*Article*

# Structural Insight into a Yeast Maltase—The *Ba*AG2 from *Blastobotrys adeninivorans* with Transglycosylating Activity

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**Abstract:** An early-diverged yeast, *Blastobotrys* (*Arxula*) *adeninivorans* (*Ba*), has biotechnological potential due to nutritional versatility, temperature tolerance, and production of technologically applicable enzymes. We have biochemically characterized from the *Ba* type strain (CBS 8244) the GH13-family maltase *Ba*AG2 with efficient transglycosylation activity on maltose. In the current study, transglycosylation of sucrose was studied in detail. The chemical entities of sucrose-derived oligosaccharides were determined using nuclear magnetic resonance. Several potentially prebiotic oligosaccharides with $\alpha$-1,1, $\alpha$-1,3, $\alpha$-1,4, and $\alpha$-1,6 linkages were disclosed among the products. Trisaccharides isomelezitose, erlose, and theanderose, and disaccharides maltulose and trehalulose were dominant transglycosylation products. To date no structure for yeast maltase has been determined. Structures of the *Ba*AG2 with acarbose and glucose in the active center were solved at 2.12 and 2.13 Å resolution, respectively. *Ba*AG2 exhibited a catalytic domain with a $(\beta/\alpha)_8$-barrel fold and Asp216, Glu274, and Asp348 as the catalytic triad. The fairly wide active site cleft contained water channels mediating substrate hydrolysis. Next to the substrate-binding pocket an enlarged space for potential binding of transglycosylation acceptors was identified. The involvement of a Glu (Glu309) at subsite +2 and an Arg (Arg233) at subsite +3 in substrate binding was shown for the first time for $\alpha$-glucosidases.

**Keywords:** $\alpha$-glucosidase; glycoside hydrolase; isomalto-oligosaccharides; acarbose; crystal structure; molecular replacement; nuclear magnetic resonance; erlose; isomelezitose; trehalulose

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*Supplementary Materials*

Tables S1, S2, S3

Figures S1, S2, S3, S4, S5, S6, S7

**Table S1.** Substrates of α-glucosidases and their potential transglycosylation products. Oligosac- charides containing glucose and fructose residues, their composition and linkage types are shown. The substrates for *Ba*AG2 were determined in [1] and in this study. Sucrose isomers are shown in bold and sucrose moiety in trisaccharides is underlined.

| Saccharide | Composition and linkage type | Description | Substrate for *Ba*AG2 |
|---|---|---|---|
| Nigerose* | Glc-α1,3-Glc | Maltose-type disaccharide | ND |
| Maltose* | Glc-α1,4-Glc | Maltose-type disaccharide | + |
| Isomaltose* | Glc-α1,6-Glc | Isomaltose-type disaccharide | − |
| Trehalulose* | **Glc-α1,1-Fru** | Substrate; disaccharide | + |
| Sucrose | Glc-α1,2-Fru | Substrate; disaccharide | + |
| Turanose* | **Glc-α1,3-Fru** | Maltose-type disaccharide | + |
| Maltulose* | **Glc-α1,4-Fru** | Maltose-type disaccharide | + |
| Isomaltulose (palatinose)* | **Glc-α1,6-Fru** | Isomaltose-type disaccharide | − |
| Maltotriose | Glc-α1,4-Glc-α1,4-Glc | Maltose-type trisaccharide | + |
| Panose | Glc-α1,6-Glc-α1,4-Glc | Mixed-type trisaccharide | − |
| Esculose* | Glc-α1,3-Glc-α1,2-Fru | Maltose-type trisaccharide | ND |
| Erlose* | Glc-α1,4-Glc-α1,2-Fru | Maltose-type trisaccharide | + |
| Theanderose* | Glc-α1,6-Glc-α1,2-Fru | Isomaltose-type trisaccharide | ND |
| Melezitose* | Glc-α1,3-Fru-β2,1-Glc | Maltose-type trisaccharide | + |
| Isomelezitose* | Glc-α1,6-Fru-β2,1-Glc | Isomaltose-type trisaccharide | + |

Glc — glucose; Fru — fructose; + hydrolysed by *Ba*AG2; − not a substrate for *Ba*AG2; ND — not determined.

