Engineering of β-fructofuranosidase from *Aspergillus kawachii* for effective synthesis of 1-kestose

Fuyuhiko Sato[1], Takumi Tochio[1], Naomi Ito[1], Keisuke Tamura[2], Tadashi Fujii[2]

[1]B Food Science Co., Ltd., R&D, [2]MicroBiopharm Japan Co., Ltd.

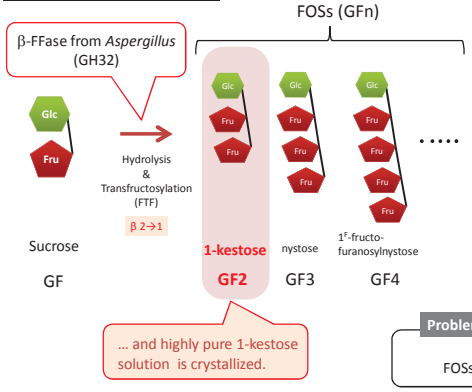
1. Abstract

1-Kestose is a component of **fructooligosaccharides (FOSs)** and a food ingredient with a significant commercial value. 1-Kestose is produced by **β-fructofuranosidase (FFase)**, a **transfructosylating (FTF)** and hydrolytic enzyme. β-FFases of *Aspergillus* are generally used for the production of FOSs. However, these enzymes are not suitable for 1-kestose production because it undergoes rapid polymerization to FOSs.

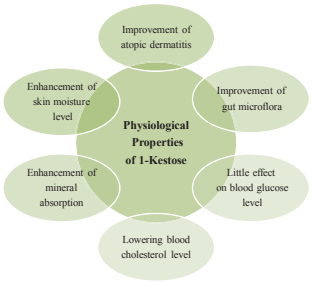
In this study, we adapted β-FFase derived from ***Aspergillus kawachii*** , belonging to **glycoside hydrolase family 32 (GH32),** as an experimental model and engineered this β-FFase for efficient production of 1-kestose using evolutionary molecular engineering techniques. Our study will lead to construction of an industrial enzyme producing 1-kestose efficiently.

2. Introduction

●What is 1-kestose ?



● Characteristic properties of 1-kestose



Problem in industrial manufacture of 1-kestose

β-FFase of *Aspergillus* produces FOSs with higher degree of polymerization.

3. Outline of the study

Our previous study

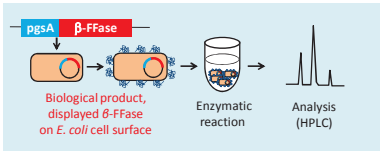
β-FFase (from *Beijerinckia indica*) glycoside hydrolase family 68

This study

β-FFase (from *Aspergillus* genus) glycoside hydrolase family 32 (GH32)

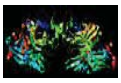
【Screening Method】

Screening method for FFase

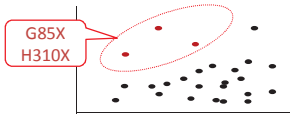


Screening (experimental model) → Derivation: ***Aspergillus kawachii***

Evolutionary molecular engineering



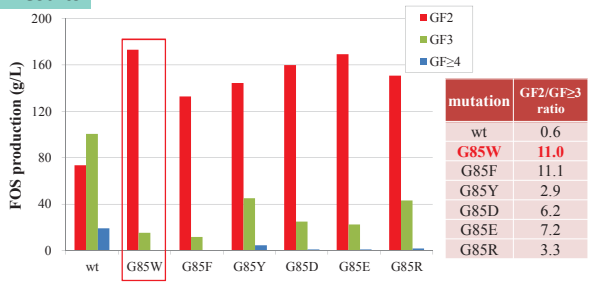
Screening: Selection of FFase variant producing more 1-kestose than other FOSs



4. Purpose

To engineer β-FFase producing 1-kestose efficiently.

5. Results

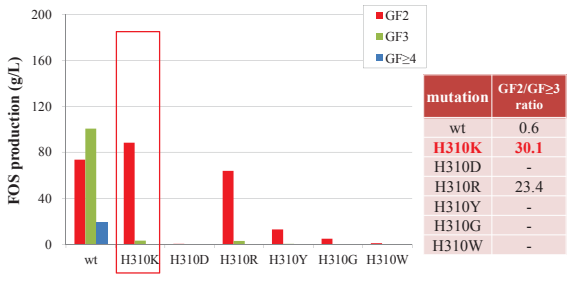


mutation	GF2/GF≥3 ratio
wt	0.6
G85W	**11.0**
G85F	11.1
G85Y	2.9
G85D	6.2
G85E	7.2
G85R	3.3

Fig. 1 Evaluation of FOS production in *A. kawachii* FFase G85X mutants

[Sucrose conc. 30% [w/w], 0.35 mL, 30℃, pH7, 3 h]

- The production of GF2 and GF≥3 was higher and lower in the G85X mutants than in the wild-type, respectively.
- G85W mutants effectively produced more GF2 and less GF≥3.

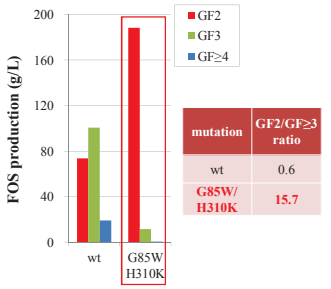


mutation	GF2/GF≥3 ratio
wt	0.6
H310K	**30.1**
H310D	-
H310R	23.4
H310Y	-
H310G	-
H310W	-

Fig. 2 Evaluation of FOS production in *A. kawachii* FFase H310X mutants

[Sucrose conc. 30% [w/w], 0.35 mL, 30℃, pH7, 3 h]

- The production of GF2 and GF≥3 was higher and lower in H310K mutant than in the wild-type, respectively.
- GF≥3 was hardly produced in all H310X mutants.

