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Continuous backslopping cycles result in genome evolution in Trappist beer yeasts

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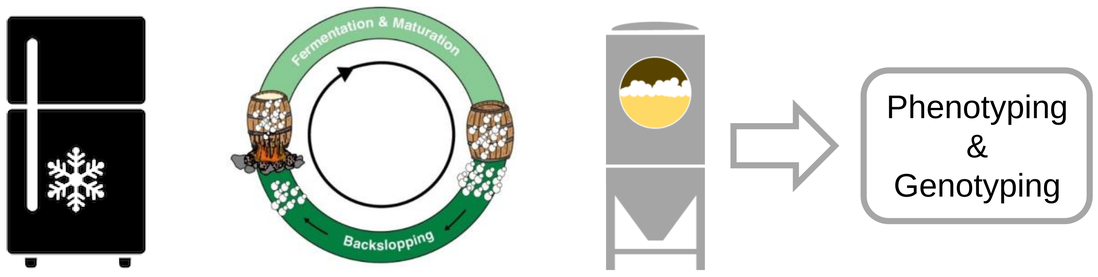
Project Overview

For thousands of years, humans have exploited the ability of baker yeast *Saccharomyces cerevisiae* to convert sugars into ethanol for brewing alcoholic beverages. Ancient brewers were already aware that using leftover yeast to start the next fermentation (backslopping) would result in more consistent quality of the fermentation. Continuous backslopping cycles until the end of 1800s resulted in the domestication of the baker yeast and its adaptation to the brewing environment. With the isolation of pure yeast strains and the introduction of frozen yeast stocks, the evolution of the yeast within the brewing environment was interrupted. In modern brewing the fermentation is started from a frozen stock of yeast and the cycles of backslopping are limited so that fermentation performances and quality of the brew are systematically maintained. The genetic alterations associated with continuous backslopping have not yet been characterized. However, understanding how backslopping shapes yeast genomes and brewing performances would allow to tailor superior yeast strains and result in economic benefits for the brewers. We have investigated the evolution a Trappist yeasts used for more than one year of continuous backslopping in the brewery. Yeast populations and individual clones were sampled from the yeast slurries during the initial brew and after one year of continuous backslopping. Populations genomics analyses were performed with cutting-edge sequencing technologies, and all samples were phenotyped. Our results indicated that the initial populations were heterogeneous and experienced an initial selective pressure during the first year of backslopping, with the emerging of a non-flocculant phenotype. Altogether, our results provide key insights on the evolution of yeast genome in the brewing environment and provided the foundation for breeding superior industrial yeasts.

Experimental Setup



Year 0 (Y0)

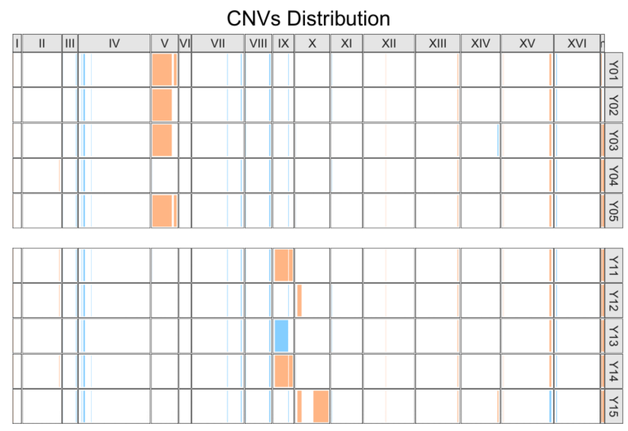
1 **population** sample
5 **individual clones**

Year 1 (Y1)

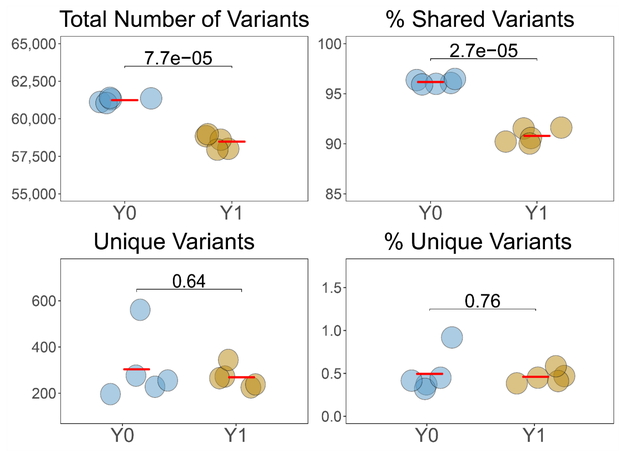
1 **population** sample
5 **individual clones**

Genotyping

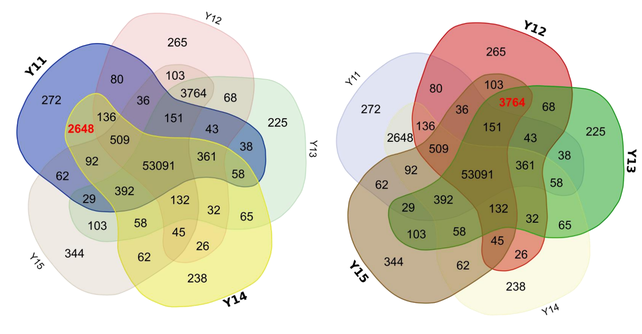
Clones Y0 have a chr V duplication absent in Y1.
Clones Y11, Y14 (**flocculant**) have duplication of chr IX
Clones Y12,Y15 (**efficient fermentation**) have partial duplication of chr X.



