

## Enzymes with Novel Properties

### Description

## Enzymes with unexpected, novel properties

### $\beta$ -Glucanotransferase Glt20 from *Bacillus japonicus*



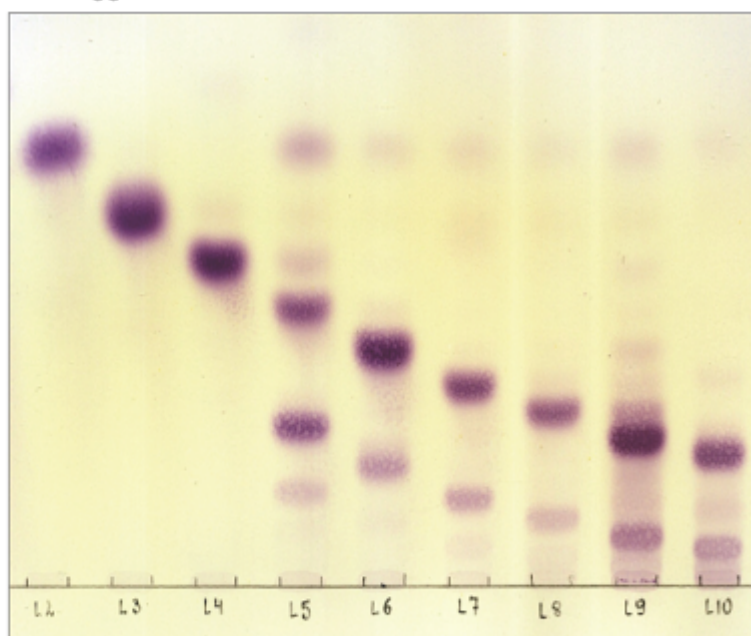
Gudmundur  
Hreggvidsson



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Incubation of ( $\beta$ 1 $\rightarrow$ 3)  
oligosaccharides, Lam  
with Glt20 at pH 6.5

TLC  
MALDI-TOF-MS  
1D/2D NMR

MALDI-TOF-MS [M+K]<sup>+</sup>

Lam-Glc<sub>5</sub>  $\rightarrow$  Pro-Glc<sub>8</sub>, Pro-

Lam-Glc<sub>6</sub>  $\rightarrow$  Pro-Glc<sub>10</sub>, Pro-

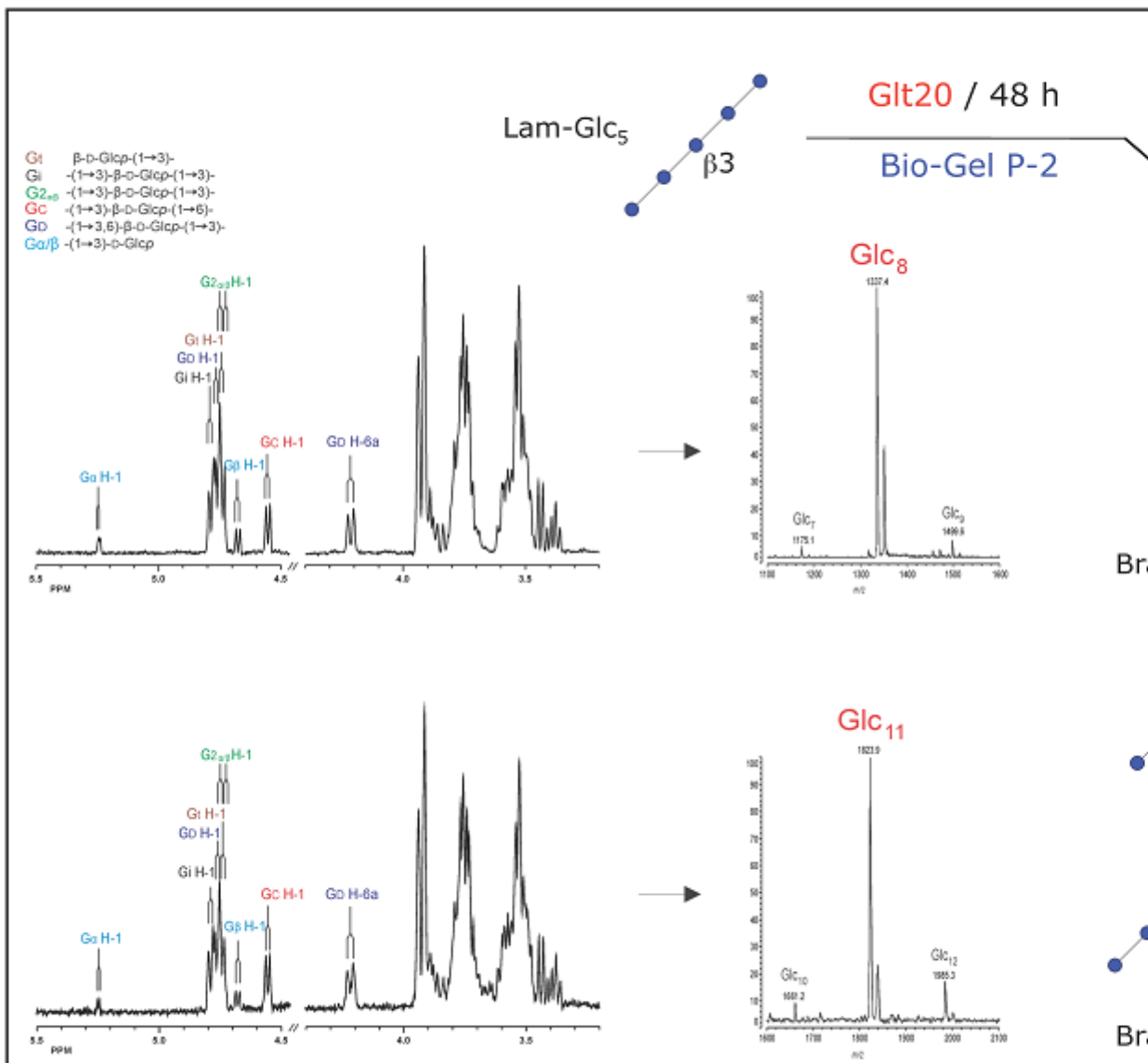
Lam-Glc<sub>7</sub>  $\rightarrow$  Pro-Glc<sub>12</sub>, Pro-

