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Supplemental Information to:

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

Hans Leemhuis, Willem P. Dijkman, Justyna M. Dobruchowska, Tjaard Pijning, Pieter Grijpstra, Slavko Kralj, Johannes P. Kamerling, Lubbert Dijkhuizen

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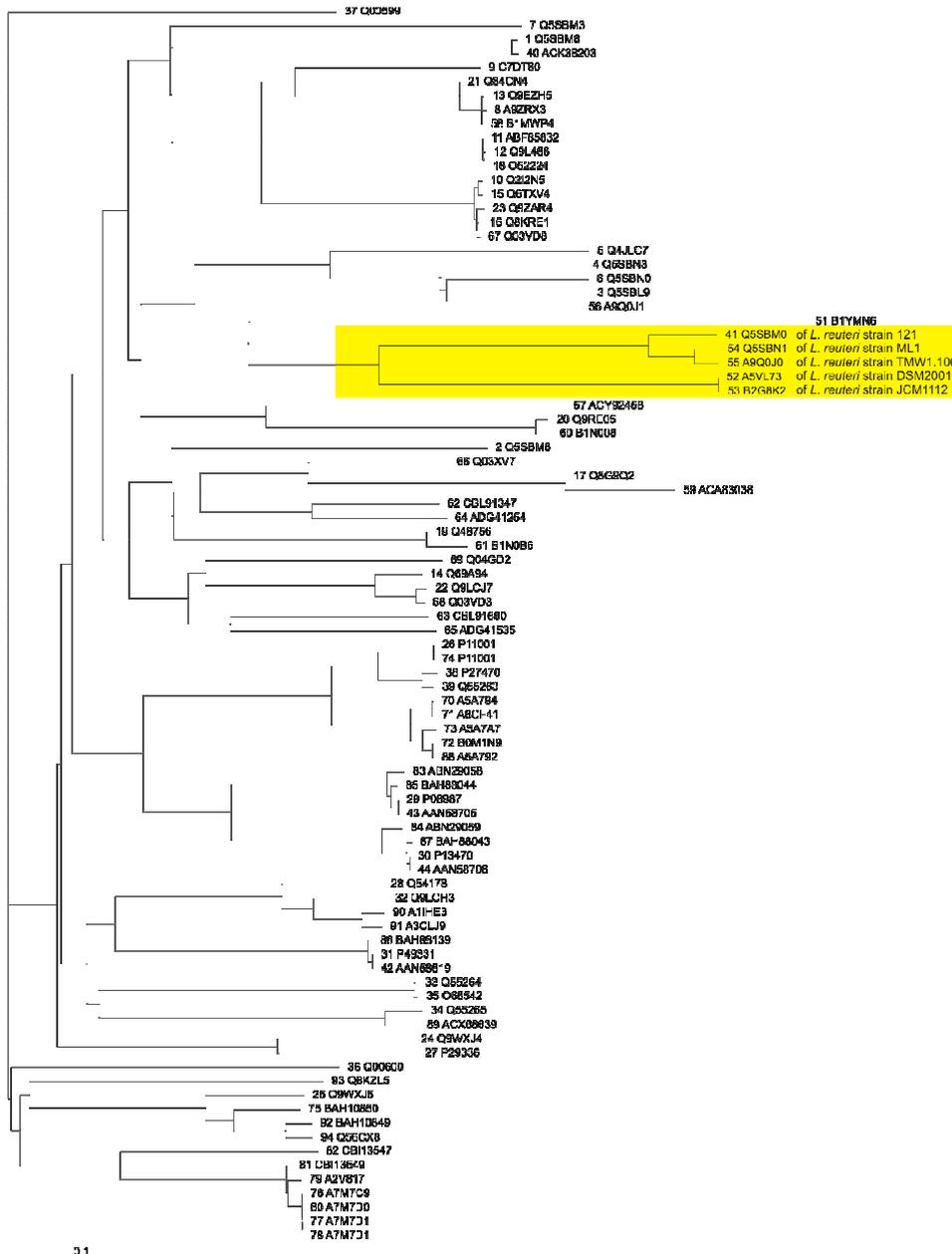


Fig. S1 Dendrogram of the GH70 protein sequences available via the Carbohydrate-Active Enzymes Database CAZy at <http://www.cazy.org> (Cantarel et al., 2009). The (putative) 4,6- α GT sequences form a cluster in the dendrogram and are highlighted with a yellow background. The proteins are indicated with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

