4,6-alpha-Glucanotransferase activity occurs more widespread in Lactobacillus strains and constitutes a separate GH70 subfamily

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Supplemental Information to:

4,6-α-Glucanotransferase activity occurs more widespread in *Lactobacillus* strains and constitutes a separate GH70 subfamily

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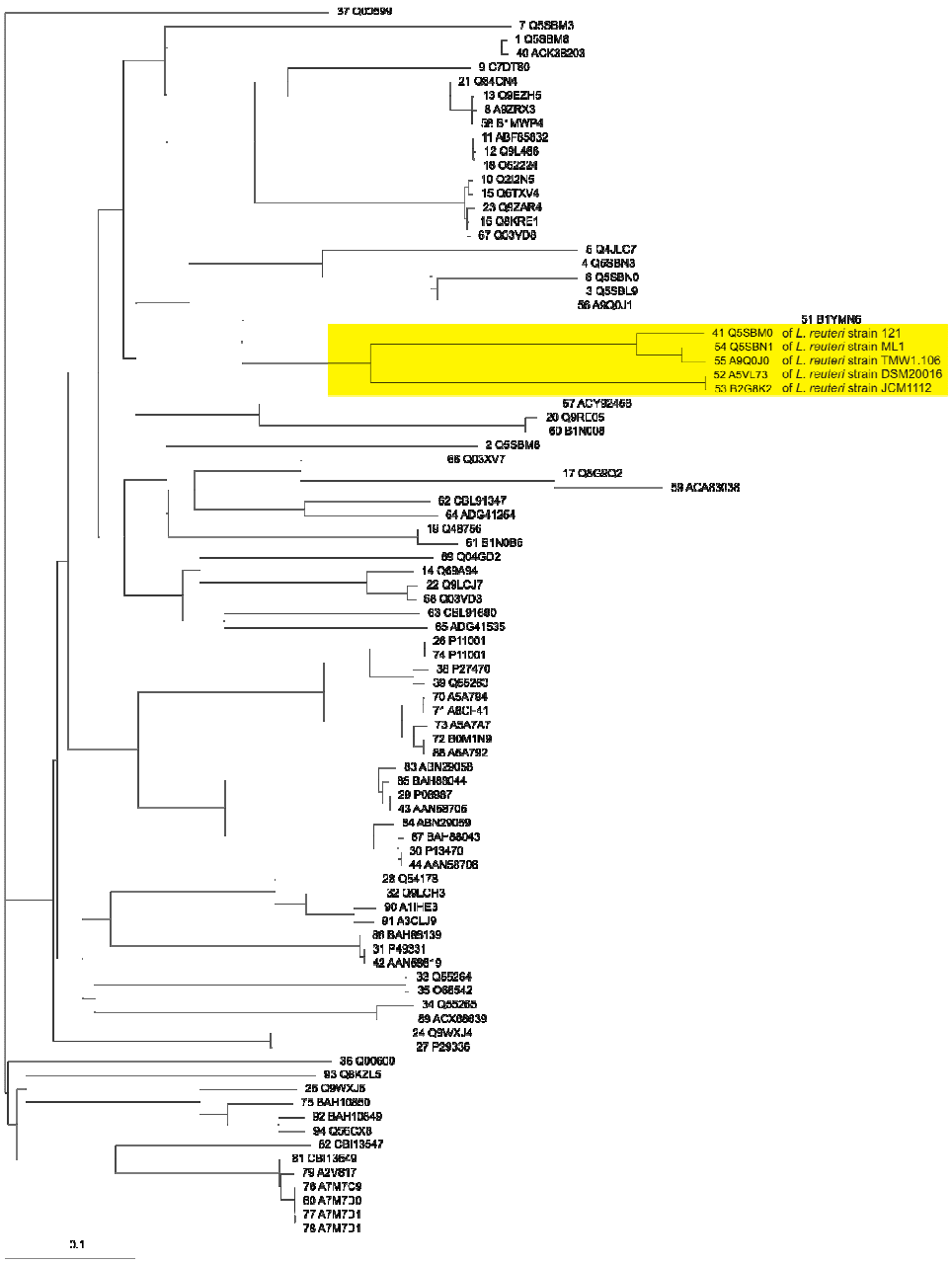

Fig. S1 Dendrogram of the GH70 protein sequences available via the Carbohydrate-Active Enzymes Database CAZy at http://www.cazy.org (Cantarel et al., 2009). The (putative) 4,6-αGT sequences form a cluster in the dendrogram and are highlighted with a yellow background. The proteins are indicated with their Uniprot code and for the (putative) 4,6-αGTs also the organisms name is provided.