* Products of *Ba*AG2 detected in this study by NMR from transglycosylation reaction on sucrose

**Table S2.** Data collection and refinement statistics of *Ba*AG2 structure determination.

| | ***Ba*AG2 + acarbose** | ***Ba*AG2 + glucose** |
|---|---|---|
| **PDB ID** | **7P01** | **7P07** |
| **Data collection** | | |
| Space group | P 1 21 1 | P 1 21 1 |
| Cell dimensions | | |
| *a, b, c* (Å) | 68.03 78.08 121.93 | 66.88 77.73 121.11 |
| α, β, γ (°) | 90 94.1 90 | 90 92.9 90 |
| Resolution (Å) | 48.14-2.12 (2.18-2.12)* | 47.73-2.13 (2.19-2.13)* |
| *R*merge | 0.023 (1.152) | 0.097 (1.204) |
| $I/\sigma I$ | 7.68 (1.54) | 8.61 (1.19) |
| Completeness (%) | 98.9 (100.0) | 98.9 (99.8) |
| Redundancy | 3.55 (3.55) | 3.55 (3.58) |
| **Refinement** | | |
| Resolution (Å) | 48.14-2.12 | 47.73-2.13 |
| No. reflections | 72345 | 69408 |
| *R*work/*R*free | 0.1842 /0.1952 | 0.2150 / 0.2318 |
| No. non-hydrogen atoms | 10177 | 9770 |
| Protein | 9345 | 9355 |
| Ligand/ion | 155 | 40 |
| Water | 677 | 375 |
| *B*-factors | 44.6 | 53.6 |
| Protein | 44.9 | 52.5 |
| Ligand/ion | 46.1 | 53.0 |
| Water | 40.1 | 80.2 |
| R.m.s. deviations | | |
| Bond lengths (Å) | 0.019 | 0.017 |
| Bond angles (°) | 1.89 | 1.89 |

* Statistics for the highest-resolution shell is shown in parentheses.

**Table S3.** NMR assignment of Glc-α1,1-Fru-β2,1-Glc.

| **Signal/Position** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| α-Glc*p*-1,1- | | | | | | |
| 1H | 4.953 | 3.488 | 3.683 | 3.371 | 3.596 | 3.709/3.732 |
| 13C | 98.49 | 71.29 | 72.85 | 69.42 | 72.24 | 60.11 |
| α-Glc*p*-1,2- | | | | | | |
| 1H | 5.371 | 3.479 | 3.670 | 3.379 | 3.772 | 3.713/3.778 |
| 13C | 92.54 | 71.024 | 72.548 | 69.23 | 72.31 | 60.48 |
| β-Fru*f*-2,1- | | | | | | |
| 1H | 3.607/3.816 | - | 4.210 | 3.989 | 3.861 | 3.747 |
| 13C | 65.99 | 103.58 | 76.46 | 73.68 | 81.24 | 62.16 |