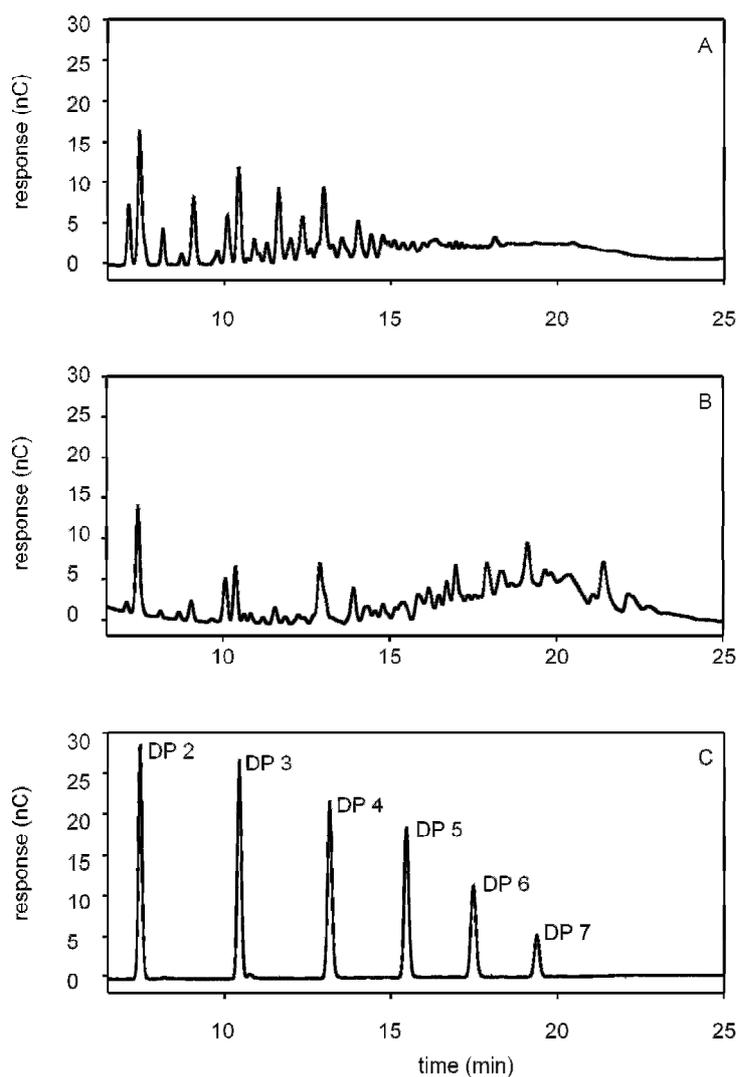


Fig. S2. HPAEC elution profiles of reaction mixtures obtained from maltoheptaose following incubation with (A) 4,6- α GT-W and (B) 4,6- α GT-ML4. Panel C shows the elution pattern of a (1 \rightarrow 4)- α -D-glucooligosaccharide standard from DP 2 to DP 7.

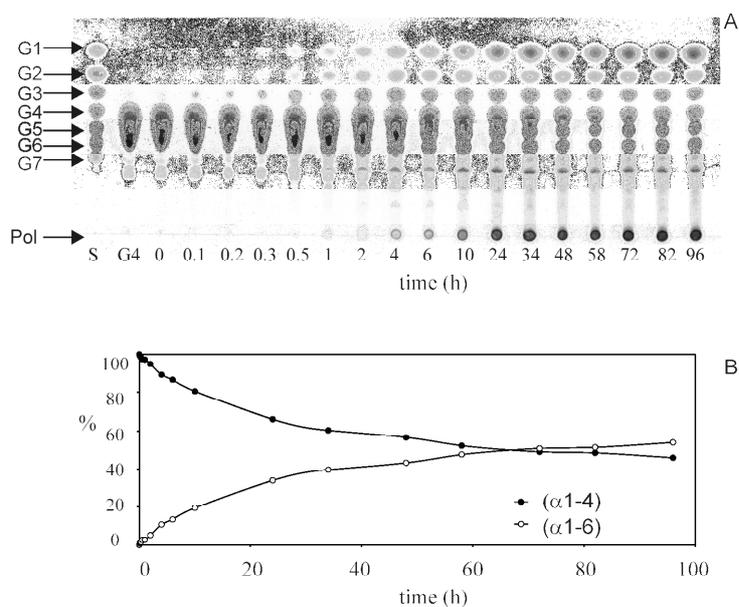


Fig. S3 Maltotetraose conversion by 4,6- α GT-W in time.

