Engineering the glucansucrase GTFR enzyme reaction and glycosidic bond specificity

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Supporting Information

Engineering the Glucansucrase GTFR enzyme reaction- and glycosidic bond specificity: towards tailor-made polymer and oligosaccharide products

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MATERIALS AND METHODS

Table 1. Oligodeoxynucleotides used. All primers are shown as used in the PCR.

Name	Sequence (5'-3')
pETR3+5179	CGGGATCCGAATTCGAGCTCCGTCGACAAGC
gtfR-1908M3	GTCAGCAATAACAGTTTG**TACTTCACT**GTC**ATGAGCCCG**AACAAAGATATAGTTAGC[a]
gtfR+1895	CTGTTATTGCTGACATCATCCG
gtfR-2783	GCAATAACTTTATTGGTATATTGGTCATCG
gtfR+1566	CGCAGACTTGCTCCAAATCGC
gtfR-2192	GCTACATACTTAATACGAGCACGC
gtfR-R624G	CTGTCATGAGCGCCAACAAAGATATAGTTAGC
gtfR-V630I	GTCAGCAATAACAGTTTGAATTTCACTGTCATG
gtfR-R624GV630I	GTCAGCAATAACAGTTTGAATTTCACTGTCATGAGCGCCAACAAAGATATAGTTAGC
gtfR+OE	GGCTCATGACAGTGAAGTAC

[a] The WT sequence is shown. Degenerate NTs are in bold and underlined. The D717A amino acid replacement is located outside the mutagenized region and probably introduced due to a PCR error (GAT/C→GC/T/C).

Description of library screen

Screening of GTFR enzyme mutant libraries displaying strong variation in oligosaccharide product synthesis

Enzyme variants were expressed and produced in *E. coli* in a 96 well format (Figure 1) and released via chemical lysis (BPER) of the cells. As an example, 4 microtitre plates are shown after growth of *E. coli* and before cell disruption. Growth and appearance in each well is similar.

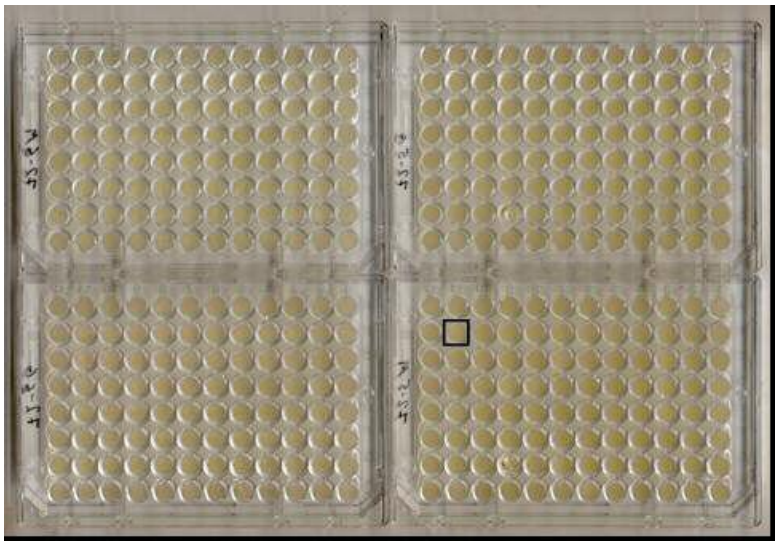


Figure 1: Part of a GTFR enzyme library screened after cell growth and before cell disruption. Each well contains one enzyme variant (here: library 72, modified with primer gtfR-1908M3, shown above). Reaction analysis by TLC highlighted variant Nr. 72P5B11 (framed), which was identified as S628D.

After lysis, the transferred extracts were used in a reaction with sucrose and analysed via TLC. As an example, a TLC-analysis of 10 variants is shown. Activity was detected by the release of glucose and fructose from sucrose, detected by TLC-analysis. Polymer production can be detected as small dots (insoluble) or circles (soluble) at the concentration zone of the TLC sheet(Figure 2).

