PS5: 4

Identification of *Saccharomyces cerevisiae* genes involved in the resistance to multiple stresses during Very-High-Gravity and lignocellulosic biomass industrial fermentations

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Most of the current processes of bioethanol production are based on the use of Very-High-Gravity (VHG) technology and the processing of lignocellulosic biomass hydrolysates. However, inVHG processes the initial high osmotic pressure and the accumulation in the growth medium of high concentrations of ethanol often result in slow and incomplete fermentations. On the other hand, biomass-based fermentations are limited by inhibitors resulting from biomass pre-treatments, including furfurals, weak acids and phenolics.

Aiming the optimization of strains and conditions for industrial bioethanol production a set of *Saccharomyces cerevisiae* genes were identified, in this study, as required for maximal fermentation performance under industrial conditions. The integration of previous chemogenomics data [1,2,3,4,5] was used to identify eight genes whose expression confers simultaneous resistance to high concentrations of glucose, acetic acid and ethanol, chemical stresses relevant for VHG fermentations; and eleven genes conferring simultaneous resistance to stresses relevant during lignocellulosic fermentations. These eleven genes were identified based on two different sets: one with five genes granting simultaneous resistance to ethanol, acetic acid and furfural, and the other with six genes providing simultaneous resistance to ethanol, acetic acid and vanillin. The expression of *BUD31* and *HPR1* was found to lead to the increase of both ethanol yield and fermentation rate, while *PHO85*, *VRP1* and *YGL024w* expressionis essentially required for maximal ethanol production in VHG fermentations. Five genes, *ERG2*, *PRS3*, *RAV1*, *RPB4* and *VMA8* were found to contribute to the maintenance of cell viability in wheat straw hydrolysate and/or for maximal fermentation rate of this substrate. The identified genes stand as preferential targets for genetic engineering manipulation in order to generate more robust industrial strains, able to cope with the most significant fermentation stresses and, thus, to increase ethanol production rate and final ethanol titers.

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