P-007

Breeding new yeasts for efficient release of thiol aromas from hops

Kristoffer Krogerus, VTT Technical Research Centre of Finland, Eugene Fletcher, Escarpment Laboratories; Nils Rettberg, VLB; Brian Gibson; Technische Universität Berlin; Richard Preiss, Escarpment Laboratories

Introduction

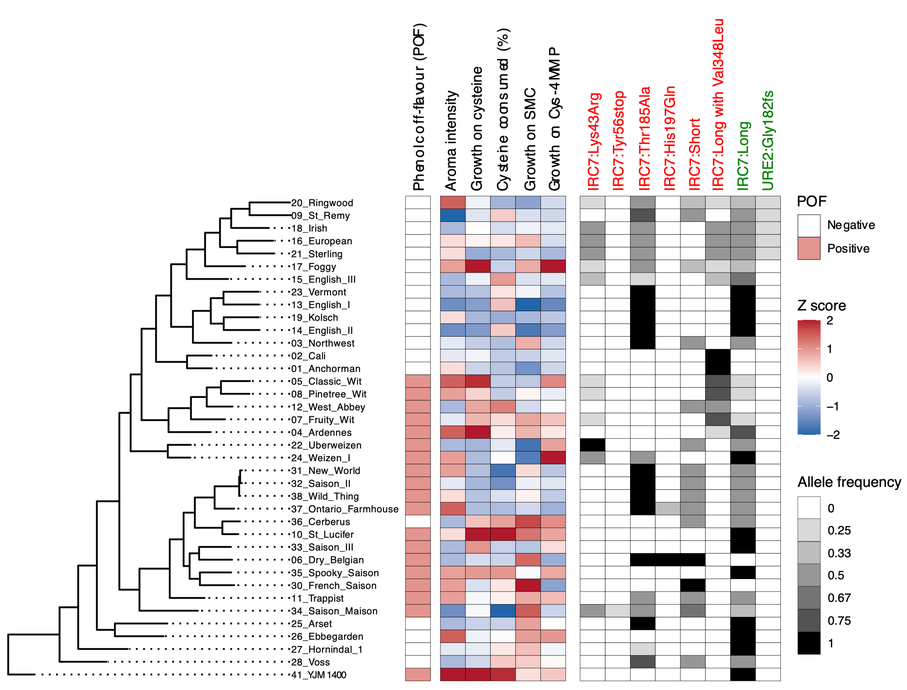
Yeast breeding is a powerful tool for developing and improving brewing yeast in a number of industry-relevant respects. However, breeding of industrial brewing yeast can be challenging, as strains are typically sterile and have large complex genomes. To facilitate breeding, we used the CRISPR/Cas9 system to generate double-stranded breaks in the MAT locus, generating transformants with a single specified mating type. The single mating type remained stable even after loss of the Cas9 plasmid, despite the strains being homothallic, and these strains could be readily mated with other brewing yeast transformants of opposite mating type. As a proof of concept, we applied this technology to generate yeast hybrids with an aim to increase *IRC7* β-lyase enzyme activity for fermentation of beer with enhanced hop flavour through release of thiols (4MMP, 3MH, 3MHA).

Methods

1. Genetic and phenotypic pre-screening of 38 yeast strains.
2. Mating competent transformants generated using CRISPR/Cas9 and used to generate >60 hybrids
3. Hybrids screened for β-lyase activity, POF and other brewing traits
4. Top performing hybrids tested for stability during repitching

To see full paper, scan QR code:

Results



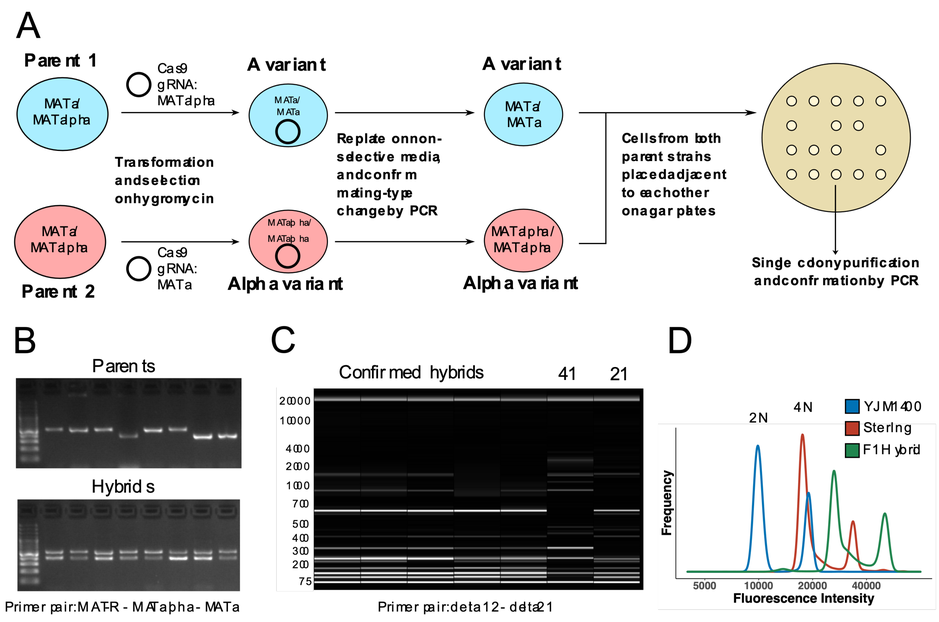


Figure 1 – Genetic and phenotypic screening of β-lyase activity in the 38 *Saccharomyces cerevisiae* included in the study. Strains are ordered based on phylogenetic relationship (maximum likelihood phylogenetic tree based on SNPs at 114700 sites, rooted with S. cerevisiae YJM1400 as outgroup). The phenotypic heatmap is colored blue to red based on Z-scores. Phenolic off-flavour status (white: negative, red: positive) of the strains is also indicated. The genotypic heatmap is colored from white to black based on allele frequency of the different mutations. The mutations that are colored red have been shown to decrease β-lyase activity, while the mutations colored green have been shown to increase β-lyase activity.

Figure 2 – Overview of hybrid construction and confirmation. (A) Scheme of how parent strains were converted to mating-competent variants, which were then mated to form hybrids. (B) Mating type PCR to confirm hybridization. Parents produced a single band for either MATa or MATα, while hybrids produced both bands. (C) Interdelta fingerprints to confirm hybridization. Hybrids produce fingerprints containing all the bands of the parent strains. (D) Flow cytometry and SYTOX Green staining reveal an increased ploidy of the F1 hybrid formed between Sterling and YJM1400 compared to the parent strains.

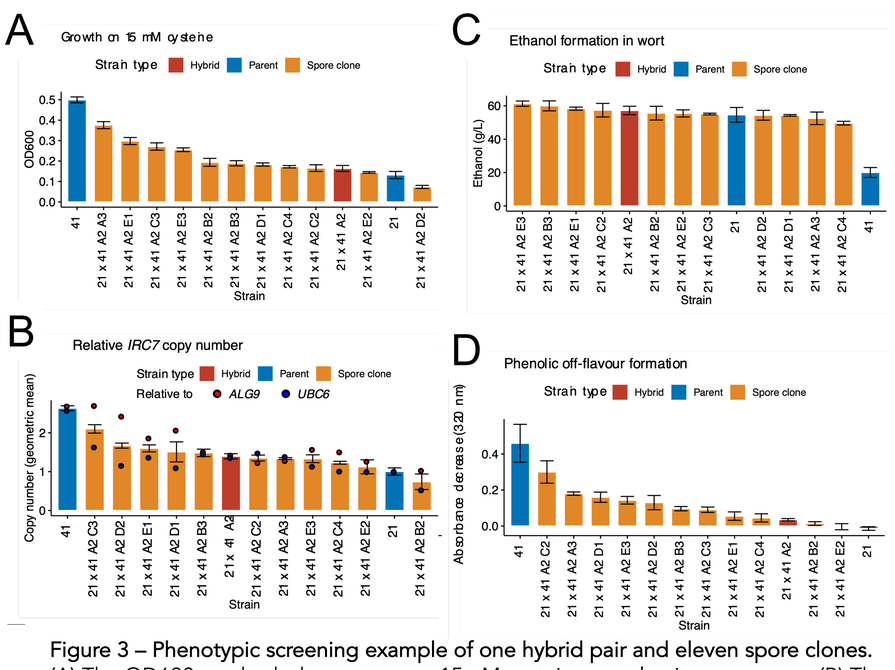


Figure 3 – Phenotypic screening example of one hybrid pair and eleven spore clones. (A) The OD600 reached when grown on 15mM cysteine as sole nitrogen source (B) The relative IRC7 copy number normalized to ALG9 and UBC6, as determined by quantitative PCR. (C) The amount of ethanol (g/L) produced from 15 °P wort in microplate fermentations. (D) The decrease in absorbance at 320 nm after cultivations in 100 mg/L ferulic acid. A larger decrease in absorbance indicates more conversion of ferulic acid to 4-vinylguaiacol. Assays were done in triplicate, and error bars represent standard deviation. 21: Sterling. 41: YJM1400.