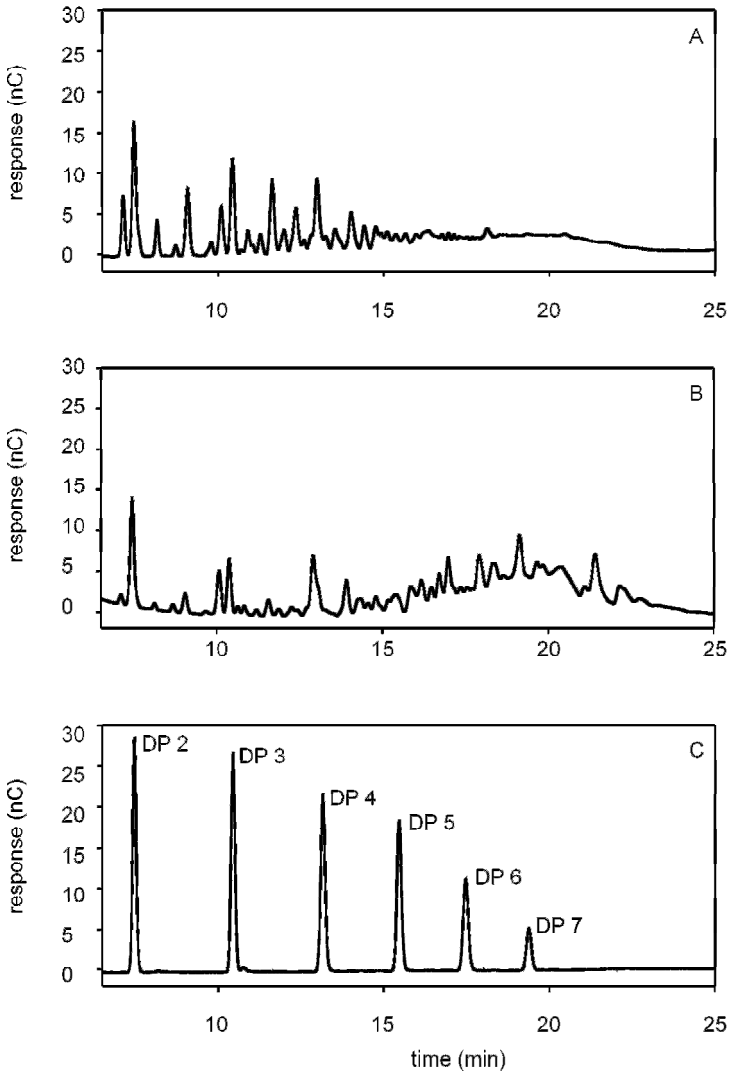

Fig. S2. HPAEC elution profiles of reaction mixtures obtained from maltoheptaose following incubation with (A) 4,6-αGT-W and (B) 4,6-αGT-ML4. Panel C shows the elution pattern of a (1→4)-α-D-glucooligosaccharide standard from DP 2 to DP 7.

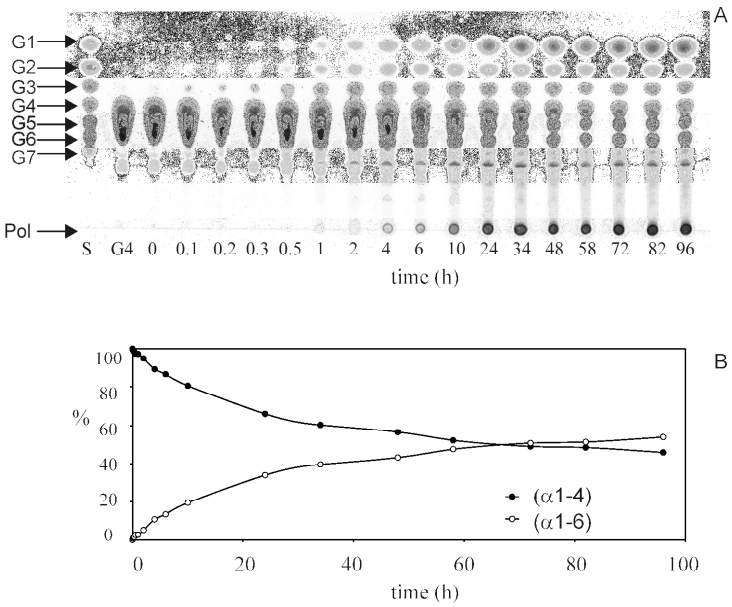

Fig. S3 Maltotetraose conversion by 4,6-αGT-W in time.