Lam-Glc<sub>8</sub>  $\rightarrow$  Pro-Glc<sub>14</sub>, Pro-

Lam-Glc<sub>9</sub>  $\rightarrow$  Pro-Glc<sub>16</sub>, Pro-

Lam-Glc<sub>10</sub>  $\rightarrow$  Pro-Glc<sub>18</sub>, Pro-

<sup>1</sup>H NMR analysis; <sup>1</sup>H NMR



## Bio-active (1→3,1→6)-β-D-glucans

The immunostimulating properties of (β1→3)-glucans with varying numbers of (β1→6) branches have been recognized by decades.

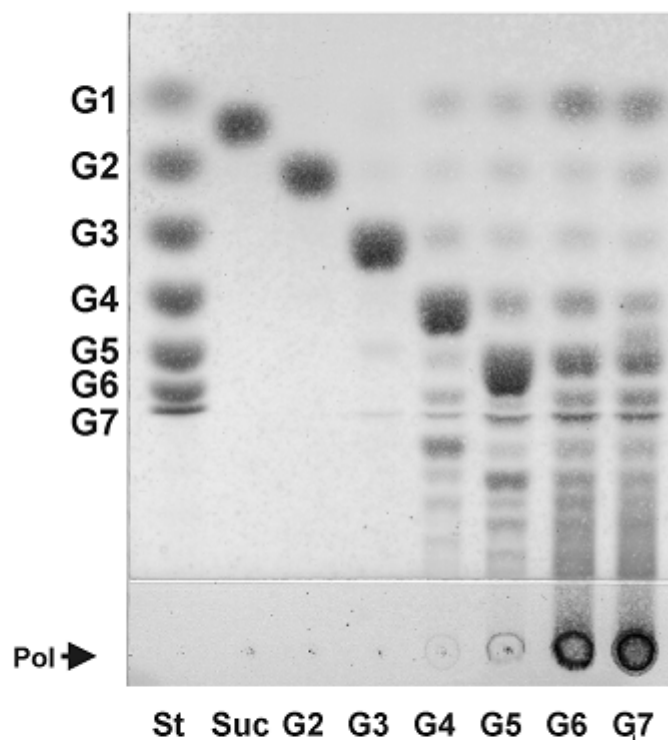
The wide of affinities appears to be due to the different sizes and numbers of branches in the (β1→3,β1→6)-glucans.

Using Glt20, linear (β1→3)-glucans can be converted into mixtures of (β1→3,β1→6)-glucans, with major amount of multiple branched structures.

Immunological evaluations of the product(s)(mixture) are underway.

## Enzymes with unexpected, novel properties

### 4,6- $\alpha$ -Glucanotransferase GTFB of *Lactobacillus reuteri*



Incubation of 90  
with 25 mM sucro  
mM malto-oligos

TLC analysis of th  
products.