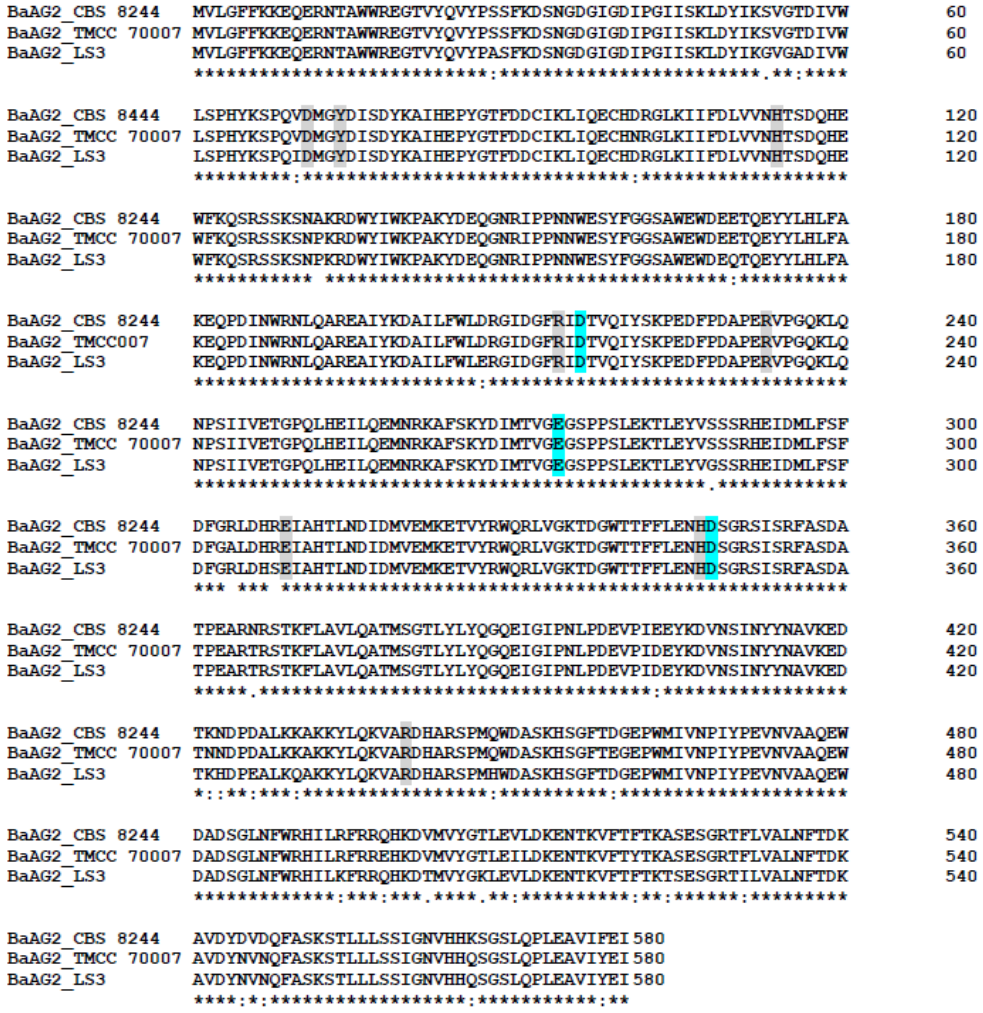


**Figure S1.** Clustal Omega alignment of *Ba*AG2 homologues from strains of three *Blastobotrys* (*Arx- ula*) sp. BaAG2_CBS 8244, a maltase from the *Ba* type strain which is a source of the *Ba*AG2 in the current work and our earlier studies [1,2]. BaAG2_TMCC 7007, a putative maltase of a *Ba* strain isolated from fermented Pu-erh tea [3]; BaAG2_LS3, a putative maltase of the first strain of *Blastobotrys* sp. with sequenced genome [4] that is currently allocated to *Blastobotrys raffinosifer- mentans* [5]. Amino acids constituting the catalytic triad are shown by cyan background and those interacting with substrate at −1, +1, +2 and +3 subsites are marked using grey background. Clustal consensus is marked below the sequence indicating conservation — * positions with fully con- served residue; : positions with residues of strongly similar properties; . positions with residues of weakly similar properties.