The progress of the reaction is followed in time by (A) TLC analysis and (B) ^{1}H NMR analysis (the percentages of the α1→4 and α1→6 glycosidic linkages are indicated with filled and open circles, respectively). Reaction conditions: 90 mg maltotetraose; 13 μg 4,6-αGT-W enzyme; pH 4.7 and 37°C. At the end of the incubation the fraction of α1→6 glycosidic bonds had increased from 0 to 0.57. The enzyme has a low hydrolytic activity as the α- plus β-anomeric signals increased by 15% relative to the total of the H-1 signals that reflect the α1→4 and α1→6 glycosidic bonds (^{1}H NMR spectra not shown).

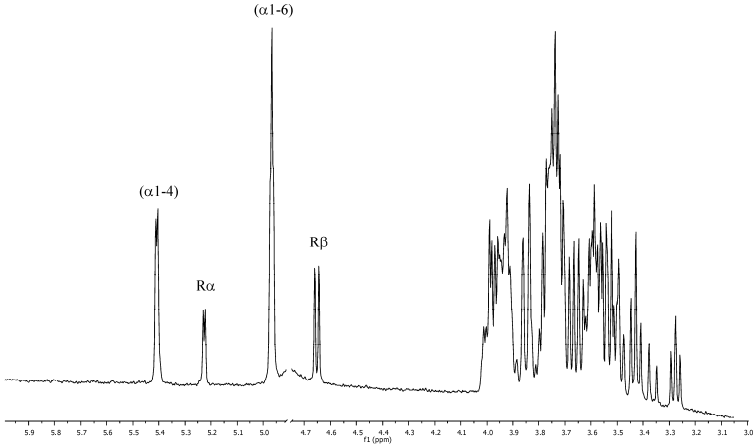

Fig. S4a ^{1}H NMR spectrum of HPAEC peak 1 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6-αGT-W. The spectrum is identical to the ^{1}H NMR spectrum of α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p* (Dobruchowska et al. 2012).

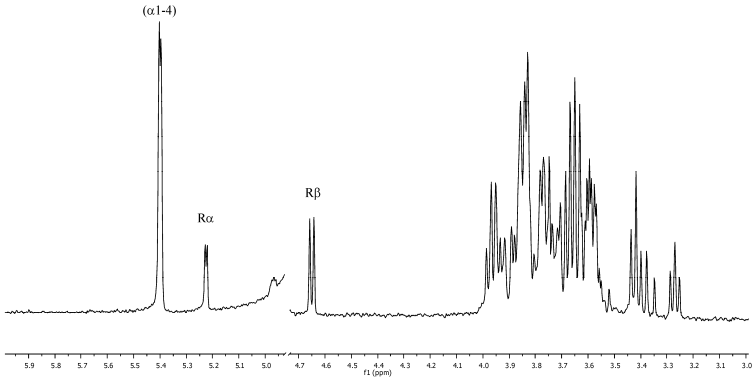

Fig. S4b ^{1}H NMR spectrum of HPAEC peak 2 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6-αGT-W. The spectrum is identical to the ^{1}H NMR spectrum of α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p* (maltotriose) (Dobruchowska et al. 2012).

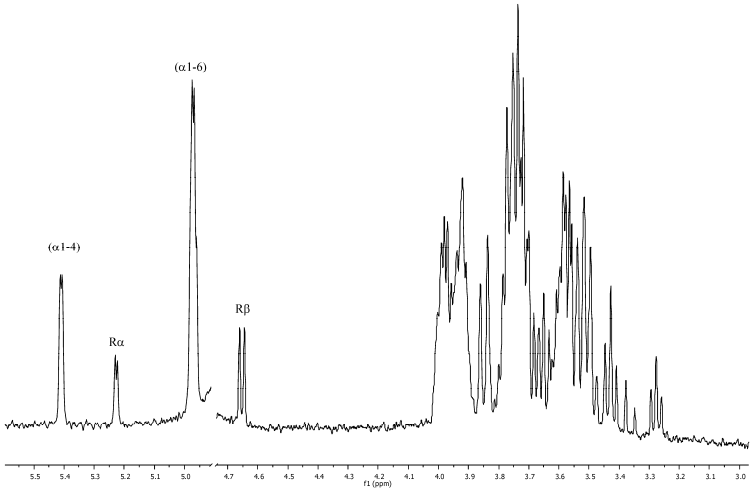

Fig. S4c ^{1}H NMR spectrum of HPAEC peak 3 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6-αGT-W. The spectrum is identical to the ^{1}H NMR spectrum of α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p* (Dobruchowska et al. 2012).

