



EVALUATION OF AN INDUSTRIAL STRAIN *Saccharomyces cerevisiae* MIG1 Δ FOR SUCROSE AN GLUCOSE CONSUMPTION

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Introduction. Glucose repression in *Saccharomyces cerevisiae* is a complex regulatory system that involves several different ways. There are two main pathways involved in signal transduction. One of this has a role on glucose sensing and regulation in the transport of glucose, while the other, it is involved on suppressing a wide range of genes such as GAL, SUC and MAL which encode proteins that are specifically concerned on the absorption and consumption of alternative carbon sources. ⁽¹⁾⁽²⁾

The major route of glucose repression is the main effector Mig1 gene, which protein with two zinc fingers (Cys₂ His₂) binds specifically to DNA GC rich sequences, corresponding to promoters of many genes, thus repressing their transcription ⁽³⁾. In the presence of elevated glucose levels, Mig1 moves rapidly into the nucleus, where it binds to the promoters of the glucose repressible gene as MAL, GAL and SUC2. SUC2 expression is repressed about 200-times when there are high glucose levels in the media ⁽⁴⁾. By contrast, when cells are deprived of glucose, Mig1 is rapidly transported to the cytoplasm, thereby inhibiting its activity as a repressor.

At industrial level some substrates used to produce alcoholic drinks have mixtures of carbohydrates, which are used by the cells in a certain order with intermittent delay stages, between the exhaustion of the initial glucose and sucrose consumption, which is often in a lower overall yield and reduced volumetric ethanol productivity (amount of ethanol produced per unit of time). ⁽⁵⁾

The main aim of this study was to development a strain of *Saccharomyces cerevisiae* isolated from industrial sources, to which MIG1 gene was deleted, to achieve a consumption, without catabolic repression, of glucose and sucrose in a media with a mixture of those sugars.

Methods. An industrial strain obtained from agave must to produce Tequila, was kept at -70 °C in 30 % glicerol.

The method used to delete MIG1 was that reported by Gietz, R.D. and R.H. Schiestl ⁽⁵⁾. The strain was treated with lithium acetate. A geneticin resistant gene replaced the MIG1 gene.

The transformed yeast was used in fermentations where YP with glucose and sucrose as carbon source was used in an equimolar mixture (2% and 10%w/v). The amount of sugars consumed by the yeast was measured using HPLC with an AMINEX HPX-87H column. The ethanol produced in the fermentation was measured by Gas Chromatograph in an Agilent 6890N, with a JW19091N-133 capillary column.

Results. It was observed that the wild *S. cerevisiae* strain did not consume the same amount of glucose and sucrose during the fermentation whereas, the transformed yeast had an efficient consumption of both sugars and catabolic repression was not observed. A faster ethanol production was observed in those media where the transformed strain was used than that obtained with the wild industrial strain.

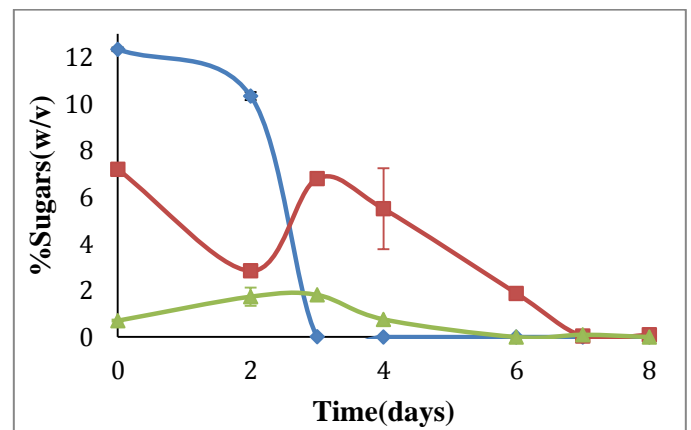


Fig.1 Concentration of glucose (■), sucrose (◆) and fructose (▲) in batch cultures under aerobic conditions and glucose-sucrose equimolar mixture with a concentration around 10% for strain Tc2, graphically represented as a function time.

Conclusions. MIG1 Δ consumed glucose and sucrose at the same time when a mixtures of these sugars were present in the media, The transformation of *S. cerevisiae* strain isolated from a tequila fermentation showed a fast sucrose consumption compare to the wild type, this allow the microorganism to increase glycolysis in less time and consequently ethanol production in less time.

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References.

1. Rolland, F. (2002). *FEMS Yeast Research* 2:183-201.
2. Westergaard, S.L. (2007). *Biotechnology and Bioengineering*. 96:134-145
3. Orzechowski, W. J. (2008). *BMC Genomics*. 9:1-15.
4. Lutfiyya, L. L. (1996). *Mol. Cell. Biol.* 16:4790-4797.
5. Rin, K. S. (2012). *CELL PRES Trends in Biotechnology*. 30:274-282.
6. Gietz, R. D. and R. H. Schiestl (1995) *Transforming Yeast with DNA. Methods in Molecular and Cellular Biology*. 5:255-269