Synthetic biology approaches to improve the production of fructooligosaccharides using *Zymomonas mobilis* as a chassis

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Abstract

Fructooligosaccharides (FOS) are promising prebiotics in the increasing market of functional food. Industrially, FOS are produced from sucrose using fructosyltransferase (FTase) or β-fructofuranosidase enzymes (FFase). To increase its economic competitiveness, cost-effective FOS production processes with high titers, yields and productivities must be designed. *Zymomonas mobilis* (ZM) is a well-known ethanologenic bacterium with outstanding characteristics which makes it a promising chassis for the biotechnological production of relevant industrial compounds. This bacterium does not possess a FTase/FFase enzyme to perform the transfructosylation of sucrose. Nevertheless, the overexpression of these enzymes from well-recognized FOS producing microorganisms using metabolic engineering and synthetic biology tools could be an excellent approach to increase the production of FOS in ZM. Hence, ZM was genetically modified to produce FOS using FTase/FFase genes from *Schwanniomyces occidentalis* and *Aspergillus fijiensis*. Since this bacterium does not contain a sucrose transporter, a native signal sequence from ZM acid phosphatase gene (*phoC*) was inserted before the FTase/FFase genes for their expression in ZM. Moreover, synthetic ribosome binding sites with high translation initiation rate (TIRs) were designed using the RBS Calculator (v2.1) tool developed by Salis Lab (Penn State University) to improve protein production. This study demonstrates the potential of use ZM as chassis to produce significant amounts of prebiotics with relevant health benefits.

Introduction

Fructooligossacharides (FOS)

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

Market:
USD 3173.3 million by 2025

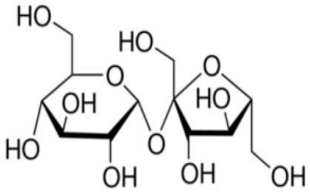
Industrial production:
Expensive
Not eco-friendly
Complex

Heterologous production using a chassis

Zymomonas mobilis

Able to produce ethanol, levan, sorbitol, gluconic acid and **FOS**

Methods



Sucrose

Fructosyltransferase (FTase)
ß-fructofuranosidase (FTase)

FOS } **Several microorganisms**

- S196 and L196 from *Schwanniomyces occidentalis*
- FopA from *Aspergillus fijiensis*

Expression of Ftases/Ffases

1. Ribosome binding sites (RBS) optimization → RBS Calculator (v2.1) tool developed by Salis Lab (Penn State University)
2. Green fluorescent protein (GFP) expression → Monitoring the transformation efficiency in *Z. mobilis*
3. FTases/FFases with native signal sequence from ZM acid phosphatase gene (*phoC*) expression to export enzymes to the extracelular media since *Z. mobilis* does not have a sucrose transporter → Increase FOS yield and titer

} **Restriction cloning: pZ7C expression vector**

Results

Optimized RBS sequence design

1. GFP expression

Initial RBS sequence: GGAG
Translation initiation rate: 532.91

Optimization

Target Translation initiation rate: 100000

The designed RBS sequence has a predicted translation initiation rate of 99986.78 after 6000 optimization iterations.

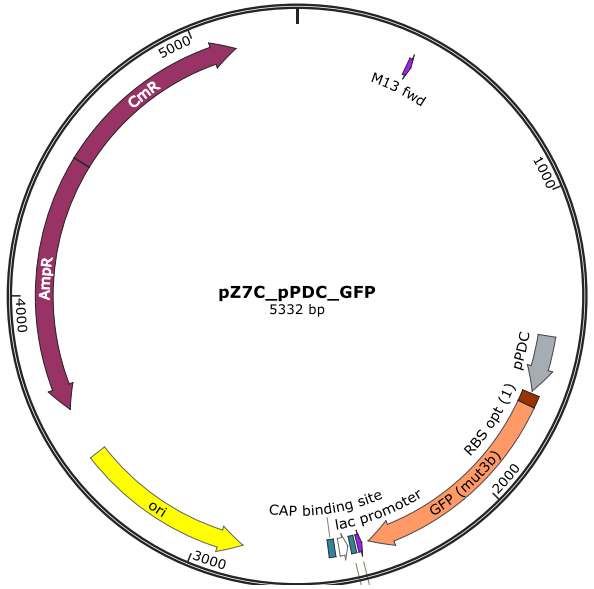
AATTTTTTGTTTATATTTGTCGTTTGTTAACAAAGATTAA

2. FTase/FFase expression

Target Translation initiation rate: 100000

Your designed RBS Sequence is TACCCATTCCATACGAGGTAAGGTATTAT with a predicted translation initiation rate of 99985.92 after 6000 optimization iterations.

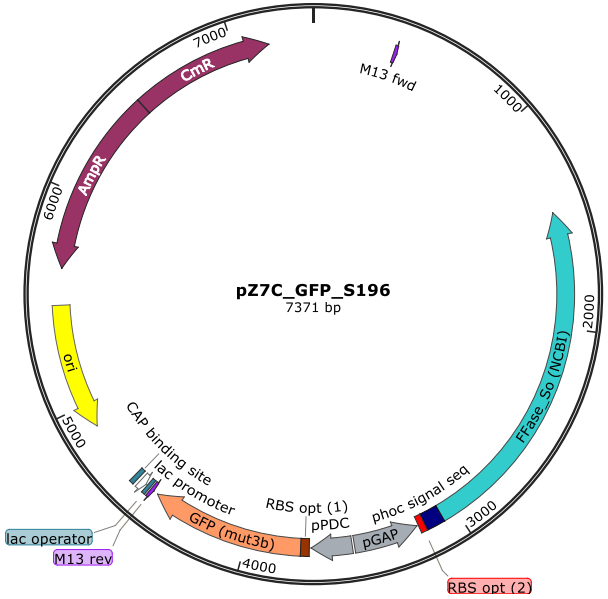
GFP expression



Transformation in *Z. mobilis* ZM4

Fluorescence assay

FTase/FFase expression



Transformation in *Z. mobilis* ZM4

Fluorescence assay

FOS production experiments

Conclusions

- This study will contribute for the development of an efficient and faster methodology for the screening of *Z. mobilis* transformants.
- In the future, the production of FOS in sucrose media by *Z. mobilis* strains carrying the Ftases/Ffases expression vectors will be assessed.

It is expected that this strain will be able to produce FOS with high yield and titers.

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