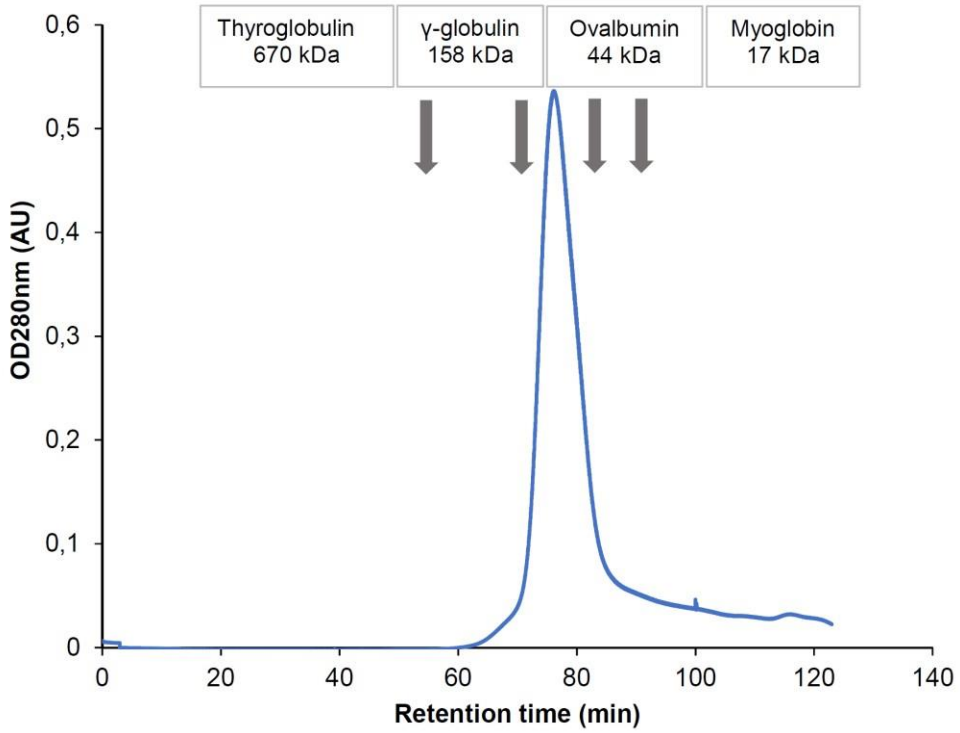


**Figure S2.** The chromatogram of size-exclusion chromatography of *Ba*AG2. The chromatography was performed on Hiload 16/60 Superdex 200 pg column (GE Healthcare, Uppsala, Sweden) as shown in chapter 2.1. in the main text. Gel filtration standard from Bio-Rad (Hercules, CA, USA) was used for calibration. The reference proteins with their molecular weights are shown and re- spective retention times are indicated by arrows. According to the calibration curve, the peak max- imum of *Ba*AG2 corresponds to 75.7 kDa.

