Genetic manipulation of an industrial bioethanol Saccharomyces cerevisiae strain based in gene expression data.

Author(s)
Osmar Vaz de Carvalho-Netto [1], Luciana S. Mofatto [1], Marcelo F. Carazzolle [1], Paulo J.P.L. Teixeira [1], Piotr A. Mieczkowski [3], Juan Lucas Argueso [2], Goncalo A. G. Pereira [1]

Institutions
[1] Unicamp - Campinas State University (Laboratory of Genomics and Expression. Department of Genetics.), [2] CSU - Colorado State University (Department of Environmental and Radiological Health Sciences), [3] UNC - University of North Carolina (Department of Genetics)

The bioethanol production system used in Brazil is based on the alcoholic fermentation of sucrose derived from sugarcane feedstock by highly adapted strains of the yeast Saccharomyces cerevisiae. We have recently described the complex genome structure of one of the most productive and widely adopted strains: PE-2 (Argueso, Carvalho-Netto et al. 2009, Genome Research, 19:2258). PE-2 is a heterothallic diploid, and its genome is characterized by a high degree of hetorozygosity, both at the nucleotide sequence and karyotype levels. This intrinsic genetic diversity is likely a key factor in PE-2s extraordinary ability to thrive in the stressful environment found in large industrial fermentation tanks. However, the molecular mechanisms that contribute to this adaptation are largely unknown. This gap in basic biological knowledge about these strains represents a significant barrier to their genetic improvement and the full exploitation of their biotechnological potential. In this study we are taking advantage of the available PE-2 genome sequence to investigate the molecular physiology of sugarcane bioethanol fermentation through genome-wide transcription profiling using sequencing-based methods (RNA-Seq), under industrial scale fermentation. The results presented here offer new insight into the biology of the PE-2 strain by the identification of stress response mechanisms active in PE-2 that could explain its superior fitness and competitiveness during bioethanol production. Based in this assumption, initially we selected 7 promoters and 10 genes to manipulate genetically. In our first screen, performing a fermentation competition assay, 9 of 29 strains that had the original promoter region altered showed a positive growth behavior compared to the parent unmodified PE-2 strain and 16 of 29 strains showed a neutral effect. We are currently conducting validation assays with the four most promising promoter substitution lines identified in our screen. These tests include competitions during high temperature and high gravity fermentations, as well as fermentation kinetics and stress tolerance assays.

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