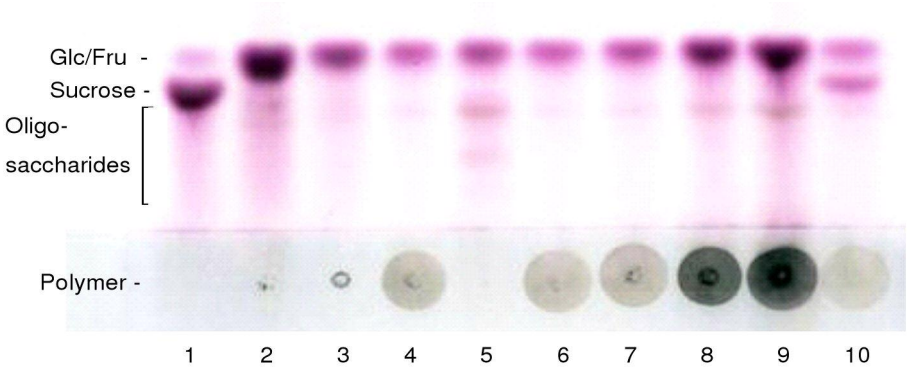
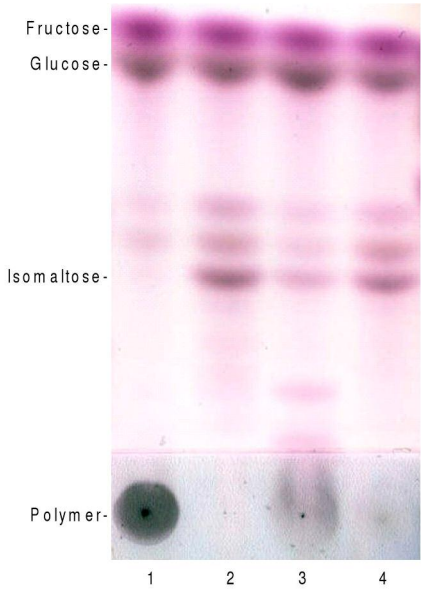


Figure 2: TLC analysis of 10 GTFR enzyme variants incubated with sucrose (292 mM sucrose, 1/10 dilution, 3 µl per lane). Variants in Lanes 2 and 3 (insoluble polymer or reduced amount of polymer produced) and in Lane 5 (loss of polymer production and enhanced oligosaccharide production) were chosen for further analysis. The mutant applied in Lane 5 (72P5B11) was identified as S628D.

Further screening of interesting variants was carried out by TLC analysis of reaction products obtained following incubation with sucrose, with or without additional acceptor substrates (See Figure 3 for an example).



Figuret 3: TLC analysis of reaction products of 4 GTFR variants selected for further characterisation, following incubation with 146 mM sucrose and 292 mM glucose, and enzyme (1/10 diluted). Wild-type (lane 1), S628D (lane 2: no polymer production, enhanced oligosaccharide production), triple mutant R624G/V630I/D717A (lane 3: insoluble polymer), S628R (lane 4: no polymer production, enhanced oligosaccharide production).

Screening of GTFR glucansucrase mutants with changed glycosidic bond specificity in polysaccharides produced

Additionally, polymer production was monitored visually for changes in appearance and solubility (Figure 4).