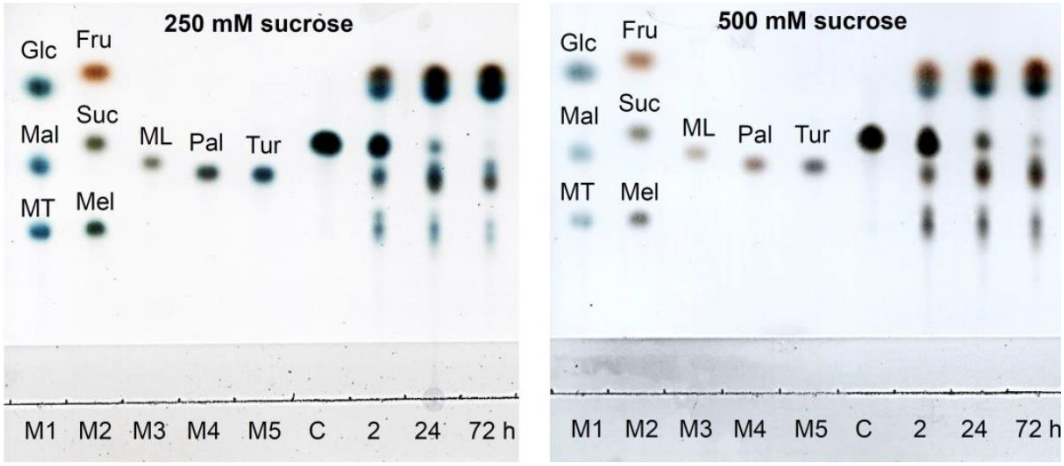


**Figure S3.** TLC analysis of transglycosylation on 250 mM or 500 mM sucrose by *Ba*AG2. The re- sults on quantification of saccharides by HPLC is shown on Figure 2 in the main text. The final concentration of the enzyme in the reaction mixture was 20 μg/mL. The reactions were carried out at 30 °C up to 72 h as shown in chapter 2.4. in the main text. Samples were 5 times diluted in mQ water before applying to TLC plates in 0.5 μL spots. The sugars were separated by TLC and visu- alized as shown in chapter 2.6. in the main text. Marker sugars were analysed as references. M1: Glc – glucose (30 mM), Mal – maltose (10 mM), MT – maltotriose (10 mM); M2: Fru – fructose (30 mM), Suc – sucrose (10 mM), Mel – melezitose (10 mM); M3: ML – maltulose (10 mM); M4: Pal – palatinose (isomaltulose, 10 mM); M5: Tur – turanose (10 mM). C – Control without enzyme but with 0.5% BSA and substrate (sucrose) incubated 72 h alongside to the samples. The compositions of reference sugars are shown in Table S1.

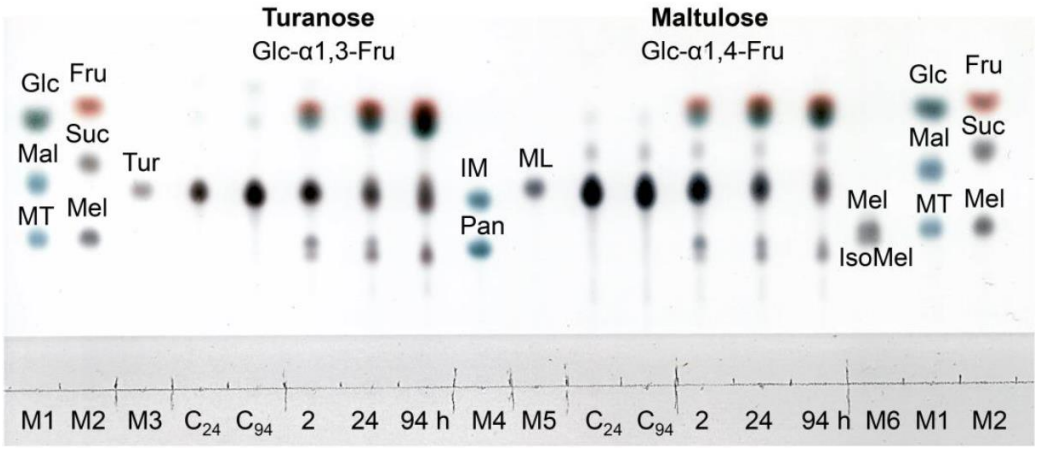


**Figure S4.** Transglycosylation assay of 500 mM turanose and maltulose by *Ba*AG2. The final con- centration of the enzyme was 20 μg/mL. The reactions were carried out at 30 °C up to 94 h simi- larly as shown in chapter 2.4. in the main text. Samples were 5 times diluted in mQ water before applying to TLC plates in 0.5 μL spots. The sugars were separated by TLC and visualized as shown in chapter 2.6. in the main text. M1: Glc – glucose (30 mM), Mal – maltose (10 mM), MT – maltotriose (10 mM); M2: Fru – fructose (30 mM), Suc – sucrose (10 mM), Mel – melezitose (10 mM); M3: Tur – turanose (10 mM); M4: IM – isomaltose (10 mM), Pan – panose (10 mM); M5: ML – maltulose (10 mM); M6: Mel – melezitose (10 mM), IsoMel – isomelezitose (*Op*MAL1 DP 3 prod- uct); $C_{24}$ and $C_{94}$ – Control without enzyme but with 0.5% BSA and substrate (turanose or maltu- lose) incubated alongside to the transglycosylation samples up to 24 h and 94 h, respectively. The compositions of reference sugars are shown in Table S1

