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Fluorescence-based approach for the effective development of industrial Saccharomyces cerevisiae strains

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Abstract
The laboratory evolution based on sexual hybridization – haploids breeding – is a powerful approach to study and develop Saccharomyces cerevisiae strains applied to the production of renewable fuels and chemicals. In this context, we developed a simple and efficient method based on reporter proteins expression and fluorescence-activated cell sorting (FACS). Exploring the natural genetic variability of two industrial Brazilian strains we obtained haploids showing superior biomass production under tree industrially relevant stress condition. In conclusion, this approach enables the isolation of an unlimited number of recombinants haploids requiring minimal and transient engineering, providing a powerful tool for create new superior hybrids through breeding, map genes related to industrially relevant traits, improvement of commercial strains and development of new ones.

Key words: *Saccharomyces cerevisiae, Fluorescent proteins, Flow cytometry.*

Introduction

In the context of developing more efficient yeasts for production of renewable fuels and chemicals, the laboratory sexual evolution is a powerful approach to explore genetic variability. As demonstrated by McDonald and colleagues[1] the sex speeds adaptation and alters its molecular signature by allowing natural selection to more efficiently sort beneficial from deleterious mutations. Yeasts naturally adapted to industrial environment – industrial strains – are considered appropriate platforms for the development of commercial strains[2], however, combine industrially relevant features by breeding require the isolation of a large number of recombinant haploids, typically obtained by laborious manual dissection of the tetrads. For this reason, the goal of this study was to develop a very simple and efficient approach to isolate recombinant haploids using flow cytometry.

Results and Discussion

The coding sequences for two fluorescent proteins were fused respectively to MATα-specific STE3 and MATa-specific STE2 promoters resulting in the pMF002. To allow the use of equipment containing the usual lasers configuration we decided to use an orange fluorescent protein (cyOFP) with a large stokes shift that can be excited simultaneously with but detected separately from EGFP (Ex. 488 nm; Em. 600 and 505 nm respectively)[3]. To confirm its functionality the pMF002 was transformed into industrial strain JAY270 (MATa/α) and its derived haploids JAY289 (MATa) and JAY290 (MATα)[4]. In the visual inspection analysis, transformed haploid cells MATa and MATα showed green and orange fluorescence, respectively. As expected, the diploid cells showed no fluorescence. Using optimized cytometry parameters was possible to detect and distinguish the both fluorescence signals. As a proof of concept, cell-pool carrying pMF002 obtained from the random lysis of industrial strain JAY270 tetrads were analyzed. At least one thousand haploid cells expressing green or orange fluorescence were collected and after growth analysis new superior haploids were selected. Compared to parental strain JAY270, the new haploids are significantly more tolerant to low pH treatment and oxidative stress induced by high concentrations of peroxide, two relevant conditions for industrial first and second-generation ethanol production.

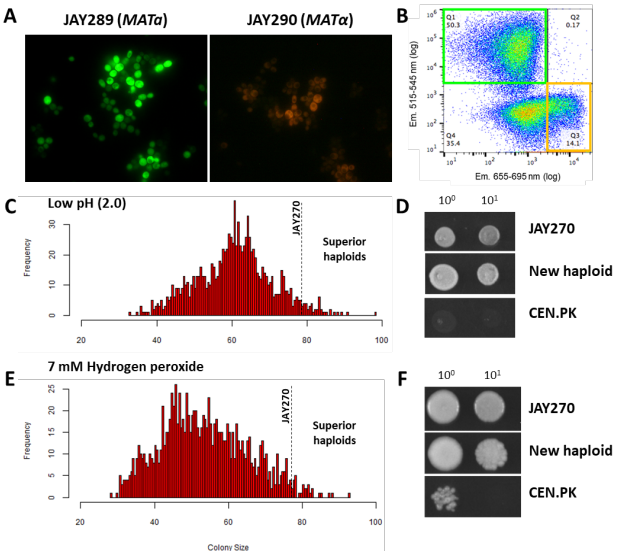

Image 1.A. Visual inspection analysis on epifluorescence microscopy. **B.** Cytometry analysis. **C-D.** Growth assay on low pH and selection of superior haploid. **E-F.** Growth assay on high concentration of hydrogen peroxide and selection of superior haploid.

Conclusions

In conclusion, this approach enables the isolation of an unlimited number of recombinants haploids requiring minimal and transient engineering, providing a powerful tool for creating new superior hybrids through breeding, map genes related to industrially relevant traits, improvement of commercial strains and development of new ones.

Acknowledgement

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