Clones Y0 have more variants than clones Y1, and are more similar between each other **(% shared variants**). Clones Y1 have less shared variants, but the amount of unique variants in Y0 and Y1 are comparable.



In Year 1, two distinct clonal lineages can be identified (Y11, Y14 and Y12, Y13, Y15, respectively), which have ~5% of variants unique to the clones. In Y11, Y14 lineage (flocculant), unique variants are enriched in cell wall organization genes.

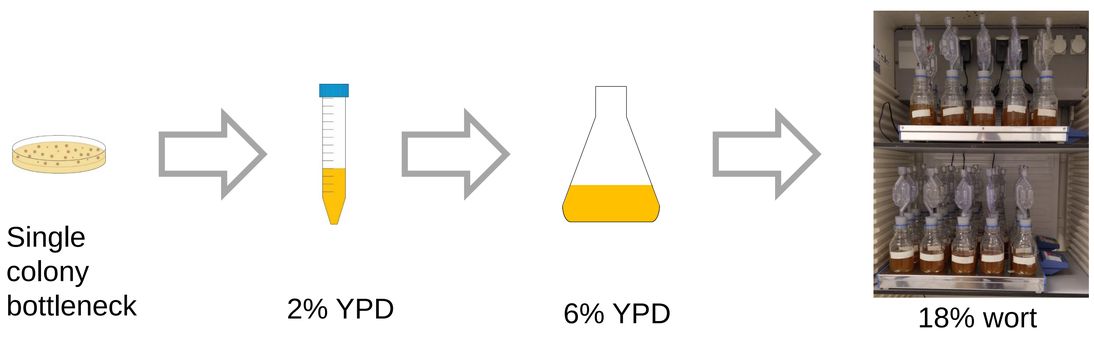


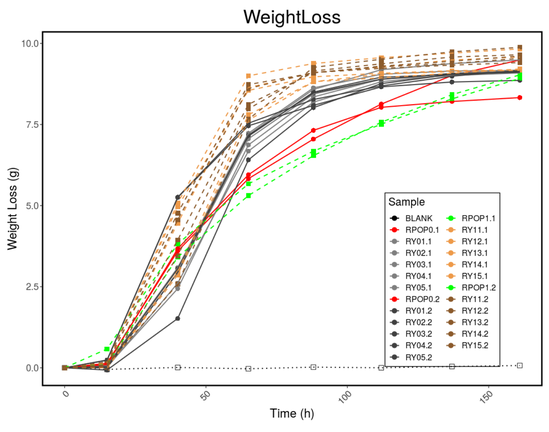
Y11, Y14 **Y12, Y13, Y15**

GO Term	P-value	FDR q-value	Enrichment	Description
GO:0071554	0.00000504	0.0264	2.19	cell wall organization or biogenesis
GO:0007165	0.00000517	0.0135	2.08	signal transduction
GO:0071555	0.0000106	0.0185	2.18	cell wall organization
GO:0045229	0.0000106	0.0138	2.18	external encapsulating structure organization
GO:0035556	0.0000138	0.0145	2.28	intracellular signal transduction
GO:0071852	0.0000452	0.0395	2.18	fungal-type cell wall organization or biogenesis

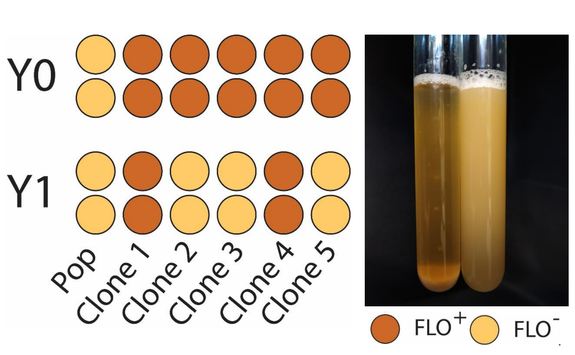
Phenotyping

Phenotyping of clones and population samples was performed on lab-scale fermentations





Fermentation efficiency



Flocculant phenotype

Conclusions

After one year of continuous backslopping, at least two distinct clonal lineages emerged in the yeast population, with specific phenotypic and genetic traits. One lineage have acquired a partial duplication of chr X and a higher fermentation efficiency. Moreover, this lineage has lost its flocculant behavior. The second lineage, instead, acquired a duplication in chr IX, and a subset of variants affecting genes involved in cell wall organization. All the sequenced clones Y1 seems to have lost chr V duplication, a genomic signature of Y0 clones. A general decrease in variants identified in Y1 when compared to Y0 can indicate a pressure toward loss of heterozygosis. The divergence of the two lineages suggests diverse trajectories of evolution of the Trappist yeast beer in the brewing environment. The two lineages can reach an equilibrium and coexist, or eventually one will outcompete the other.