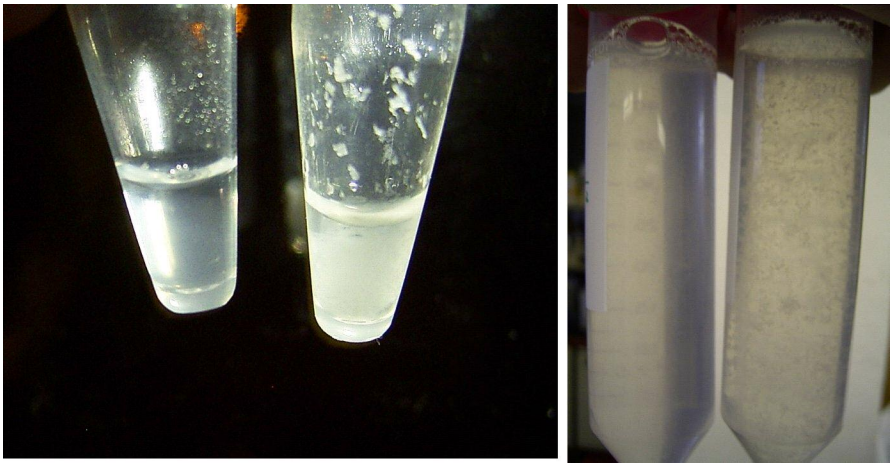


Figure 4: Polymer formed by GTFR wild type (left) and triple mutant R624G/V630I/D717A. While the polymer of the wild type is mainly soluble and clear, the polymer of the triple mutant has a milky, fluffy appearance.

Additional supplemental data

Table 1: **Transglycosylation yields.** Yields of different acceptor products, measured after sucrose depletion, formed in reactions with (A) sucrose or with sucrose (146 mM) and (B) glucose or (C) fructose as acceptor substrate (292 mM). For isomaltose, only one glucose is accounted. (errors are shown in parentheses). (n.d. - = not detected)

A) 146 mM sucrose:

	WT	S628D	S628R	triple	R624G	V630I	double	D717A
%								
Hydrolysis	4.8 (3.8)	22.7 (0.0)	22.4	27.3 (7.4)	19.5 (0.7)	1.1 (1.1)	10.9 (1.8)	0.7 (0.7)
Leucrose	10 (0.4)	21.8 (0.2)	28.0	10.6 (0.6)	10.3 (0.1)	15.7 (0.8)	11.5 (0.6)	9.7 (0.0)
Isomaltose	n.d.	11.4 (0.7)	12.0	1.8 (0.7)	0.8 (0.8)	n.d.	n.d.	n.d.
Palatinose	0.6 (0.2)	13.6 (0.8)	3.0	2.2 (0.3)	0.5 (0.5)	n.d.	n.d.	0.6 (0.1)

B) 146 mM sucrose, 292 mM glucose

	WT	S628D	S628R	triple	R624G	V630I	double	D717A
%								
Leucrose	5.9 (1.7)	12.9 (0.1)	14.0 (0.5)	5.6 (0.6)	4.4 (0.5)	7.2 (0.8)	6.4 (0.8)	5.3 (0.7)
Isomaltose	1.9 (0.1)	46.5 (0.3)	49.0 (0.5)	14.9 (2.9)	2.7 (0.4)	1.6 (0.1)	8.1 (1.2)	1.4 (0.1)
Palatinose	1.2 (0.1)	4.5 (0.2)	2.0 (0.4)	n.d.	n.d.	n.d.	n.d.	0.6 (0.6)

C) 146 mM sucrose, 292 mM fructose

	WT	S628D	S628R	triple	R624G	V630I	double	D717A
%								
Hydrolysis	n.d.	11.2 (1.0)	13.0 (0.8)	12.6 (0.7)	19 (0.6)	13.5 (0.2)	1.1 (1.1)	1.7 (1.7)
Leucrose	42.6 (2.3)	64.0 (0.1)	66.7 (0.9)	38.8 (0.5)	43.3 (2.1)	48.4 (0.7)	48.2 (0.8)	39.8 (2.8)
Isomaltose	n.d.	2.8 (0.7)	7.3 (0.3)	0.8 (0.1)	n.d.	n.d.	n.d.	n.d.
Palatinose	1.9 (0.2)	20.6 (0.3)	5.8 (0.6)	8.6 (0.9)	4.8 (1.2)	2.4 (0.4)	3.9 (0.8)	4.3 (0.9)

Polymer production. Determination of polymers produced by different GTFR variants and the amount of insoluble polymer (errors are shown in parentheses).

	WT	S628D	S628R	triple mutant	R624G	V630I	double mutant	D717A
total polymer (% wt)	100 (4)	0	11 (1)	61 (0)	79 (4)	91 (7)	52 (2)	91 (8)
insoluble polymer (% poly.)	26	0	n.d.	44	30	33	46	24

Analysis of oligosaccharides. HPAEC analysis of oligosaccharides produced by GTFR WT and mutant S628D incubated with sucrose (146 mM) in comparison with dextran digested with dextranase. (L= leucrose, IM= isomaltose, GO = glucooligosaccharides) (Figure 5).

