

# *Zymomonas mobilis*: an emerging microbial cell factory to produce prebiotics

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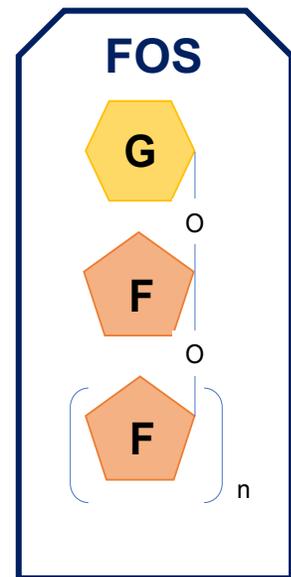
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# CONTEXT

Fructooligosaccharides (FOS) are promising prebiotics in the increasing market of functional food

## Microbial production



**Largely used in the food industry:**  
Low-calorie sugar substitutes  
Prebiotic properties

**Market:**  
USD 3173.3 million by 2025

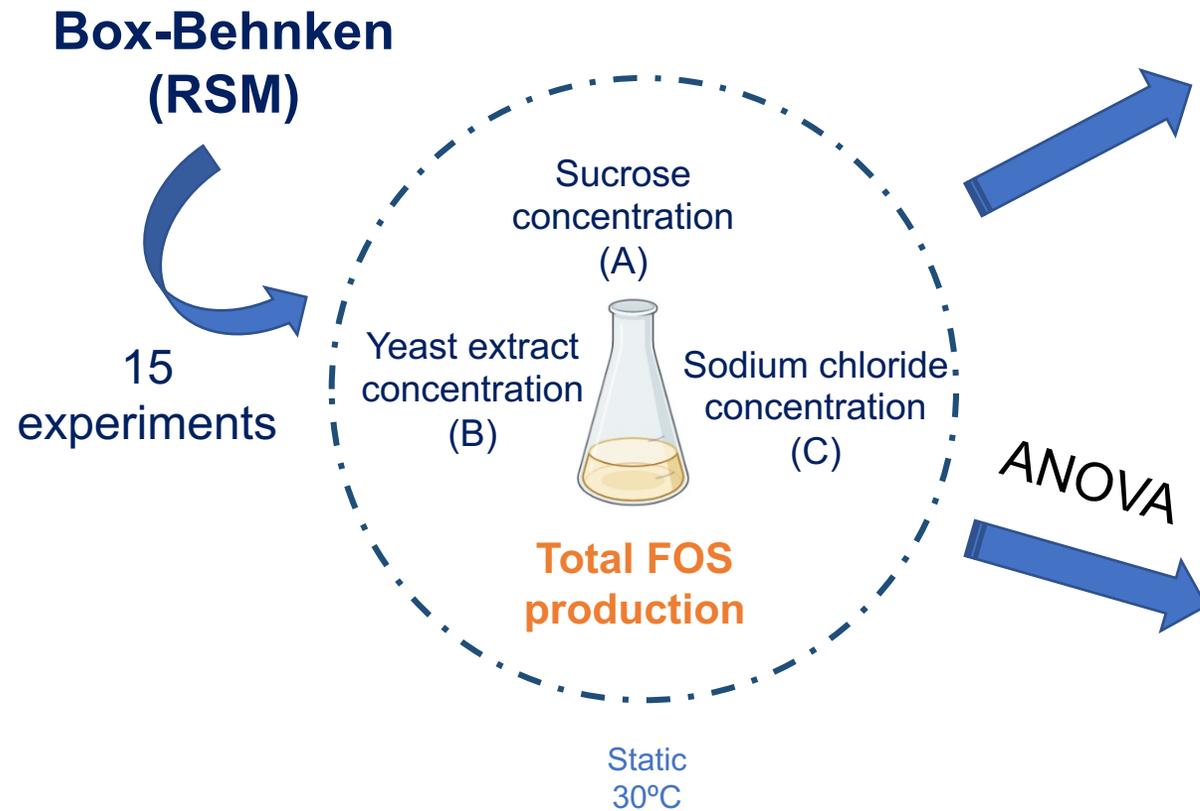
**Industrial production:**  
Expensive  
Not eco-friendly  
Complex



