Supporting Information

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

Hendrik Hellmuth, Sabine Wittrock, Slavko Kralj, Lubbert Dijkhuizen, Bernd Hofer, Jürgen Seibel

MATERIALS AND METHODS

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

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>pETR3+5179</td>
<td>CGGGATCCGAATTCGAGCTCCGTCGACAAGC</td>
</tr>
<tr>
<td>gtfR-1908M3</td>
<td>GTCAGCAATAACAGTTTGTTTTTCACTGTCATGAGCCCAAAACAGATAGTGTTAGC</td>
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<td>gtfR+1895</td>
<td>CTGGTTATGTGCTGACATCATGCG</td>
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<td>gtfR-2783</td>
<td>GCAATAACTTTATTGGTTATATGTCATCG</td>
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<td>gtfR+1566</td>
<td>CGCAGACTTGCTCCAAATCGC</td>
</tr>
<tr>
<td>gtfR-2192</td>
<td>GCTACATACCTAATACGAGCACG</td>
</tr>
<tr>
<td>gtfR-R624G</td>
<td>CTGTCATGCGGCAAACAAAGATAGTGTTAGC</td>
</tr>
<tr>
<td>gtfR-V630I</td>
<td>GCCAGCAAAACTTGGAACTTTTCACTGTCATG</td>
</tr>
<tr>
<td>gtfR-R624G V630I</td>
<td>GCCAGCAAAACTTGGAACTTTTCACTGTCATG GCCAGCAAAACTTGGAACTTTTCACTGTCATG</td>
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<tr>
<td>gtfR+OE</td>
<td>GGCTCATGACAGTGAAGTAC</td>
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</table>

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.
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).

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).
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).
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:

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

B) 146 mM sucrose, 292 mM glucose

<table>
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<tr>
<th></th>
<th>WT</th>
<th>S628D</th>
<th>S628R</th>
<th>triple</th>
<th>R624G</th>
<th>V630I</th>
<th>double</th>
<th>D717A</th>
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<tbody>
<tr>
<td><strong>%</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Leucrose</td>
<td>5.9</td>
<td>12.9</td>
<td>14.0</td>
<td>5.6</td>
<td>4.4</td>
<td>7.2</td>
<td>6.4</td>
<td>5.3</td>
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<tr>
<td>(1.7)</td>
<td>(0.1)</td>
<td>(0.5)</td>
<td>(0.6)</td>
<td>(0.5)</td>
<td>(0.8)</td>
<td>(0.8)</td>
<td>(0.7)</td>
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<tr>
<td>Isomaltose</td>
<td>1.9</td>
<td>46.5</td>
<td>49.0</td>
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<td>1.6</td>
<td>8.1</td>
<td>1.4</td>
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<tr>
<td>(0.1)</td>
<td>(0.3)</td>
<td>(0.5)</td>
<td>(2.9)</td>
<td>(0.4)</td>
<td>(0.1)</td>
<td>(1.2)</td>
<td>(0.1)</td>
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</tr>
<tr>
<td>Palatinose</td>
<td>1.2</td>
<td>4.5</td>
<td>2.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>0.6</td>
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<tr>
<td>(0.1)</td>
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<td>(0.4)</td>
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</table>
C) 146 mM sucrose, 292 mM fructose

<table>
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<th>S628D</th>
<th>S628R</th>
<th>triple R624G</th>
<th>V630I</th>
<th>double R624G</th>
<th>D717A</th>
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<tbody>
<tr>
<td><strong>Hydrolysis</strong></td>
<td>n.d.</td>
<td>11.2</td>
<td>13.0</td>
<td>12.6</td>
<td>19</td>
<td>13.5</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>(1.0)</td>
<td>(0.8)</td>
<td>(0.7)</td>
<td>(0.6)</td>
<td>(0.2)</td>
<td>(1.1)</td>
<td>(1.7)</td>
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<tr>
<td><strong>Leucrose</strong></td>
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<td>64.0</td>
<td>66.7</td>
<td>38.8</td>
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</tr>
<tr>
<td></td>
<td>(2.3)</td>
<td>(0.1)</td>
<td>(0.9)</td>
<td>(0.5)</td>
<td>(2.1)</td>
<td>(0.7)</td>
<td>(0.8)</td>
</tr>
<tr>
<td><strong>Isomaltose</strong></td>
<td>n.d.</td>
<td>2.8</td>
<td>7.3</td>
<td>0.8</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>(0.7)</td>
<td>(0.3)</td>
<td>(0.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Palatinose</strong></td>
<td>1.9</td>
<td>20.6</td>
<td>5.8</td>
<td>8.6</td>
<td>4.8</td>
<td>2.4</td>
<td>3.9</td>
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<tr>
<td></td>
<td>(0.2)</td>
<td>(0.3)</td>
<td>(0.6)</td>
<td>(0.9)</td>
<td>(1.2)</td>
<td>(0.4)</td>
<td>(0.8)</td>
</tr>
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</table>

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

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>S628D</th>
<th>S628R</th>
<th>triple R624G</th>
<th>R624G</th>
<th>V630I</th>
<th>double R624G</th>
<th>D717A</th>
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</thead>
<tbody>
<tr>
<td><strong>total polymer</strong></td>
<td>100</td>
<td>0</td>
<td>11</td>
<td>61</td>
<td>79</td>
<td>91</td>
<td>52</td>
<td>91</td>
</tr>
<tr>
<td>(% wt)</td>
<td>(4)</td>
<td>(1)</td>
<td>(0)</td>
<td>(4)</td>
<td>(7)</td>
<td>(2)</td>
<td>(8)</td>
<td></td>
</tr>
<tr>
<td><strong>insoluble polymer</strong></td>
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<td>44</td>
<td>30</td>
<td>33</td>
<td>46</td>
<td>24</td>
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<tr>
<td>(% poly.)</td>
<td></td>
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</tbody>
</table>
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.