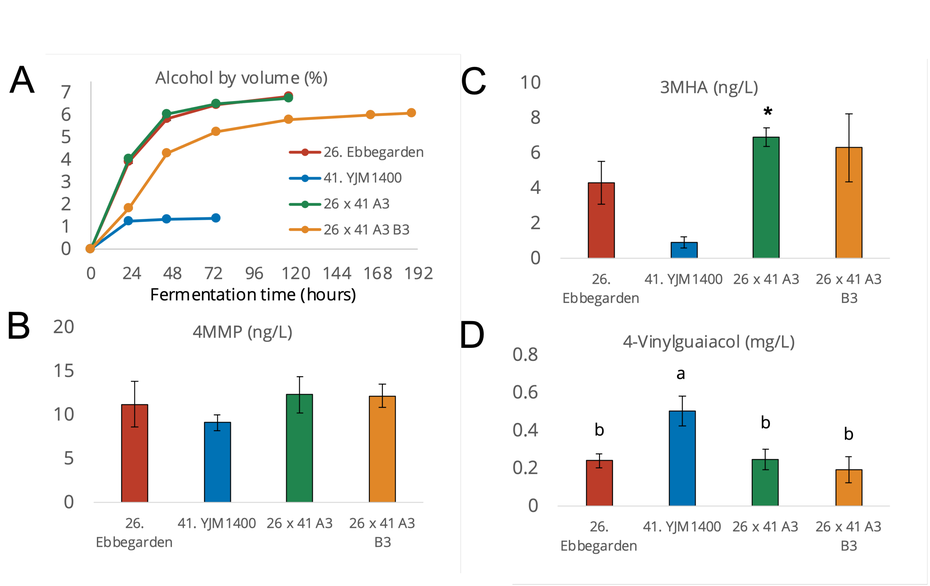


Figure 4 – Fermentation performance and concentrations of thiols and 4-vinylguaicol in 2L-scale fermentations of one hybrid pair. (A) Alcohol by volume (%) during fermentations. Concentrations (ng/L) of (B) 4-mercapto-4-methyl-2-pentanone (4MMP), and (C) 3-mercaptohexylacetate (3MHA) in the beers. An asterisk (*) indicates a concentration significantly higher ($p < 0.05$) than both parent strains as determined by unpaired two-tailed t-test. (D) Concentrations of 4-vinylguaicol (mg/L) in the beers. Different letters indicate significant differences ($p < 0.05$) as determined by one-way ANOVA and Tukey's post-hoc test. Fermentations were performed in triplicate.

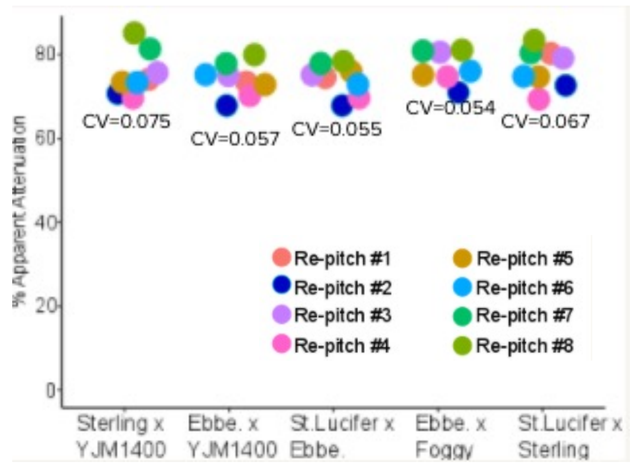


Figure 5 – Fermentation stability of selected hybrids during serial yeast repitching. Yeast strains were propagated in wort and inoculated at 10 million cells/mL in 400mL of standardized wort. Fermentation was monitored for 7 days at which point the yeast was collected and inoculated into the subsequent fermentation for a total of 8 generations of beer fermentation. Coefficient of variation (CV) = stdev/mean. A CV >0.1 (10%) indiciates high variation.

Summary

- CRISPR/Cas9-based mating type switching was applied to industrial brewing yeast
- Transformed strains could be readily mated to form new hybrids
- Hybrids exhibited hybrid vigor for traits including 3-MHA (guava, passionfruit)
- Hybrids were stable for multiple generations of beer brewing

Support

ESCARPMENT LABORATORIES
Canada NRC·CNRC
BUSINESS FINLAND
VTT
eurostars™

Selected References

Cordente AG, Borneman AR, Bartel C, Capone D, Solomon M, Roach M, Curtin CD (2019) Inactivating Mutations in Irc7p Are Common in Wine Yeasts, Attenuating Carbon-Sulfur β-Lyase Activity and Volatile Sulfur Compound Production. Appl Environ Microbiol 85.

Dufour M, Zimmer A, Thibon C, Marullo P (2013) Enhancement of volatile thiol release of *Saccharomyces cerevisiae* strains using molecular breeding. Appl Microbiol Biotechnol 97:5893–5905.

Xie Z-X, Mitchell LA, Liu H-M, Li B-Z, Liu D, Agmon N, Wu Y, Li X, Zhou X, Li B, Xiao W-H, Ding M-Z, Wang Y, Yuan Y-J, Boeke JD (2018) Rapid and Efficient CRISPR/Cas9-Based Mating-Type Switching of *Saccharomyces cerevisiae*. G3 Genes|Genomes|Genetics 8:173–183.