BIOCATALYSIS OPEN DAY 2020
26 NOVEMBER 2020 | VIRTUAL

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

Cláudia Amorim*, Adelaide Braga, Joana L. Rodrigues, Beatriz B. Cardoso, João Rainha, Eduardo J. Gudiña, Sara S. Silvério, Lígia R. Rodrigues

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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 ß-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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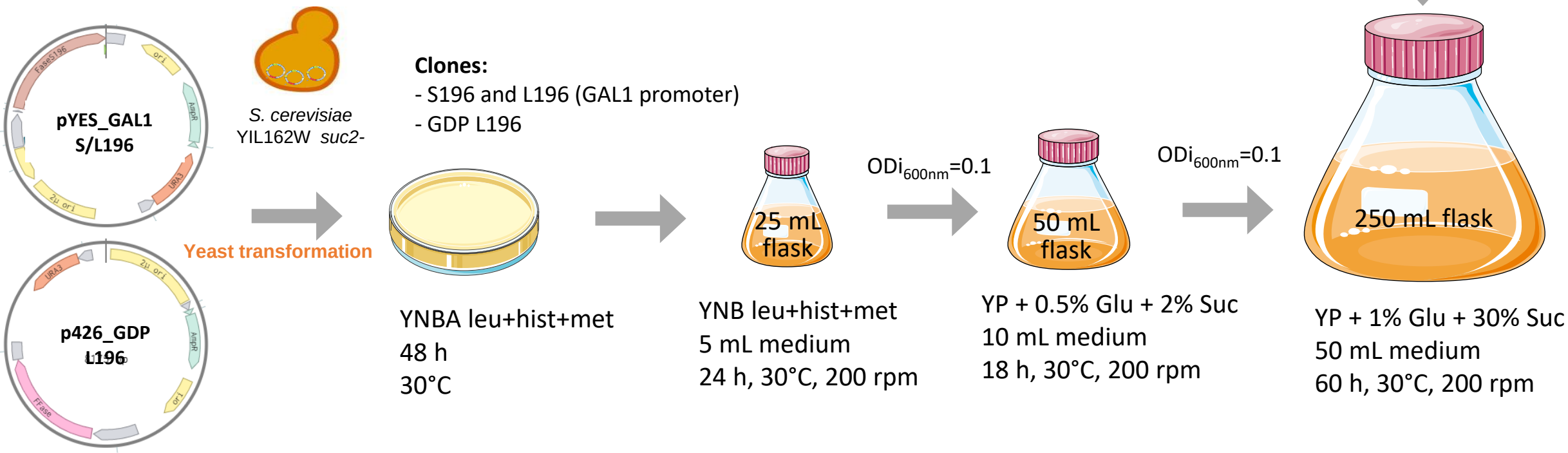
Challenge: *To develop an efficient and sustainable bioprocess for FOS production to reduce the production cost of these compounds, increasing their market potential.*

Experimental



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Results

	GAL1 S196	GAL1 L196	GDP L196
FOS (g/L)	16±2	78 ±4	61±4
Maximum extracellular sucrolytic activity (U/mL)	22±3	6±0,5	8±1
Monosaccharides (g/L)	118±14	51±7	6±1
Ethanol (g/L)	25±4	175±1	13.0±0.9
Glycerol (g/L)	6.2±0.6	4±1	3.8±0.5
Optimal time (h)	24	24	48
$Ymax_{FOS/Suc}$ (g/g)	0.05±0.01	0.23±0.04	0.21±0.06

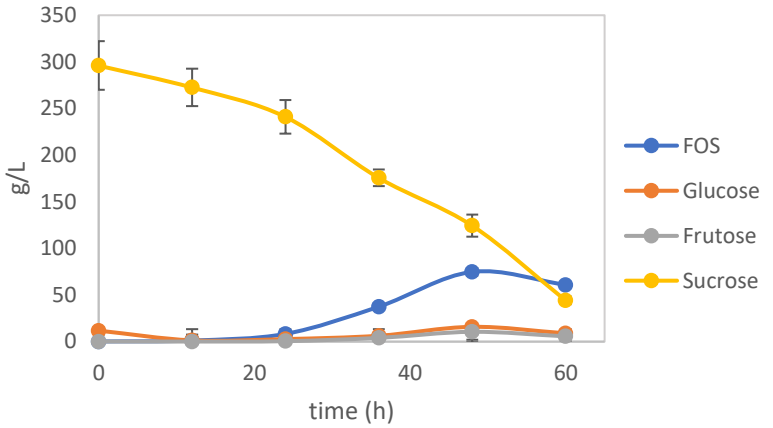

Fig. 1 – Consumption of sucrose and production of fructooligosaccharides (FOS), glucose and fructose by *Saccharomyces cerevisiae* clone GDP L196, harboring the β-fructofuranosidase (Ffase) *INV* gene from *Schwanniomyces occidentalis*. The fermentation assays were performed at 30°C and 200 rpm with an initial concentration of sucrose of 300 g/L in yeast extract synthetic media.

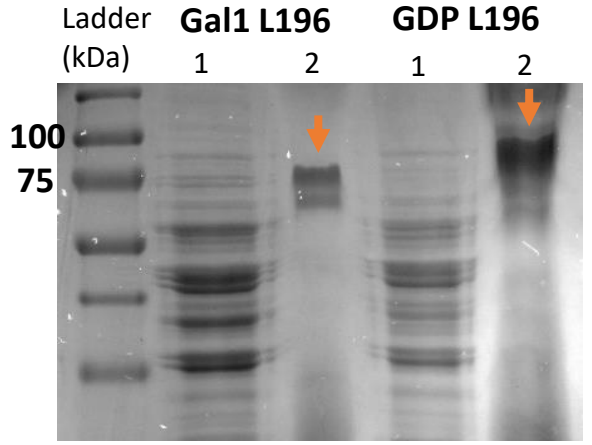

Fig. 2 – Gel analysis of the GAL1 L196 and GDP L196 clones. Biomass and cultures filtrates (25 mL) of the *S. cerevisiae* transformants were processed for protein purification. Purified proteins (5-20 µg) were subjected to SDS-PAGE. Numbers at the left indicate the positions of the ladder used as a control (in kDa). The expected band should have 95 kDa.

Conclusions

- Clone L196 presented a higher efficiency towards FOS production.
- The amount of undesired monosaccharides was 9 times lower with the GDP promoter.
- Sustainable and safe approach, suitable for food application.

Promising strategy for fructooligosaccharides production

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