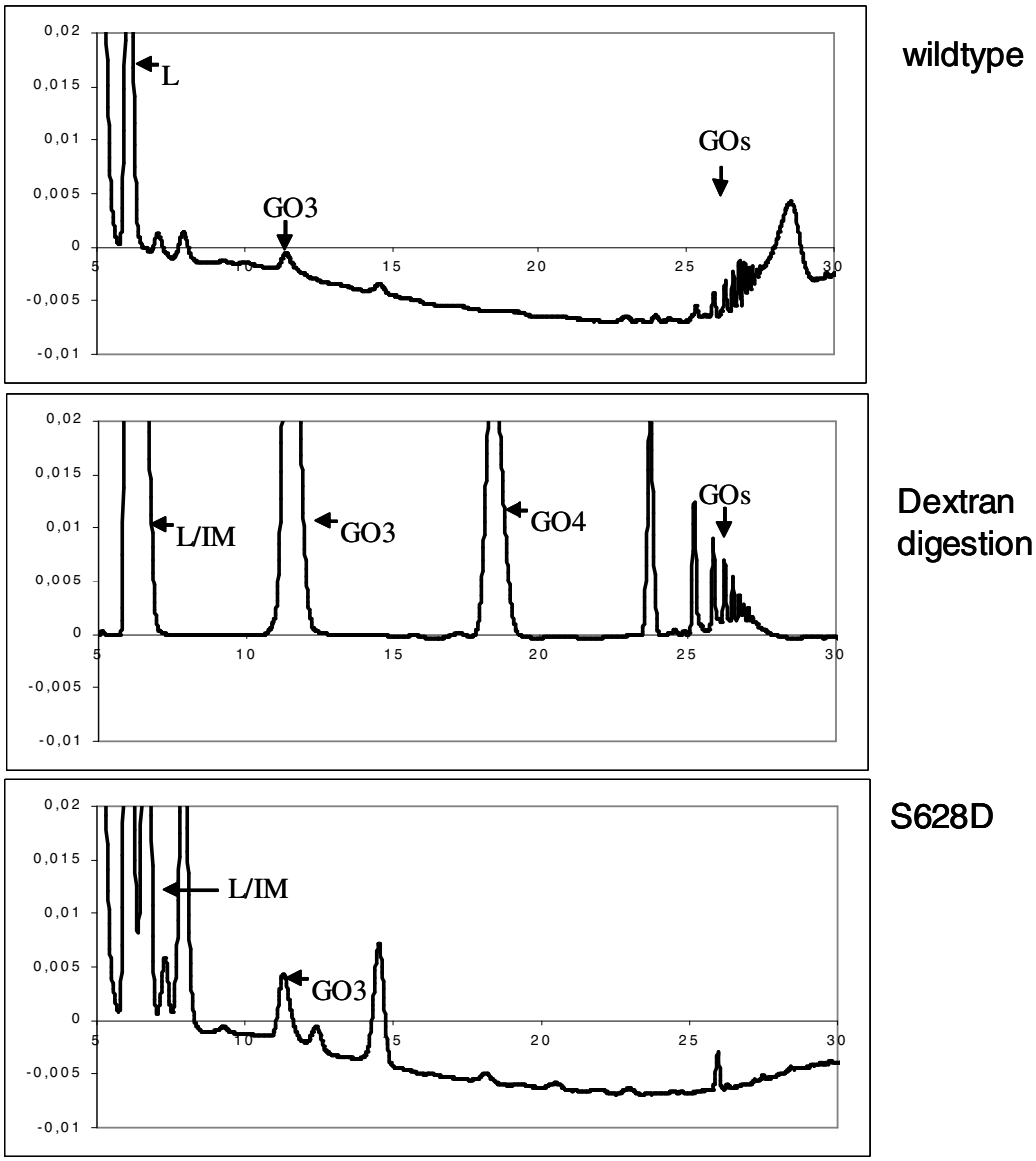


Figure 5: HPAEC analysis of oligosaccharides.