Biotechnology to build a brighter future:
Food, Health and Environmental Applications

Synthetic biology approaches to design and construct microbial cell factories for the production of fructooligosaccharides

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CONTEXT

- New products that improve the quality of life and reduce the risk of suffering from some diseases;

- Functional food: are **foods** that have a potentially **positive effect** on health **beyond basic nutrition;**

CONTEXT

Prebiotics

International Scientific Association for Probiotics and Prebiotics → **“a substrate that is selectively utilized by host microorganisms conferring a health benefit”**

A rising market!

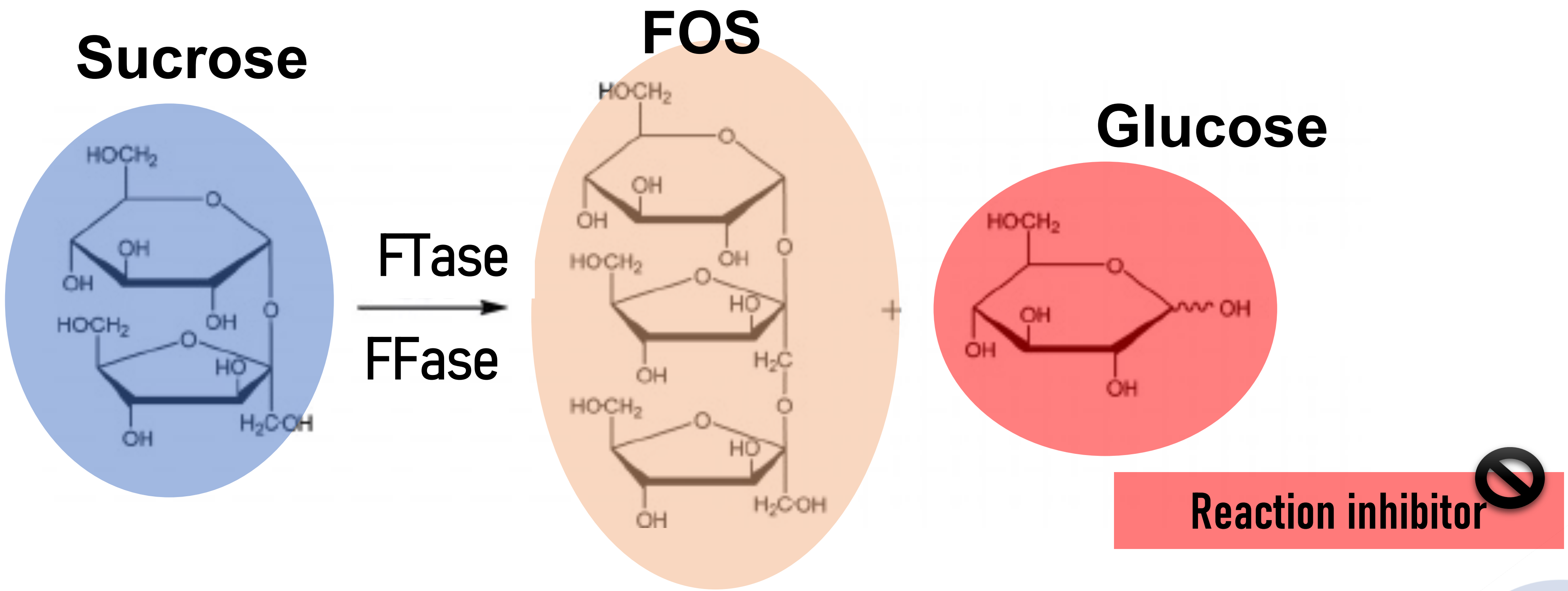
Expected to reach a value of

USD 7.37 billion by 2023

CONTEXT