The progress of the reaction is followed in time by (A) TLC analysis and (B) ^1H NMR analysis (the percentages of the $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$ glycosidic linkages are indicated with filled and open circles, respectively). Reaction conditions: 90 mg maltotetraose; 13 μg 4,6- α GT-W enzyme; pH 4.7 and 37°C. At the end of the incubation the fraction of $\alpha 1 \rightarrow 6$ 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 $\alpha 1 \rightarrow 4$ and $\alpha 1 \rightarrow 6$ glycosidic bonds (^1H NMR spectra not shown).

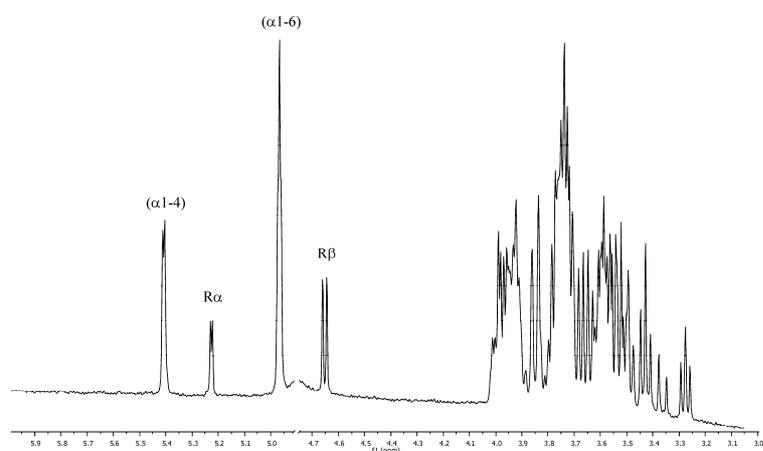


Fig. S4a ^1H NMR spectrum of HPAEC peak 1 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6- α GT-W. The spectrum is identical to the ^1H NMR spectrum of α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 4)-D-Glcp (Dobruchowska et al. 2012).

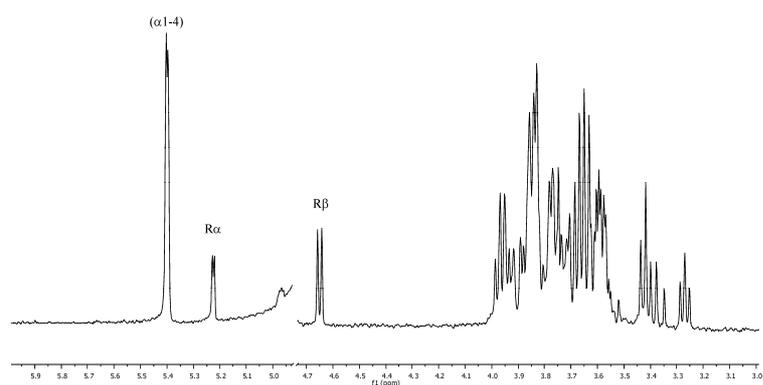


Fig. S4b ^1H NMR spectrum of HPAEC peak 2 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6- α GT-W. The spectrum is identical to the ^1H NMR spectrum of α -D-Glcp-(1 \rightarrow 4)- α -D-Glcp-(1 \rightarrow 4)-D-Glcp (maltotriose) (Dobruchowska et al. 2012).

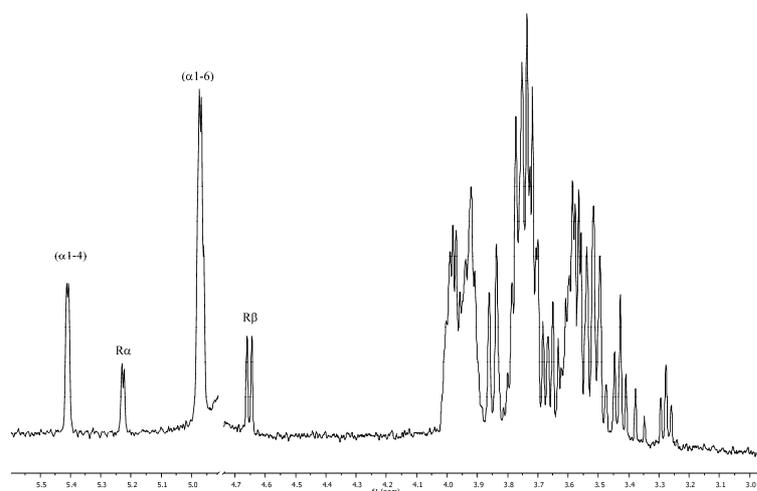


Fig. S4c ^1H NMR spectrum of HPAEC peak 3 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6- α GT-W. The spectrum is identical to the ^1H NMR spectrum of α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 4)-D-Glcp (Dobruchowska et al. 2012).

