Las levaduras: en la intersección entre la Biología de sistemas y la Biomedicina
En memoria del Profesor Julio Rodríguez Villanueva

*Yeasts: at the cross-roads of Systems biology and Biomedicine*
*In memory of Professor Julio Rodríguez Villanueva*

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# Yeast-based ethanol production: improving product yield and robustness by metabolic engineering

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'Bio'-ethanol produced by the yeast *Saccharomyces cerevisiae* is the largest-volume product of industrial biotechnology. While ethanol production still predominantly relies on corn starch and cane sugar as feedstocks, the past decade has seen the first full-scale factories that instead convert non-edible, lignocellulosic agricultural residues. In these factories, deconstruction of plant biomass is followed by fermentation of the released sugars by engineered *S. cerevisiae* strains.

In large-scale fermentation, costs of the carbohydrate feedstock account for up to 70 % of overall process costs. Achieving near-theoretical yields of ethanol on the feedstock is therefore crucial. Xylose and arabinose are abundantly present in lignocellulosic feedstocks, but cannot be fermented by wild-type *S. cerevisiae* strains. This problem has been addressed by metabolic engineering strategies that involve functional expression of heterologous, pentose-isomerase-based pathways.

Our recent research on bioethanol production focuses on strategies to further improve ethanol yields on feedstock. A key objective in this research was to minimize formation of the by-product glycerol, while redirecting electrons and carbon to ethanol production. Two such strategies, based on reduction of acetyl-CoA and on implementation of Calvin-cycle enzymes in *S. cerevisiae*, respectively, will be discussed. Research on the latter subject also illustrate how CRISPR-Cas9-mediated genome editing can accelerate and intensify yeast metabolic engineering.

Evolutionary engineering studies played a key role in yeast strain development for ethanol production from lignocellulosic feedstocks. Most of these strategies are based on prolonged cultivation under a constant or monotonously increasing selective pressure. As an example, we recently combined metabolic and evolutionary engineering to identify mutations that stimulate simultaneous fermentation of glucose and xylose. Application of dynamic selection regimes can mitigate the impact of evolutionary trade-offs and enable selection of more robust genotypes.

These advantages are illustrated by a study on improving tolerance to acetic acid, a key inhibitor of yeast performance in lignocellulosic hydrolysates.

Engineered 'generalist' *S. cerevisiae* strains, which anaerobically ferment mixtures of d-glucose, d-xylose and l-arabinose, show deteriorating fermentation kinetics during prolonged cultivation in anaerobic serial-batch cultures. This deterioration reflects a trade-off between glucose and pentose fermentation, possibly dictated by resource allocation to the responsible pathways. Results obtained with a synthetic consortium of three 'single-sugar specialist strains' has the potential to improve process robustness and, thereby, may ultimately enable biomass recycling in industrial processes for ethanol production from lignocellulosic feedstocks.