Adaptive responses of evolved Saccharomyces cerevisiae strains tolerant to acidic pH, acetic acid, and supraoptimal temperature

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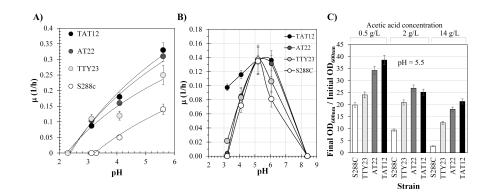
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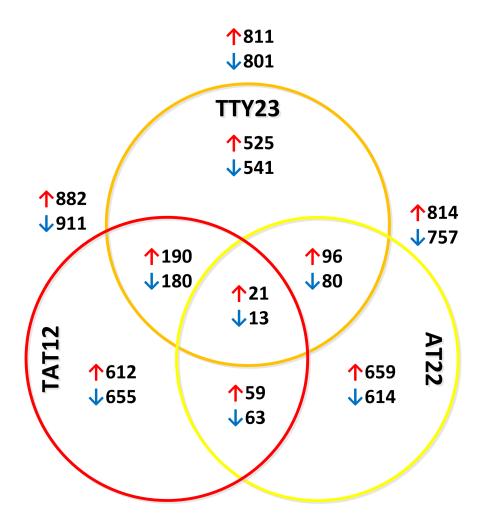
Abstract

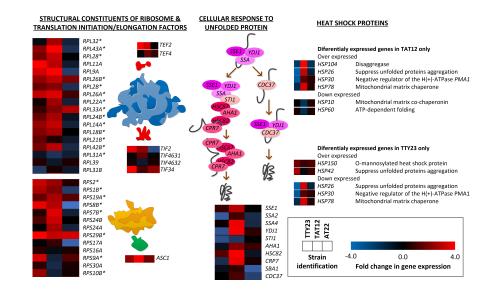
Ethanol fermentations can be prematurely halted as Saccharomyces cerevisiae faces adverse conditions, such as acidic pH, presence of acetic acid, and supraoptimal temperatures. The knowledge on yeast responses to these conditions is essential to endowing a tolerant phenotype to another strain by targeted genetic manipulation. In this study, physiological, genomic, and transcriptomic analyses were conducted to obtain insights on molecular responses which could potentially render yeast tolerant towards thermoacidic conditions. To this end, we used thermotolerant TTY23, acid tolerant AT22, and thermo-acid tolerant TAT12 strains previously generated. The results showed an increase in thermoacidic profiles in the tolerant strains. The wholegenome sequence revealed the importance of genes related to: H +, iron, and glycerol transport (i.e. FRE1/2, JEN1, VMA2, VCX1, KHA1, AQY3, and ATO2); transcriptional regulation of stress responses to drugs, ROS and heat-shock (i.e. HSF1, SKN7, BAS1, HFI1, and WAR1); and adjustments of fermentative growth and stress responses by glucose signaling pathways (i.e., ACS1, GPA1/2, RAS2, IRA2 and REG1). At 30°C and pH 5.5, more than a thousand differentially expressed genes (DEGs) were identified in each strain. The integration of results from genomics and transcriptomics revealed that, as part of their adaptive responses, evolved yeast strains aimed to adjust their intracellular pH by H ⁺ and acetic acid transport; modify their metabolism and stress responses via glucose signaling pathways; control of cellular ATP pools by regulating translation and de novo synthesis of nucleotides; and direct the synthesis, folding and rescue of proteins throughout the heat-shock stress response. Furthermore, tolerant strains did not show growth trade off under optimal ancestral conditions. Moreover, the motifs analysis in mutated TFs suggested a significant association of SFP1, YRR1, BAS1, HFI1, HSF1, and SKN7 TFs with DEGs found in thermoacidic tolerant yeast strains. This study provides insights into the molecular elements associated with yeast tolerance to acidic pH, acetic acid, and supraoptimal temperatures; information that can be used in inverse metabolic engineering.

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