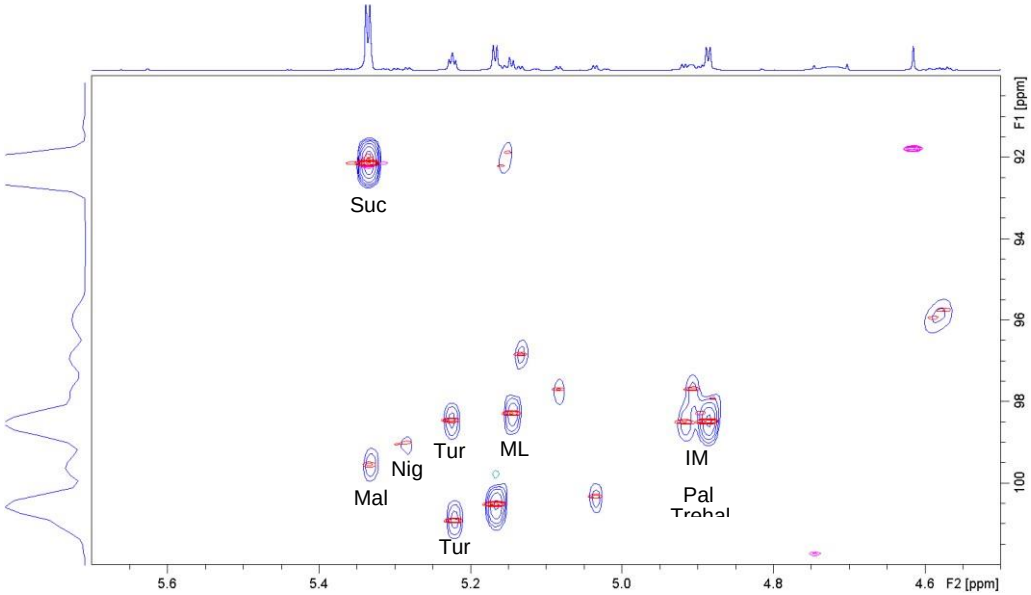


**Figure S5.** HSQC spectrum of the anomeric region (blue) overlaid with narrow HSQC (red) of the DP 2 sample prepared using 500 mM sucrose as substrate. The NMR spectra were recorded as shown in chapter 2.5. and analysed as described in chapter 3.4. in the main text. The $^{1}$H projection is from a separate experiment. The labels of non-reducing anomeric signals are as follows: Suc – sucrose, Mal – maltose, Nig – nigerose; Tur – turanose, ML – maltulose, IM – isomaltose, Pal – palatinose (isomaltulose), Trehal – trehalulose. The compositions of sugars are shown in Table S1.