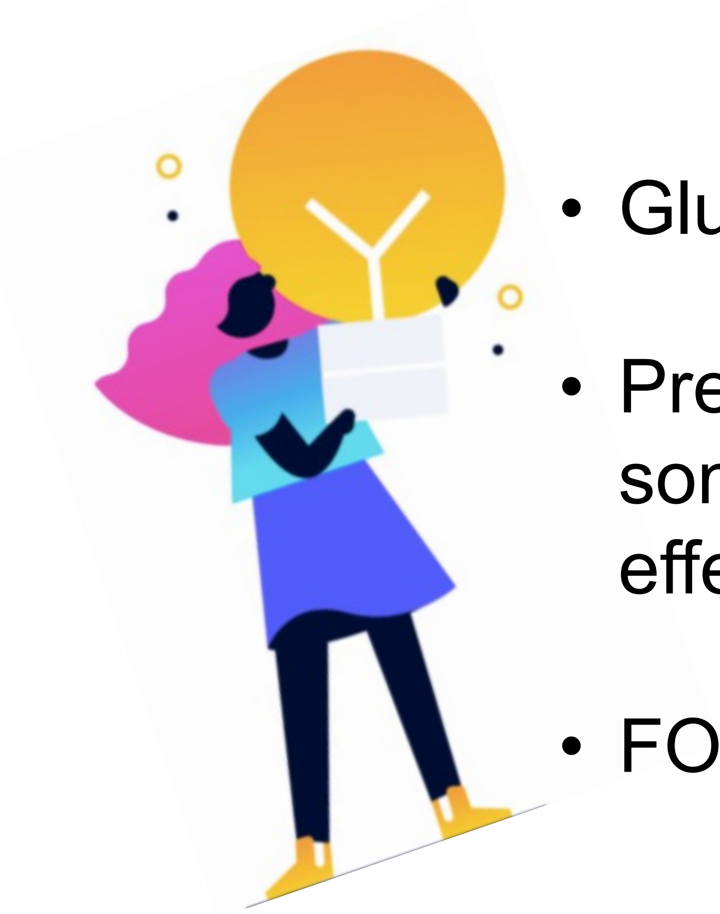
Fructooligossacharides (FOS)

Non-digestible carbohydrates that are composed by fructose residues with a terminal glucose molecule residue linked by β(2→1) glycosidic bonds



CONTEXT

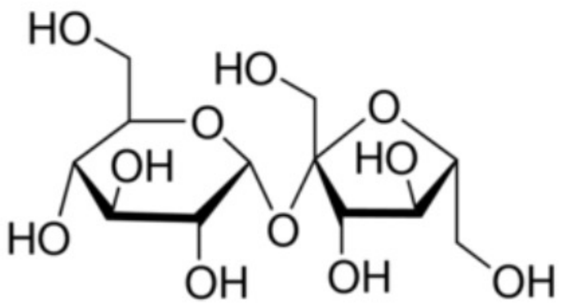
Problems



- Glucose inhibition;
- Presence of high concentrations of glucose, together with some sucrose and fructose, compromises the prebiotic effect;
- FOS purification steps → increase the production costs

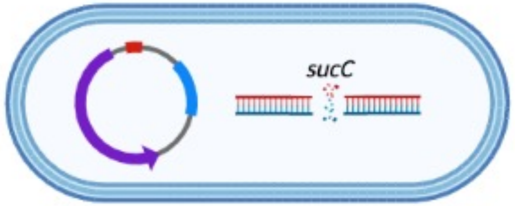
INNOVATIVE SOLUTION

Sucrose

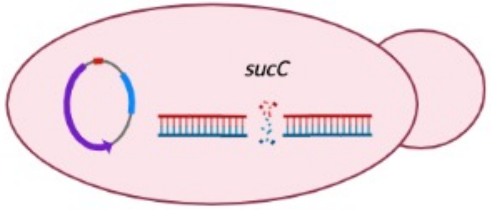


OR

Agri-food industry
by-products



Zymomonas mobilis



Saccharomyces cerevisiae

↑ FOS Purity

↓ Glucose + Fructose

↓

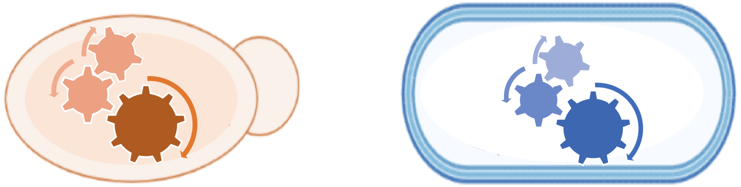
Final product with high prebiotic effect and commercial value

