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SYSTEMS METABOLIC ENGINEERING OF *S. CEREVISIAE* THERMOTOLERANCE

Luis Caspeta
Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos
Av. Universidad No. 1001, Col Chamilpa, Cuernavaca, Morelos, México. C.P. 62209
Luis.caspeta@uaem.mx

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Fermentation of lignocellulosic biomass derived feedstock using *S. cerevisiae* is very attractive for production of liquid biofuels for transportation (1). The production of ethanol and advanced biofuels benefits greatly from fermentation at temperatures higher than 40°C (2, 3), since such temperatures reduce contaminations and production costs associated to cooling operations (3). High operating temperatures also enhance the conversion of hemicellulose to fermentable sugars (4). However, temperatures equal or higher than 40°C seriously impair *S. cerevisiae* viability and growth.

S. cerevisiae thermotolerance is very difficult to engineer due to the systematic physiological stress caused by the pervasive effects of heat. We used adaptive laboratory evolution (ALE) approach, multi-omics analysis and systems biology to investigate *S. cerevisiae* evolutionary strategies orchestrated for inducing thermotolerance at temperatures higher than 40°C. With this information *S. cerevisiae* strains capable to growth faster and produce ethanol a higher productivities than the wild type strain were generated.

Nine thermotolerant yeast strains with improved growth, glucose consumption and ethanol production were isolated from ALE experiments (5). Seven out of these nine strains were sequenced. The results showed that loss of respiration capacity and changes in membrane composition were the dominant evolutionary strategies as nonsense mutations in related genes appeared in all the sequenced strains –e.g. *ATP2*, *ATP3* and *ER3*. Remarkably, the loss of cellular functions that accompanied these mutations seems to be required for survival to high temperatures as it was concluded from a thermal stability analysis of *S. cerevisiae* proteome (unpublished data). Metabolic flux analysis using genome-scale metabolic models showed that carbon fluxes increased in glycolysis, fermentation, mevalonate and sterol synthesis pathways and corroborated with changes in transcription profiles of genes from the last two pathways. Gene expression analysis also revealed that thermotolerant strains kept higher expression levels of signaling gene networks of cell responses to high osmolality, invasive growth and mating processes. Thus, genetic changes appeared during evolution generated a cross protection to osmotic stress. All these traits of thermotolerant yeast strains isolated from ALE experiments were successfully reproduced in the wild type strain by incorporating just one mutation find in the *ERG3* gene.

Results from physiological characterization in bioreactors and metabolic flux analysis of thermotolerant yeast strains were used to evaluate the cost of ethanol production from lignocellulosic biomass using industrial process flowcharts. We found than the incorporation of thermotolerant yeast reduces ethanol production costs in about 0.5 dollars per gallon (6).

Here we showed that systems metabolic engineering (7) is a valuable approach to generate complex phenotypes in industrially relevant microorganism.

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