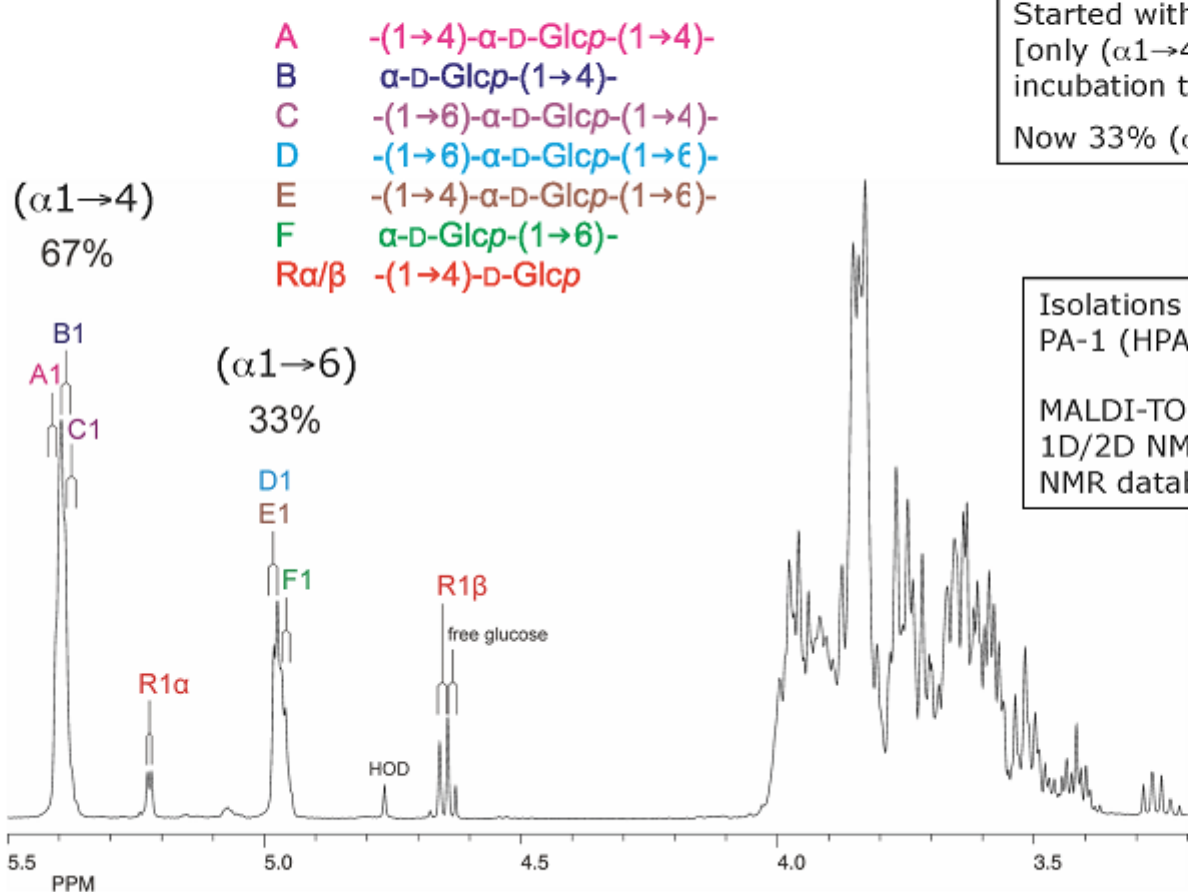
Lubbert Dijkhuizen

<sup>1</sup>H NMR analysis

Reaction conditions: 13 h, 37°C / 50 mM NaOAc buffer, pH 4.7 / 1 mM CaCl<sub>2</sub>

St = standards; Suc = sucrose; G1 = glucose; G2 = maltose; G3 = r  
G4 = maltotetraose; etc.