T1 - Design and construction of ZM and SC strains able to produce FOS

Mutant strains to prevent substrate consumption

Overexpression of a Ftase/FFase from a recognized FOS producer

T2 - Genetic optimization of ZM and SC to increase the FOS yield and purity



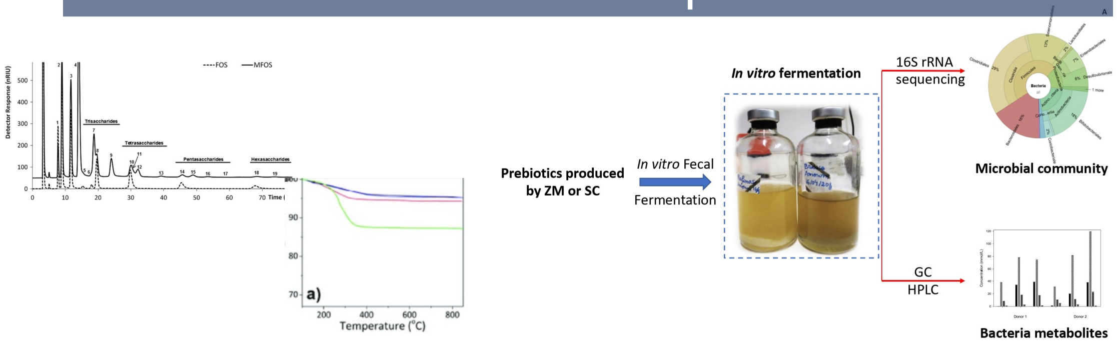
Engineer the strains to consume the excess of glucose and fructose

T3 - Design and optimization of the FOS production bioprocess

Agroindustrial by-products as alternative low-cost substrates

Process scale-up

T4 - Physicochemical and functional characterization of the produced FOS



Physicochemical and functional characterization

Prebiotic potential → *In vitro* gut model

In vivo prebiotic production using *Zymomonas mobilis*

In vivo prebiotic production using *Z. mobilis*

Box-Behnken (RSM)

15 experiments

Sucrose concentration (A)

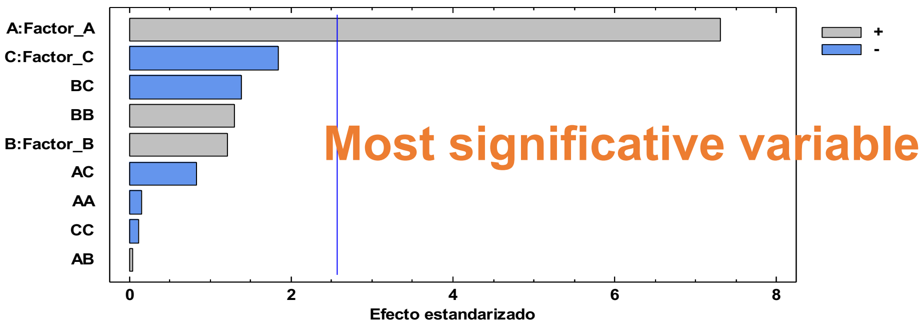
Yeast extract concentration (B)

Sodium chloride concentration (C)