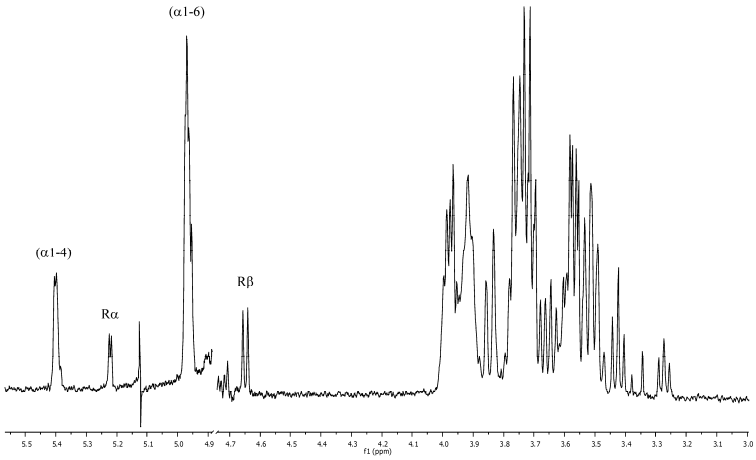

Fig. S4d ^{1}H NMR spectrum of HPAEC peak 4 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6-αGT-W. The spectrum is identical to the ^{1}H NMR spectrum of α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p* (Dobruchowska et al., 2012).

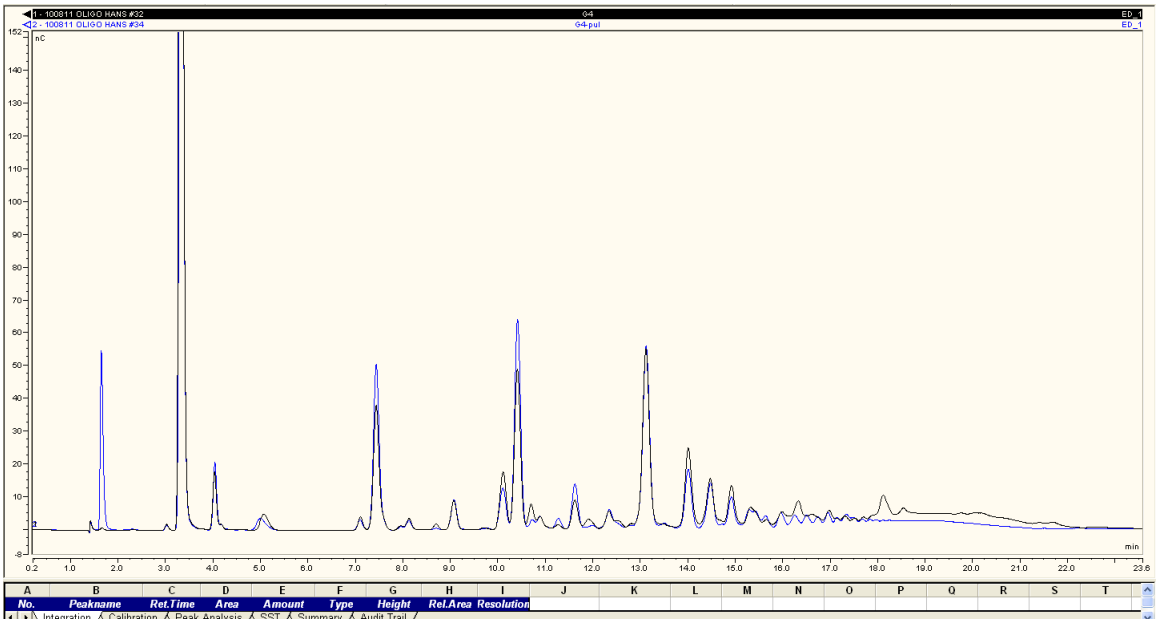

Fig. S5 HPAEC analysis of the α-glucan products of 4,6-αGT-W from maltotetraose (G4) (black line) and after (blue line) incubation with pullulanase type M1. Pullulanase type M1 hydrolyzes α1→6 glycosidic linkages at the reducing side of an α1→4 glycosidic linkage. Pullulanase hydrolysis would thus demonstrate that 4,6-αGT-W catalyzes α1→4 elongation in addition to α1→6 elongation, its main activity. The comparison shows that some of the larger products, eluting after 15 min, are hydrolyzed yielding smaller compounds proofing that 4,6-αGT-W has some α1→4 elongating activity onto non-reducing end glucose moieties linked via α1→6 bonds.

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