrogen Sulfide Captured Over 72 hours

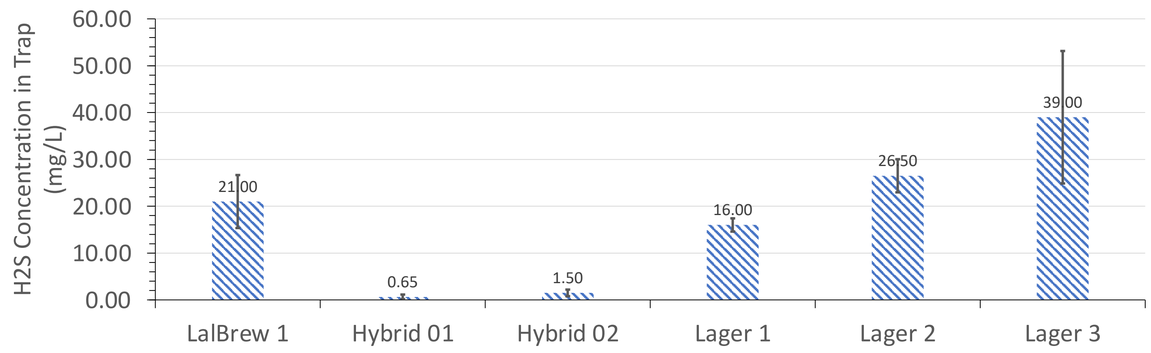
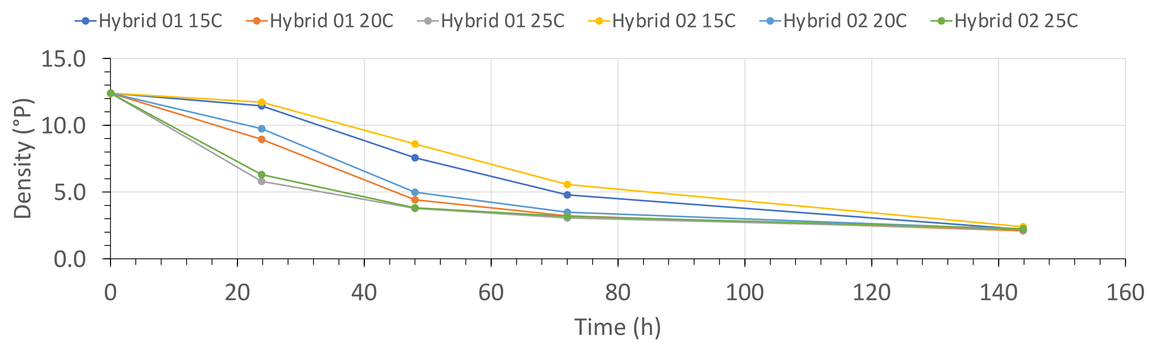


Figure 4. Fermentation Kinetics: Average Mass Loss, Normalized Pitch Rate



Discussion

The primary objective of these benchmarking trials were to showcase the viability of *de novo* hybridization techniques in the development an entirely novel *S. pastorianus* brewing strain. Genetically and phenotypically, the hybrids tested here fit the classical definitions of a lager yeast while successfully eliminating H2S production seen in other lager strains.

Other data collected (but not shown here) highlight secondary metabolite production – i.e., volatile organic compounds related to strain-specific organoleptic properties. Under controlled conditions, Hybrid 01 displays a unique characteristic when compared to other classical lager lineages. Pilot scale trials have shown that in the hands of competent brewers, a wide range of styles can be achieved with lower maturation times when compared to more traditional lager styles.

The performance showcased here represents a small slice of the total data available but remains a noteworthy addition to the industrial brewing scene.

Citations and Acknowledgments

1. Dunn B, Sherlock G. Reconstruction of the genome origins and evolution of the hybrid lager yeast Saccharomyces pastorianus. Genome Res. 2008 Oct;18(10):1610-23. doi: 10.1101/gr.076075.108. Epub 2008 Sep 11. PMID: 18787083; PMCID: PMC2556262.
2. Libkind, D., Hittinger, C. T., Valeŕio, E., Goncąlves, C., Dover, J., Johnston, M., et al. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc. Natl. Acad. Sci. U. S. A. (online). 10.1073/PNAS.1105430108/SUPPL_FILE/SD06.XLSX, 2011.
3. 1. Libkind, D., Hittinger, C. T., Valeŕio, E., Goncąlves, C., Dover, J., Johnston, M., et al. Microbe domestication and the identification of the wild genetic stock of lager-brewing yeast. Proc. Natl. Acad. Sci. U. S. A. (online). 10.1073/PNAS.1105430108/SUPPL_FILE/SD06.XLSX, 2011.

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