**New insights into the molecular mechanism behind mannitol and erythritol fructosylation by β-fructofuranosidase from *Schwanniomyces occidentalis***

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**-Table S1.** Crystallographic statistics.

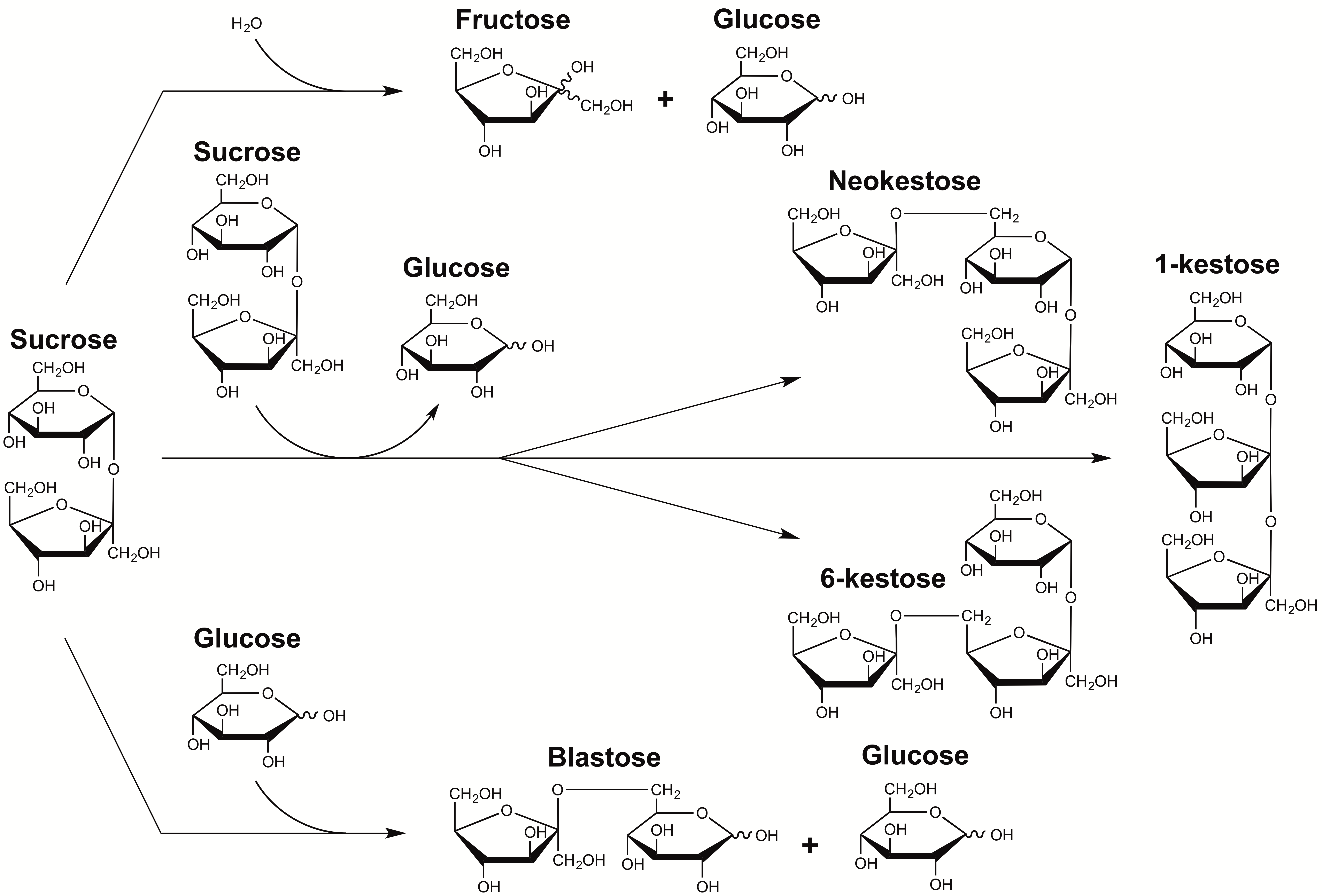
**-Figure S1.** Schematic view of the reactions catalyzed by Ffase on sucrose.

**-Figure S2.** HPLC chromatograms from transfructosylating reactions produced by N254T (a) or wild-type (b) Ffase variants.

**-Figure S3.** Mass spectrum of the reaction mixture obtained with the Ffase-N254T variant in reactions containing sucrose and erythritol.