Total FOS production

Static
30ºC

Total FOS 51.6 g/L

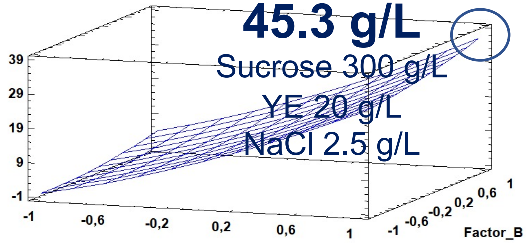


Most significative variable- Sucrose

ANOVA

Model for predicting Total FOS production

Max. Total FOS



45.3 g/L
Sucrose 300 g/L
YE 20 g/L
NaCl 2.5 g/L

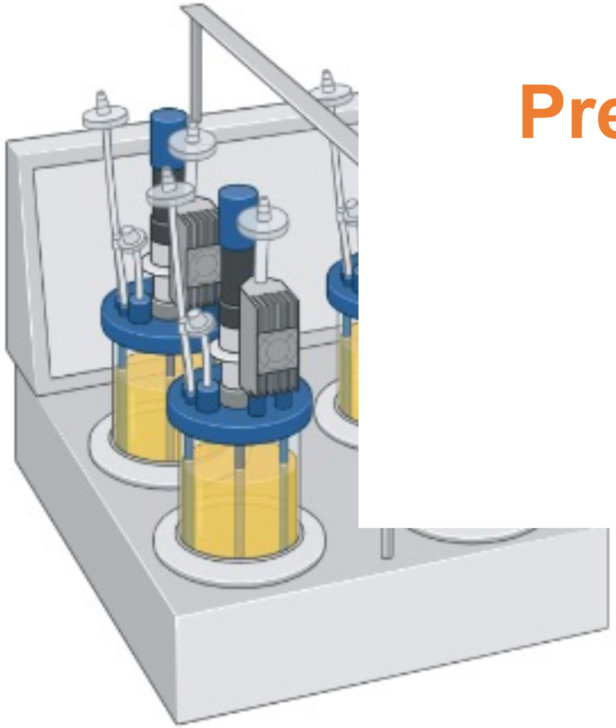
~ experimentally obtained

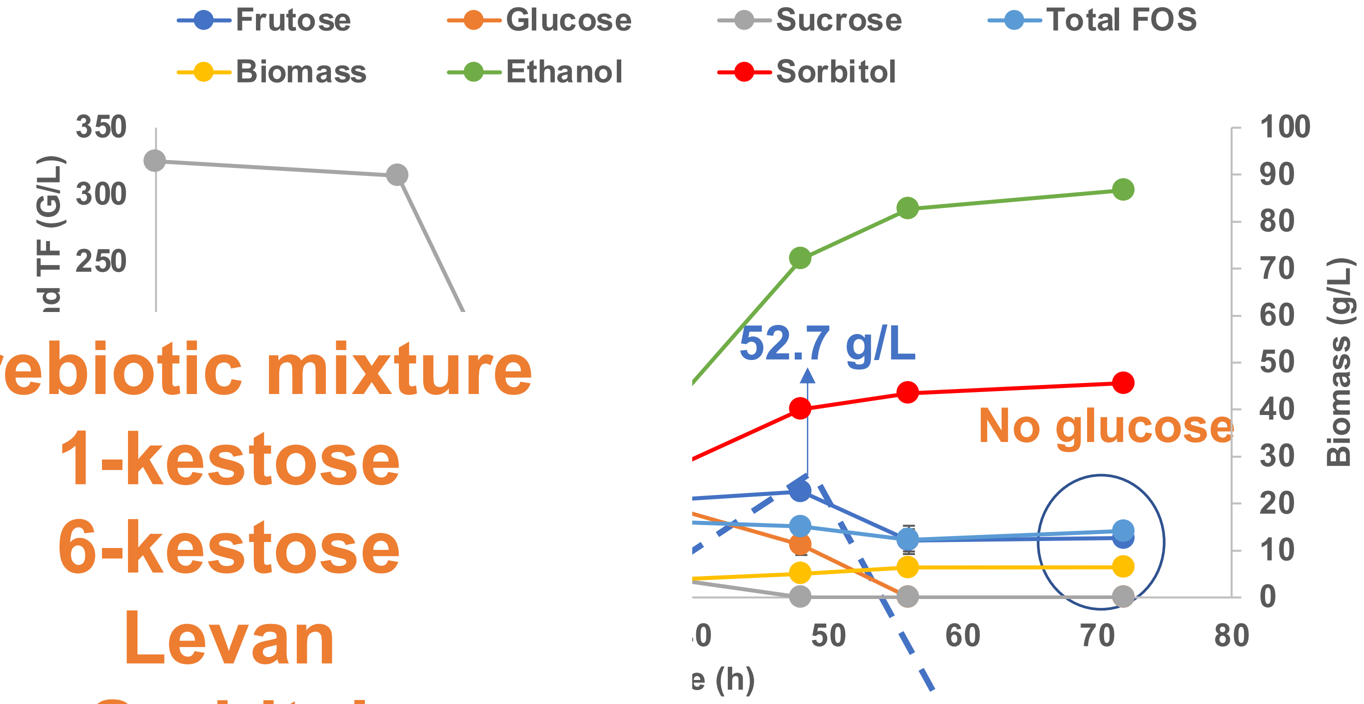
Sucrose 350 g/L
YE 20 g/L
NaCl 2.5 g/L

In vivo prebiotic production using *Z. mobilis*

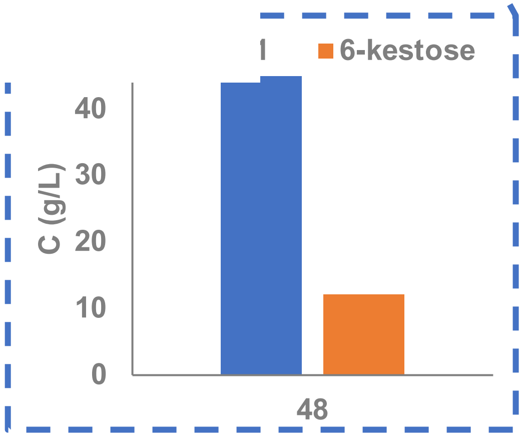
Process scale up

Sucrose 350 g/L
YE 20 g/L
NaCl 2.5 g/L
