TITLE: OVEREXPRESSION OF *MSN2* GENE IMPROVES *Saccharomyces cerevisiae* ETHANOL PRODUCTION IN VHG FERMENTATION SIMULATING PLANT CONDITIONS

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ABSTRACT:
In the Brazilian industrial ethanol production, the several types of stresses imposed to *Saccharomyces cerevisiae* yeasts are the most challenging limiting factors. Genetic manipulation of the gene *MSN2* associated with stress resistance is a promising strategy to overcome these limitations. The *MSN2* gene was previously suggested as associated with stress tolerance in *S. cerevisiae*. They encode transcription factors (Msn2/Msn4) that upregulate genes containing stress-responsive elements (STRE). The aim of the present work was to assess the potential of a genetically modified *S. cerevisiae* strain for fermentations with high sugar content and cell-recycles, simulating the industrial conditions of Brazilian distilleries. A DNA fragment containing *Kan*^{r} (conferig resistance to G418 - geneticin) - flanked by *LoxP* regions and the constitutive PADH1 promoter - was integrated into the genomic *locus* of the *MSN2* gene of CAT-1 industrial strain, deleting the N-terminal region (first 48 amino acids) of the protein. We performed 5 fermentations assays (cell-recycles/reuse of cells) and analyzed the physiological parameters of the strains CAT-1 (industrial wild type) and ATT-6 (truncated version of *MSN2* overexpression). In the molasses`s must containing 33% of total reducing sugars (TRS) at 30°C (conditions of cycle 4), strain ATT-6 showed the most interesting results, with higher ethanol production (15.87%, v/v), sugar consumption (0.41% of residual TRS), glycerol production (0.78%), and showed a high cell viability (93.53%), indicating that these would be the more suitable conditions for employing this yeast at industrial fermentation. ATT-6 presented significantly higher ethanol production and sugar utilization in the five fermentative cycles where we applied drastic increases of sugar concentrations in the sugarcane molasses. ATT-6 strain was able to resist the multiple stresses of fermentations typical of Brazilian distilleries, and therefore has a high biotechnological potential. In sum, these results suggest that the truncated overexpression of the *MSN2* gene favored *S. cerevisiae* under conditions of simultaneous stresses. ATT-6 strain could be used primarily for industrial ethanol production under conditions of osmotic stress, promoted by its higher sugar utilization and glycerol production.

Keywords: VGH fermentation, *Saccharomyces cerevisiae*, gene overexpression, *MSN2* gene, general stress response

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