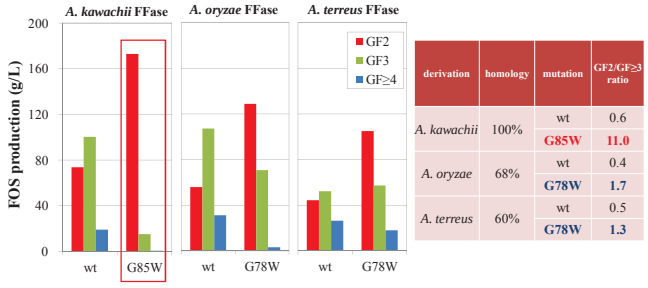


mutation	GF2/GF≥3 ratio
wt	0.6
G85W/ H310K	**15.7**

Fig. 3 Evaluation of FOS production in *A. kawachii* FFase G85W/H310K mutant

[Sucrose conc. 30% [w/w], 0.35 mL, 30℃, pH7, 32 h]

- The production of GF2 and GF≥3 was higher and lower in G85W/H310K mutant than in the wild-type and single mutants, respectively.



derivation	homology	mutation	GF2/GF≥3 ratio
A. kawachii	100%	wt	0.6
		G85W	**11.0**
A. oryzae	68%	wt	0.4
		G78W	**1.7**
A. terreus	60%	wt	0.5
		G78W	**1.3**

Fig. 4 Evaluation of FOS production in homologs of *A. kawachii* FFase, belonging to GH32 [Sucrose conc. 30% [w/w], 0.35 mL, 30℃, pH7, 3 h]

- According to sequence alignment of β-FFases, belonging to GH32, G85 of *A. kawachii* FFase was conserved as G78 of *A. oryzae* and *A. terreus* FFases, respectively.
- In *A. oryzae* and *A. terreus* FFase G78W mutants, the production of GF2 and GF≥3 was not very different from that in the wild-type, respectively.

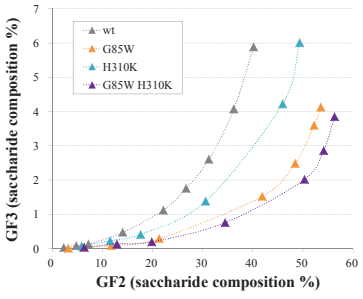


Fig. 5 GF2 and GF3 production profiles of *A. kawachii* FFase mutants

[Sucrose conc. 30% [w/w], 0.4mL, 30℃, pH 7, over time]

- According to the GF2 and GF3 production profiles in the saccharide composition, GF2 and GF3 were more and less in all mutants , especially the double mutant, than in the wild-type, respectively.

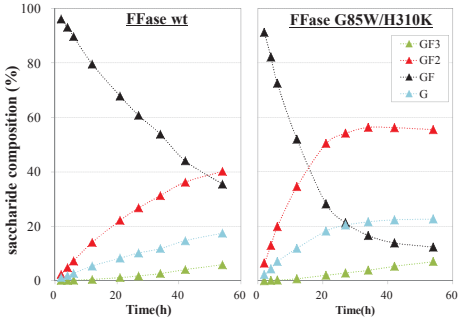
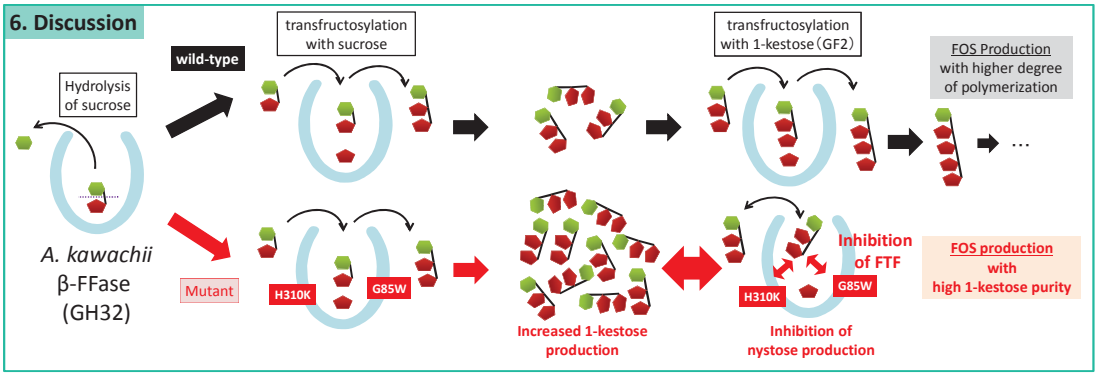


Fig. 6 Saccharide composition in *A. kawachii* FFase wt and G85W/H310K over the course of time

[Sucrose conc. 30% [w/w], 0.4 mL, 30℃, pH 7, over time]

- The specific FTF activity from sucrose to GF2 in double mutant was higher than that in the wild-type.

6. Discussion



7. Conclusion

We concluded that G85 and H310 of *A. kawachii* FFase were involved in the regulation of FTF from 1-kestose to nystose .

Their FFases may enable efficient 1-kestose production.

8. Future perspectives

Our study will enable development of an industrial enzyme producing 1-kestose efficiently, which is generally considered difficult.