Without air
Agitation 100 rpm





Prebiotic mixture
1-kestose
6-kestose
Levan
Sorbitol



FOS 1 - 1-kestose
6-kestose

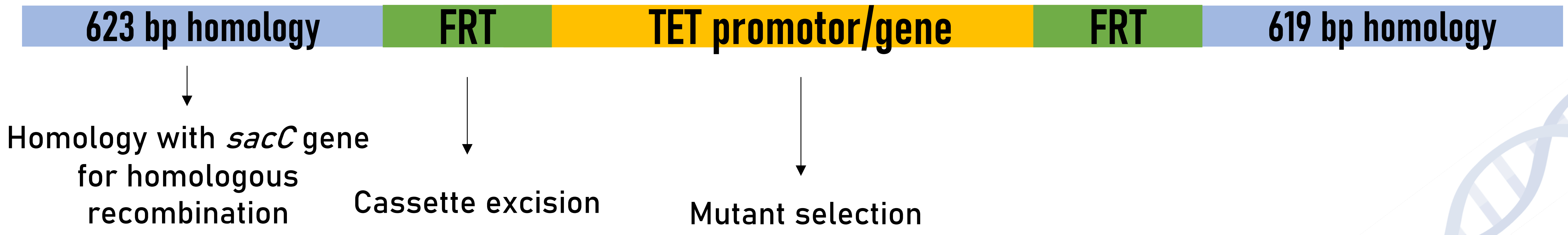
In vivo prebiotic production using *Z. mobilis*

Deletion of sucrase genes in *Z. mobilis*

Purpose: Prevent substrate consumption by the engineered *Z. mobilis*

Objective: Development of a sucrase deficient strain by *sacC* deletion

How? By Integration of a cassette carrying the Tet resistance gene and FRT sequence in *sacC* gene of *Z. mobilis* genome using homologous recombination