Holds the biochemical pathways responsible to produce several interesting compounds:

Ethanol, Levan, Sorbitol, Gluconic acid,  
**Fructooligosaccharides (FOS)**

# *In vivo* prebiotic production using *Z. mobilis*



Model for predicting Total FOS production

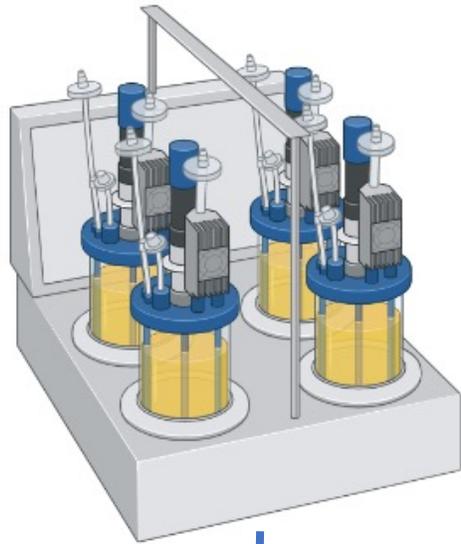
Max. Total FOS

Total FOS  
 $51.6 \pm 0.2$  g/L

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

# *In vivo* prebiotic production using *Z. mobilis*

## Process scale up



FOS: 156.50 g/L  
Yield: 0.52 g/g  
Productivity: 4.89 g/Lh

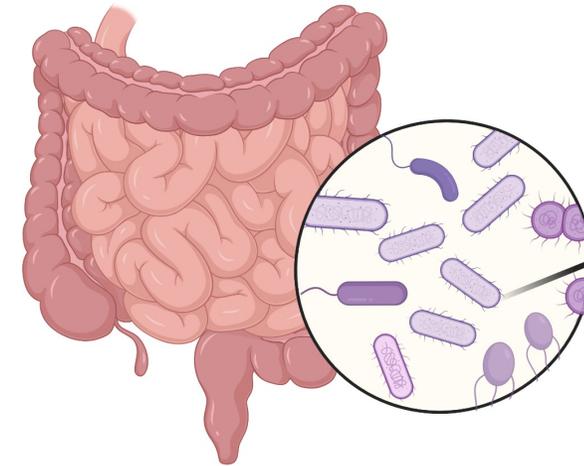
## Purification



FOS-enriched mixture  
(purity 80.2%)