**-Figure S4.** SDS-PAGE analysis of the purified Ffase variants.

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| **Table S1.** Crystallographic statistics. | | |
| **Crystal data** | Ffase-D50A / Fru-erythritol | Ffase-D50A / Sucrose |
| Space group | P 21 | P 21 |
| Unit cell parameters |  |  |
| a (Å) | 60.48 | 60.90 |
| b (Å) | 92.54 | 93.20 |
| c (Å) | 116.11 | 116.61 |
|  (º) | 104.71 | 104.90 |
| **Data collection** |  |  |
| Beamline | XALOC  (ALBA) | XALOC  (ALBA) |
| Temperature (K) | 100 | 100 |
| Wavelength (Å) | 0.97926 | 0.97926 |
| Resolution (Å) | 49.33-1.88  (1.91-1.88) | 49.69-2.09  (2.13-2.09) |
| **Data processing** |  |  |
| Total reflections | 536923 (25965) | 369570 (22936) |
| Unique reflections | 98,961 (4791) | 74544 (4621) |
| Multiplicity | 5.4 (5.4) | 5.0 (5.0) |
| Completeness (%) | 98.6 (97.9) | 99.9 (99.8) |
| Mean *I*/σ (*I*) | 9.2 (3.0) | 8.6 (2.5) |
| *Rmerge†* (%) | 12.3 (63.0) | 13.8 (56.9) |
| *Rpim††* (%) | 5.9 (30.0) | 7.0 (28.6) |
| Molecules per ASU | 2 | 2 |
| **Refinement** |  |  |
| Rwork / Rfree*†††* (%) | 15.39 /17.85 | 17.49/21.36 |
| **Nº of atoms/average B** (Å2) |  |  |
| Protein | 8341/19.21 | 8308/20.28 |
| Carbohydrate | 226/37.91 | 248/37.18 |
| Water Molecules | 849/30.72 | 794/27.06 |
| All atoms | 9416/20.69 | 9350/21.30 |
| **Ramachandran plot** (%) |  |  |
| Favoured | 96 | 96 |
| Outliers | 0 | 0 |
| **RMS deviations** |  |  |
| Bonds (Å) | 0.0072 | 0.0065 |
| Angles (°) | 1.4496 | 1.4589 |
| **PDB accession codes** | 6S2B | 6S1T |
| Values in parentheses are for the high resolution shell  †Rmerge = ∑hkl ∑i | Ii(hkl) – [I(hkl)]| / ∑hkl ∑i  Ii(hkl), where Ii(hkl) is the ith measurement of reflection hkl and [I(hkl)] is the weighted mean of all measurements.  ††Rpim = ∑hkl [1/(N - 1)] 1/2 ∑i | Ii(hkl) – [I(hkl)]| / ∑hkl ∑i  Ii(hkl), where N is the redundancy for the hkl reflection.  †††Rwork / Rfree = ∑hkl | Fo – Fc | / ∑hkl | Fo |, where Fc is the calculated and Fo is the observed structure factor amplitude of reflection hkl for the working / free (5%) set, respectively. | | |

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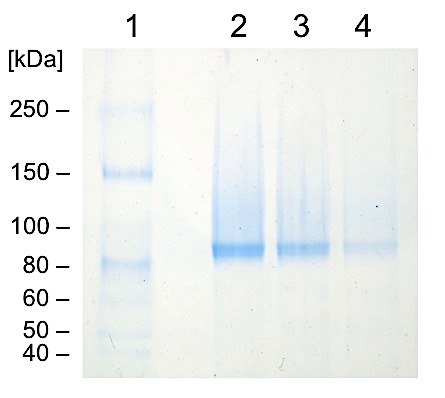
**Figure S1. Schematic view of the reactions catalyzed by Ffase on sucrose**. This enzyme cleaves the β-(2→1) linkage of sucrose (hydrolytic activity) and transfers the fructosyl moiety into a sucrose or a glucose unit (transferase activity) generating: blastose [β-D-Fru-(2→6)-Glc], neokestose [β-D-Fru-(2→6)-α-D-Glc-(1→2)-β-D-Fru], 1-kestose [β-D-Fru-(2→1)-β-D-Fru-(2→1)-α-D-Glc] and 6-kestose [β-D-Fru-(2→6)-β-D-Fru-(2→1)-α-D-Glc].

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**Figure S2.** **HPLC chromatograms from transfructosylating reactions produced by N254T (a) or wild type (b) Ffase variants.** 10 U ml-1 of pure enzyme was incubated in 200 g l-1 sucrose and 500 g l-1 erythritol. Analyses were conducted at the point of maximum fructosyl-erythritol production. Upper right corners show a close-up view of the corresponding figures including the detector response range. Peaks assignations: (1) erythritol, (2) fructose, (3) glucose, (4) fructosyl-erythritol, (5) sucrose, (6) blastose, (7) potential (fructosyl)2-erythritol, (8) neokestose, (9) 1-kestose, and (10) 6-kestose.



**Figure S3. Mass spectrum of the reaction mixture obtained with the Ffase-N254T variant in reactions containing sucrose and erythritol.** Reaction conditions: 1.5 ml mixtures containing 200 g l-1 sucrose and 500 g l-1 erythritol in 0.1 M sodium acetate at pH 5.5 were incubated at 50 ºC for 240 min. Molecular masses of chemical constituents plus sodium ion (~ 23 u) were detected in positive mode. Peaks of [M+Na]+ m/z of 365.1, 469.2, and 631.2 were assigned respectively as fructosyl-erythritol, potential (fructosyl)2-erythritol, and negligible traces of hypothetical (fructosyl)3-erythritol. Peaks of [M + Na]+ m/z of 145.0, 203.0, 365.1, 527.2, and 689.2 corresponds to erythritol, monosaccharides, disaccharides, trisaccharides, and minimal quantities of unidentified tetrasaccharides, respectively.



**Figure S4. SDS-PAGE analysis of the purified Ffase variants.** Lane 1: Unstained protein standards ranging 10-250 KDa (New England Biolabs Inc.; Ipswich, USA) used for molecular weight estimations. Lane 2-4: 0.5 µl of purified enzyme Ffase-D50A, -N254T, and -Q228E; respectively. Numbers at the left indicate the positions of molecular mass standards in KDa.