# $^1\text{H}$ NMR of total product mixture obtained from maltoheptaose (G7) and GTFB

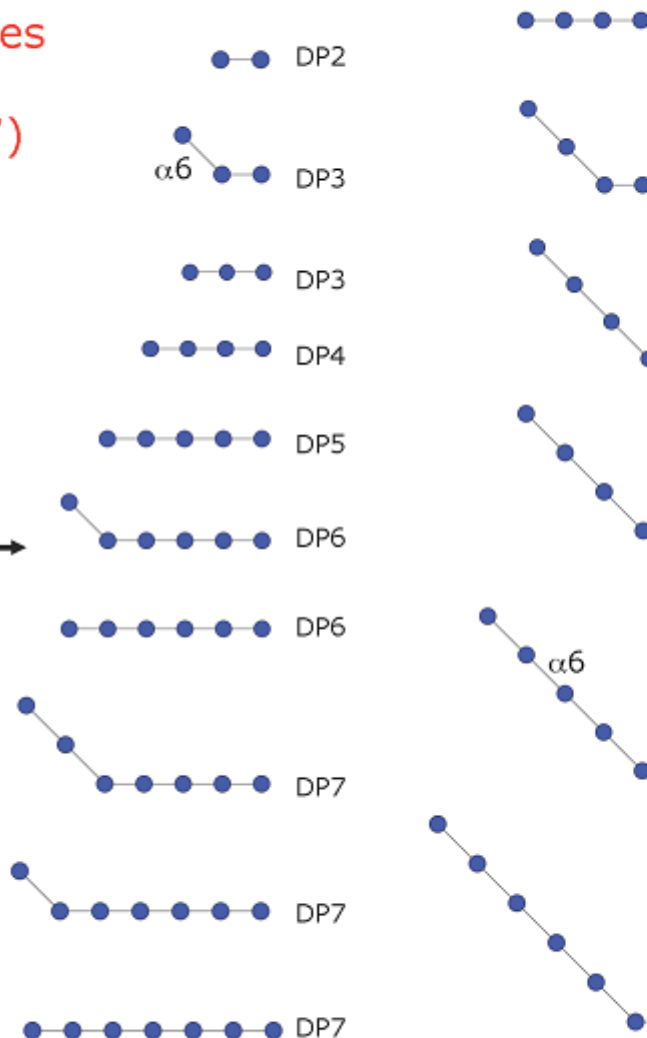


Lower glucan structures  
generated from  
maltoheptaose (DP7)

Oligosaccharide  
products formed  
range at any case  
from DP2 - DP35



GTFB



GTFB cleaves ( $\alpha 1 \rightarrow 4$ )  
linkages and elongates  
predominantly with  
( $\alpha 1 \rightarrow 6$ ) linkages; it can  
not cleave ( $\alpha 1 \rightarrow 6$ )  
linkages.

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## Structural characterization of linear isomalto-/malto-oligomer products synthesized by the novel GTFB 4 $\alpha$ -glucanotransferase enzyme from *Lactobacillus reuteri* 121

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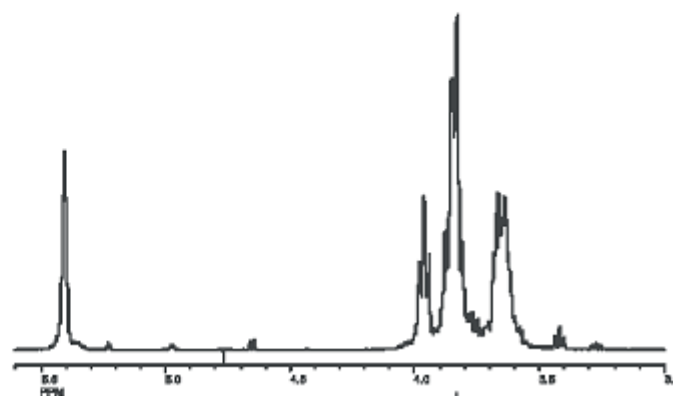
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emulsifying, sweetening, gelling or water-binding properties. LAB  $\alpha$ -glucansucrases (GSs)/glucosyltransferases (GTFs) are able to convert their natural substrate sucrose into exopolysaccharides (EPSs), being complex  $\alpha$ -D-glucose polymers. Many strains possess multiple GTF enzymes.

When searching for novel carbohydrate-degrading enzymes which may be used in industrial applications, several *gtf* genes (e.g. *gtfA*, *gtf180*, *gtf181*)



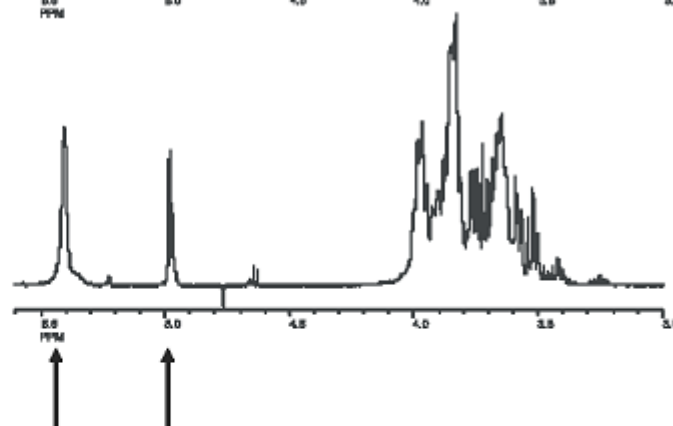
## GTFB modifies starch via a novel trans- $\alpha$ -gluc



$^1\text{H-NMR}$  spectrum of starch substrate



$^1\text{H-NMR}$  spectrum



$(\alpha 1 \rightarrow 4)$   $(\alpha 1 \rightarrow 6)$

GTFB creates  $(\alpha 1 \rightarrow 6)$  glyco

Potential food application

Soluble dietary fiber

### Category

1. News