## Prebiotic activity

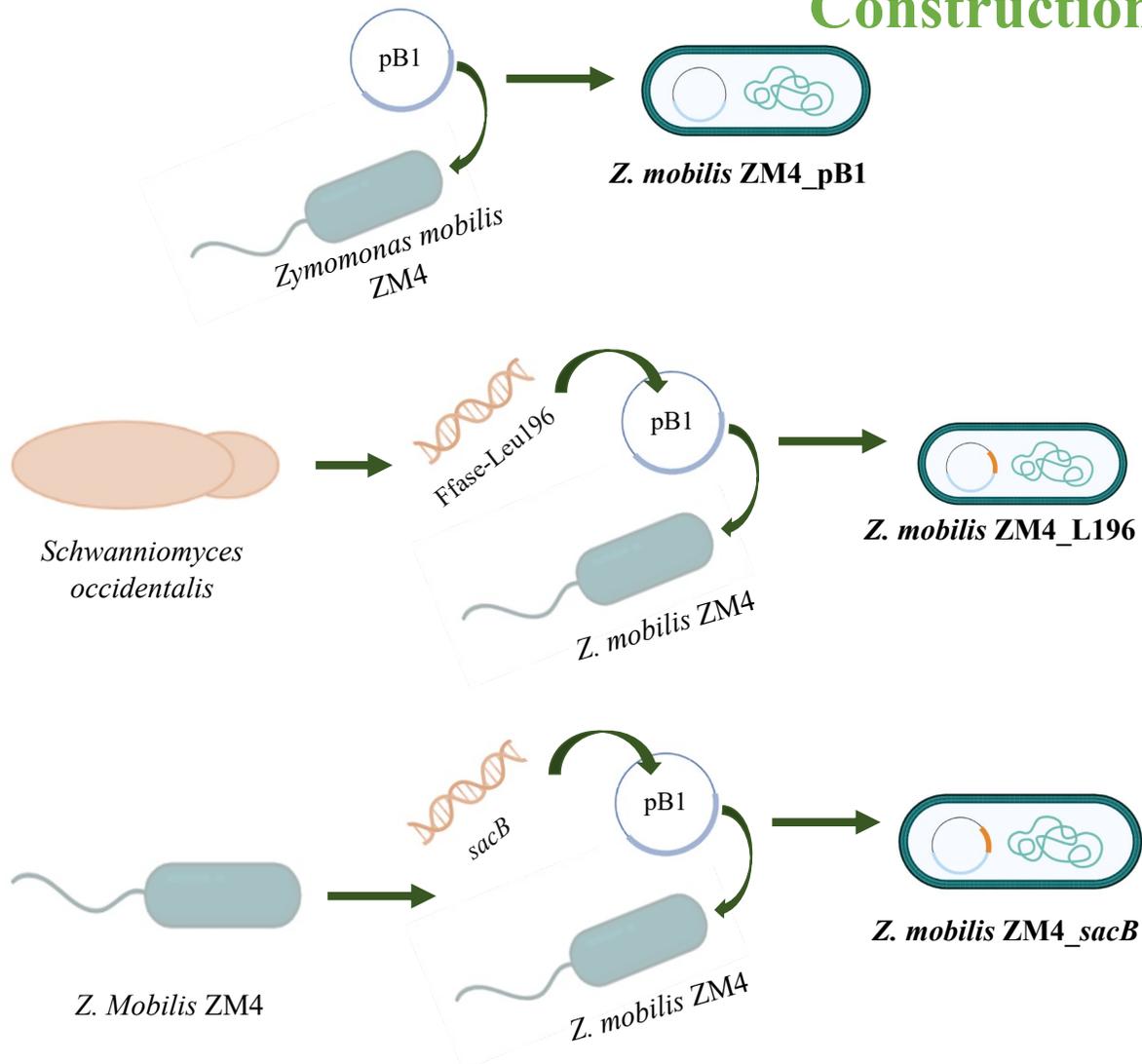
*In vitro* fermentation



↑ SCFAs  
CO<sub>2</sub>

# Engineering *Z. mobilis* to produce FOS

## Construction of mutant strains



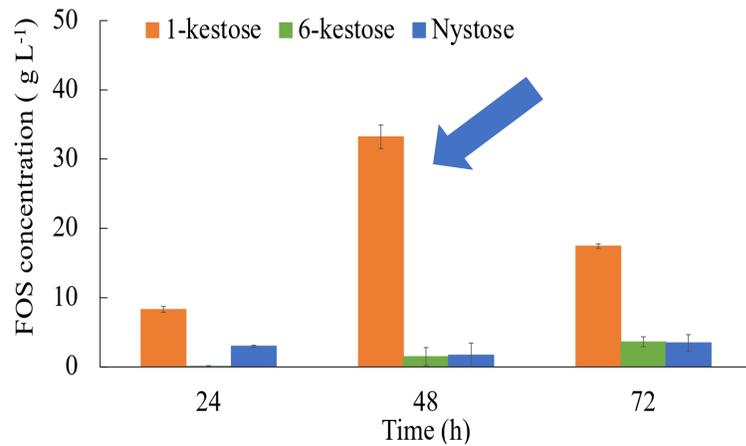
*S. occidentalis* is one of the most selective producers of 6-kestose

Native extracellular levansucrase enzyme (*SacB*) that converts sucrose into levan and FOS

