APRESENTAÇÃO DE PÔSTER - MICROBIOLOGIA

APPLICATION OF A GUIDE RNA LIBRARY BASED ON THE DCAS9 SYSTEM AIMING TO IMPROVE XYLOSE CONSUMPTION IN SACCHAROMYCES CEREVISIAE

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Texto:

Despite numerous efforts to enable the production of hemicellulosic bioethanol, the metabolism of pentoses by the yeast Saccharomyces cerevisiae remains one of the limitations of the process. Metabolic engineering towards improving this phenotype is the focus of research projects by several groups. Despite classic genetic editing efforts for gene deletion or overexpression has been shown to be quite effective, the incorporation of moderate levels of gene

expression remains underexplored, in special for essential genes that could not be fully knocked out. Therefore, this project aims to identify genetic targets that require moderate regulation for improved xylose fermentation in S. cerevisiae, thus providing new approaches to be implemented in the industry for an efficient conversion of this sugar into value-added products. For that, a methodology based on single guided RNAs (sgRNAs) libraries and the CRISPR-dCas9 system was implemented. The deactivated Cas9 (dCas9) is known to be efficiently directed to the genomic target while not having an endonuclease activity. dCas9 fused to VPR effectors are responsible for activating expression, while Mxi1 effectors act on transcription repression. Within this library, sgRNAs are designed to target up to 969 genes participating in metabolic pathways, divided in four pools: (1) carbohydrate and lipid metabolism, (2) energy and cofactor metabolism, (3) amino acid and nucleotide metabolism, and (4) maintenance/other. The strains used for this investigation are LVY34.4 and LVY27, both derived from the bioethanol yeast PE-2 and modified for xylose consumption, being the first subjected to an adaptative laboratory evolution assay and presenting an effective trait for xylose fermentation. To evaluate the effect of such libraries in xylose consumption of both strains, a high-throughput screening assay was applied. After transformation of yeast cells with the DNA from each library (8 in total, being 4 pools for each dCas9 effector), colonies were isolated, and growth monitored in microtiter plates in minimum medium with xylose. For the evolved LVY34.4 strain, adaptation time and growth rate were evaluated, while final OD was the parameter used to select transformed LVY27 colonies. For strain LVY34.4, colonies transformed with vector dCas9-Mxi1 of pool 3 and the ones expressing vectors dCas9-VPR of pool 1 caused greater variation in the parameters analyzed. Whereas for the LVY27 strain, repression of genes that are present in the carbohydrate and lipid metabolism and activation of those present in energy and cofactor metabolism had a more positive effect in the final OD. Following, the identification of sgRNA sequences that enabled top-performance will reveal the genes responsible for such feature. The identification of these genetic targets will allow a better discernment of approaches for genetic regulation, leading to the discovery of new targets that were possibly overlooked in traditional attempts for enhanced xylose metabolism in S. cerevisiae.

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