In vivo prebiotic production using *Z. mobilis*

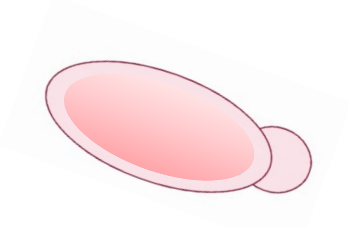
Deletion of sucrase genes in *Z. mobilis*

Cassette excision

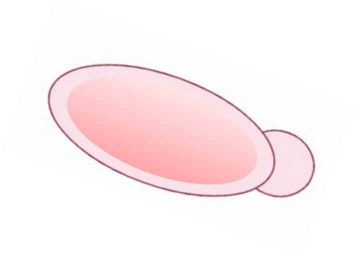
In vivo prebiotic production using *Z. mobilis*

Overexpression of a Ftase/FFase from a recognized FOS producer

Aspergillus sidowi

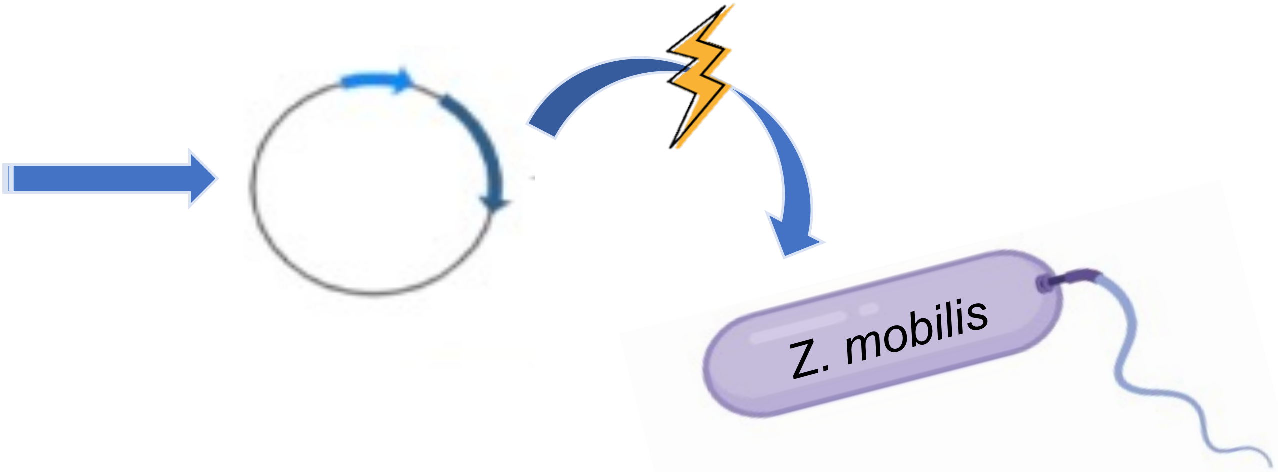


S. occidentalis
(L196)
(mutated for improved transferase activity)



Schwanniomyces occidentalis
(S196)

Aspergillus foetidus

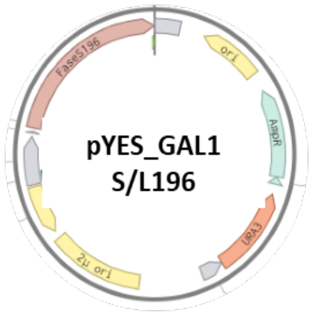


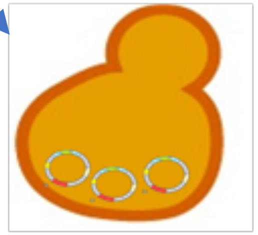
Heyer AG, Wendenburg R (2001) DOI: 10.1128/AEM.67.1.363-370.2001
Rehm J, Willmitzer L, Heyner AG (1998) DOI: 10.1128/JB.180.5.1305-1310.1998
Álvaro-Benito M, et al. (2010) DOI: 10.1128/AEM.01614-10