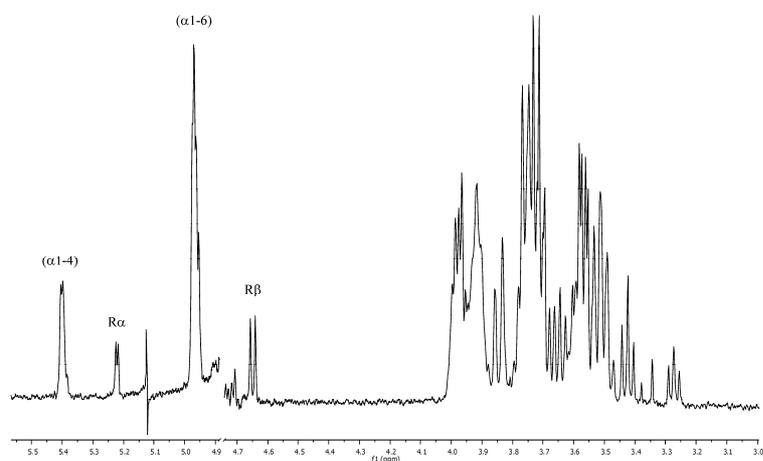


Fig. S4d ^1H NMR spectrum of HPAEC peak 4 (see Fig.4 in the manuscript), isolated from the product mixture generated from maltose by incubation with 4,6- α GT-W. The spectrum is identical to the ^1H NMR spectrum of α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 6)- α -D-Glcp-(1 \rightarrow 4)-D-Glcp (Dobruchowska et al., 2012).

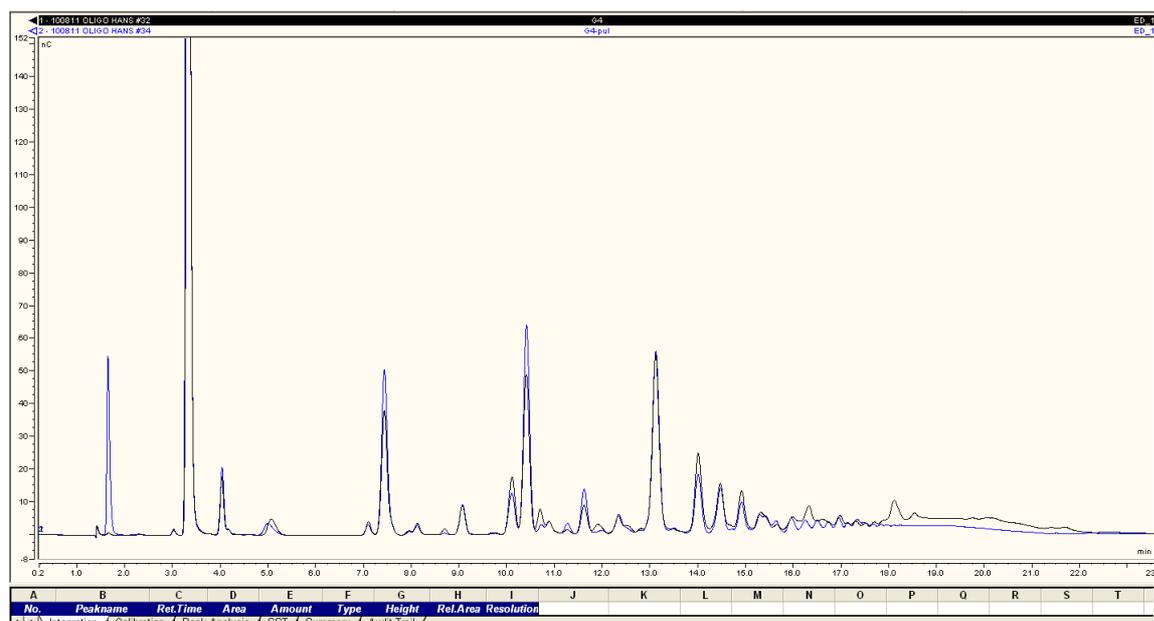


Fig. S5 HPAEC analysis of the α -glucan products of 4,6- α GT-W from maltotetraose (G4) (black line) and after (blue line) incubation with pullulanase type M1. Pullulanase type M1 hydrolyzes α 1 \rightarrow 6 glycosidic linkages at the reducing side of an α 1 \rightarrow 4 glycosidic linkage. Pullulanase hydrolysis would thus demonstrate that 4,6- α GT-W catalyzes α 1 \rightarrow 4 elongation in addition to α 1 \rightarrow 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- α GT-W has some α 1 \rightarrow 4 elongating activity onto non-reducing end glucose moieties linked via α 1 \rightarrow 6 bonds.

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