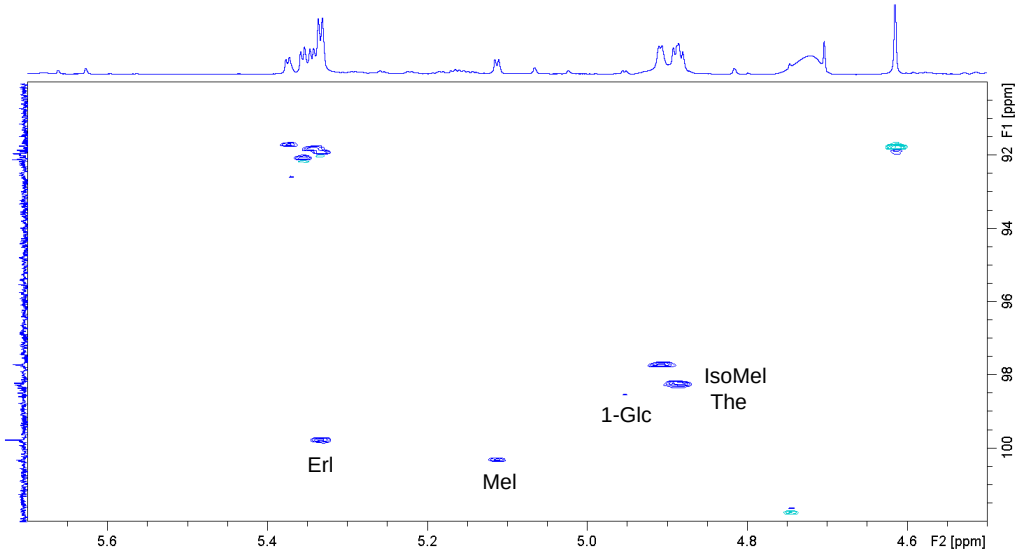


**Figure S6.** Narrow HSQC spectrum of the DP 3 sample prepared using 500 mM sucrose as sub- strate. The NMR spectra were recorded as shown in chapter 2.5. and analysed as described in chapter 3.4. in the main text. The $^{1}$H and $^{13}$C projections are from separate experiments. The labels are of non-reducing anomeric signals not arising from sucrose (Glc-$\alpha$1,2$\beta$-Fru), and are ordered by descending $^{1}$H chemical shift as follows: Erl – erlose, Mel – melezitose, 1-Glc – 1-O$^{F}$-glycosyl-su- crose, IsoMel – isomelezitose, The – theanderose.

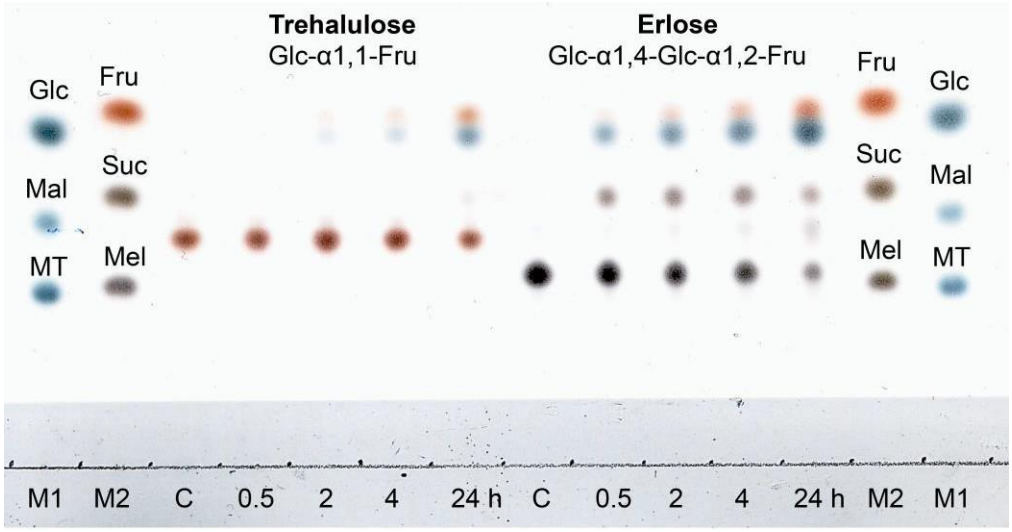


**Figure S7.** TLC analysis of *Ba*AG2 reaction products from 50 mM trehalulose and erlose. The final concentration of the enzyme was 3 μg/mL in the reaction mixture. The reactions were carried out at 30 °C up to 24 h similarly as in chapter 2.4. in the main text. Samples were 2 times diluted in mQ water before applying to TLC plates in 0.5 μL spots. The sugars were separated and visualized as shown in chapter 2.6. in the main text. M1: Glc – glucose (30 mM), Mal – maltose (10 mM), MT – maltotriose (10 mM); M2: Fru – fructose (30 mM), Suc – sucrose (10 mM), Mel – melezitose (10 mM); C – Control without enzyme but with 0.5% BSA and substrate (trehalulose or erlose, respec- tively) incubated 24 h alongside to the samples.

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