In vivo FOS production using *Saccharomyces cerevisiae*

In vivo FOS production using *S. cerevisiae*

Construction of *S. cerevisiae* Suc2$^-$ mutant harboring the pYES_SoS196 or pYES_SoL196 (frutosiltransferase gene from ***Schwanniomyces occidentalis***)

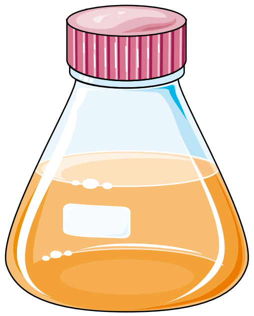




S. cerevisiae
YIL162W *suc2-*

Induction 2% Gal

OD_{600nm}=0.8



YP + 1% Glu + 30% Suc
250 mL flask
50 mL medium
48 h

S196: Higher sucrose hydrolysis
L196: Higher FOS production

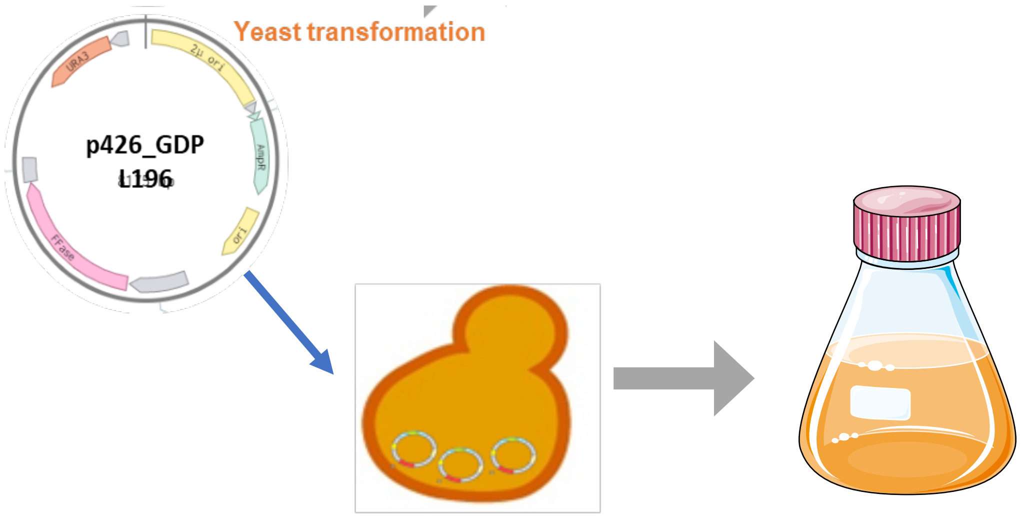
Max. FOS production at 12 h after induction:

S196 $Y_{FOS/Sac}$=54 ± 3 mg/g

L196 $Y_{FOS/Sac}$=269 ± 12 mg/g

In vivo FOS production using *S. cerevisiae*

GOAL: Transform the SoL196 gene on p426_GPD → Constitutive promotor (production cost reduction)



