In silico modular design of *Saccharomyces cerevisiae* strains for enhanced production of organic acids

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Saccharomyces cerevisiae is widely used as microbial cell factory in industrial biotechnology due to its high tolerance to harsh industrial conditions. However, despite some progress over the last years, the development of optimized yeast strains for the production of chemicals and other materials is still a costly and time-consuming process, mainly due to the unavailability of suitable *chassis* cells.

In this work we propose a conceptual framework for the design of platform strains (Fig. 1A) towards enhanced production of organic acids, which have found widespread applications in the food, chemical and pharmaceutical industries, by applying an OptGene-based metaheuristic approach [1, 2] and building upon the fact that these compounds are derived from the same metabolic precursors.

Several *chassis* strains containing common genetic deletions to fumarate, succinate and Lmalate, along with additional genetic targets to guarantee the desired phenotype, were generated and characterized in terms of its biological significance. Although wet lab experimental validation is still lacking, a chassis strain encompassing the non-intuitive inactivation of glucose-6-phosphate dehydrogenase (*ZWF1*) and 3-phosphoglycerate dehydrogenase isoenzymes (*SER3/SER33*), along with the inactivation of enzymes to disrupt the cyclic behavior of the tricarboxylic acid cycle and allow the respective compound excretion (Fig. 1B), was scored as an interesting candidate metabolic engineering strategy towards overproduction of the three C_4-dicarboxylic acids, based on a multi-criteria systematic approach.

We hereby establish a proof-of-concept showing that is possible to generate pre-optimized platform strains for enhanced production of diverse organic acids. Furthermore, we foresee that this model-driven modular design concept may constitute an important step towards realizing the full potential for the economical and sustainable production of other families of industrially relevant chemicals.

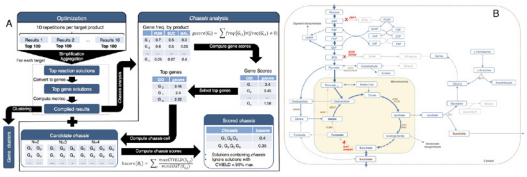

Figure 1. A - platform-strain design pipeline; B - overview of the in silico strain design for growth-coupled succinate production. Knockouts suggested by the developed strain design framework are shown in red font and reaction steps carrying no flux are shaded grey. The predicted flux is correlated with arrow width, while dashed lines represent lumped reactions.

[1] K. R. Patil, I. Rocha, J. Forster, J. Nielsen, BMC Bioinformatics 6(308) 2005.
[2] M. Rocha, P. Maia, R.Mendes, J. P. Pinto *et al. BMC Bioinformatics* 9(499). 2008