# Engineering *Z. mobilis* to produce FOS

## One-step FOS production experiments

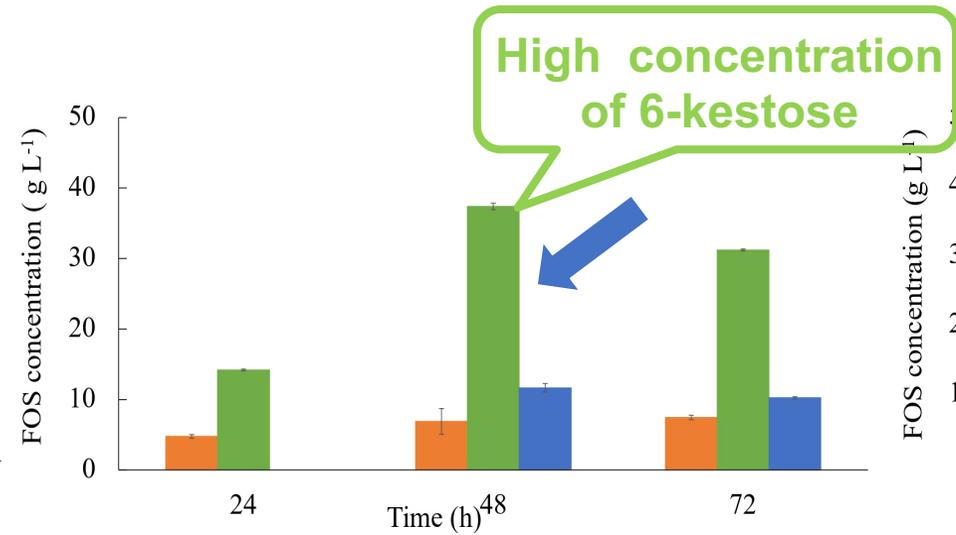
### *Z. mobilis* ZM4\_pB1



**FOS: 33.5 g/L**

**Productivity: 0.7 g/Lh**

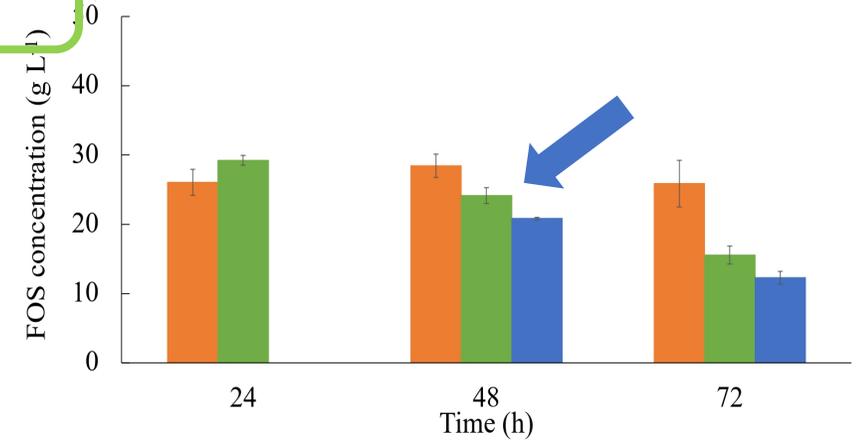
### *Z. mobilis* ZM4\_L196



**FOS: 56.0 g/L**

**Productivity: 1.2 g/Lh**

### *Z. mobilis* ZM4\_sacB



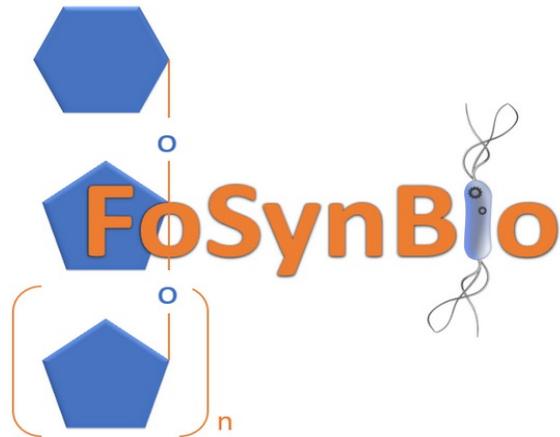
**FOS: 73.4 g/L**

**Productivity: 1.5 g/Lh**

# Perspectives and Final Remarks

- First report on the production of a prebiotic mixture with *Z. mobilis* in an *in vivo* single-step approach
- New prebiotic “mix” → Prebiotic potential
- A new route to produce tailor-made FOS mixtures was presented
- Conversion of industrial by-products (waste) and renewable raw materials into added value food ingredients (prebiotics) → EU Green Deal





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