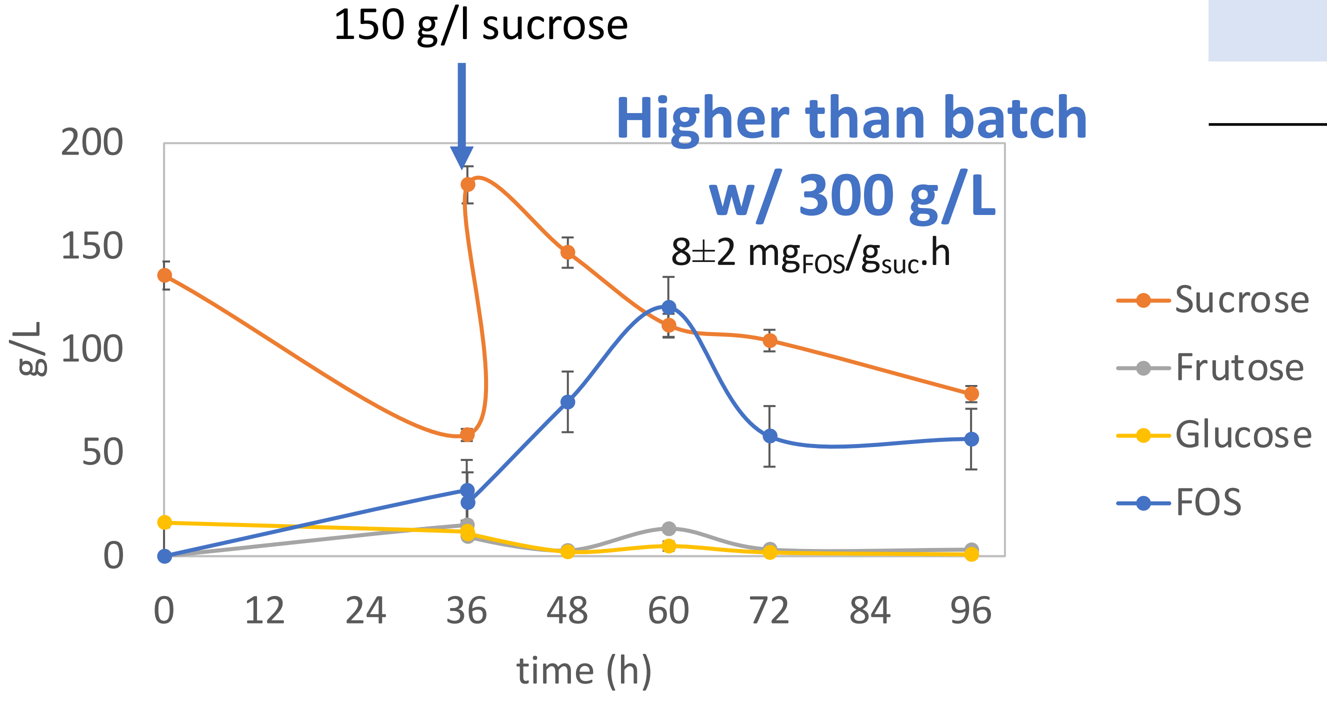
S. cerevisiae
YIL162W *suc2-*

YP + 1% Glu + 30% Suc
250 mL flask
50 mL medium
60 h

	GAL1 L196	GPD L196
FOS (g/L)	78 ±4	61±4
Maximum extracellular sucrolytic activity (U/mL)	6±0,5	8±1
Monosaccharides (g/L)	51±7	6±1
Ethanol (g/L)	17.5±1	13.0±0.9
Glycerol (g/L)	4±1	3.8±0.5
$Productivity_{max}$ (mg/g.h)	9.58±0.08	5.0±0.1

In vivo FOS production using *S. cerevisiae*

Effect of sucrose concentration



Maximum productivity and respective optimal time achieved during sucrose fermentation using S. cerevisiae GPD L196 clone.

Sucrose (g/L)	$Productivity_{max}$ (mg/g.h)	Optimal time (h)
120	4,6±0,4	36
210	5,1±0,2	48
300	5,9±0,3	48
390	5,8±0,3	60

Step-wise fed-batch

Perspectives and Final Remarks

- New prebiotic "mix" → Prebiotic potential

- Incorporation of the produced prebiotic mixture in food matrix or its production and further commercialization as a prebiotic "mix"

- Conversion of industrial by-products (waste) and renewable raw materials into added value food ingredients (prebiotics)→ EU Green Deal

Acknowledgements







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COMPETE 2020

PORTUGAL 2020

NORTE2020
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B+F H E Biotechnology to build a brighter future:
Food, Health and Environmental Applications

FoSynBio

Synthetic biology approaches to design and construct microbial cell factories for the production of fructooligosaccharides