**Recombinant *Saccharomyces cerevisiae* as a microbial biocatalyst for the one-step production of prebiotic fructooligosaccharides**

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**Abstract**

Fructooligosaccharides (FOS) are widely consumed prebiotics with proven beneficial effects on both human and animal health. As a result, alternative production processes with high-efficiency have been an increasing focus of interest by both academy and industry.

In this work, a *in vivo* bioprocess approach was successfully developed for one-step production of FOS from sucrose fermentation by recombinant yeast. *Saccharomyces cerevisiae* YIL162W lacking the gene responsible for sucrose hydrolysis (*suc*2) was transformed to express the β-fructofuranosidase (Ffase) *INV* gene from *Schwanniomyces occidentalis* (clone L196), and its mutated version containing a serine instead of a leucine at position 196 (clone S196), under the inducible GAL1 promotor. Clone S196 presented a 2.75-fold higher sucrolytic activity (22±3 $U.mL^{-1}$), while clone L196 presented a higher efficiency towards FOS production, producing mainly 6-kestose (76±3 $g.L^{-1}$) and 1-kestose (1.6±0.6 $g.L^{-1}$) after 24 h of fermentation at 30 °C and 200 rpm, in a medium containing 300 g/L of sucrose.

Attending the potential of process simplification and cost-reduction, the Ffase *INV* gene was then expressed under the glyceraldehyde-3-phosphate dehydrogenase (GPD) constitutive promoter (clone GPD L196), resulting in a maximum FOS production of 61±4 $g.L^{-1}$ ( 56±3 $g.L^{-1}$ of 6-kestose and 5±31 of fructosylnystose) after 48 h of fermentation using 300 g/L of sucrose. Interestingly, the total amount of undesired glucose and fructose present in the media whenever the maximal FOS production was achieved, was 9 times lower with the GDP promoter (5.5±0.9 $g.L^{-1}$).

The present work demonstrates the high potential of this bioprocess approach for industrial production of prebiotic FOS in a single step. Nevertheless, there is still room for yield improvement in future work, namely through bioprocess optimization.

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