

## Enzymes with Novel Properties

### Description

## Enzymes with unexpected, novel properties $\beta$ -Glucanotransferase Glt20 from *Bacillus japonicus*



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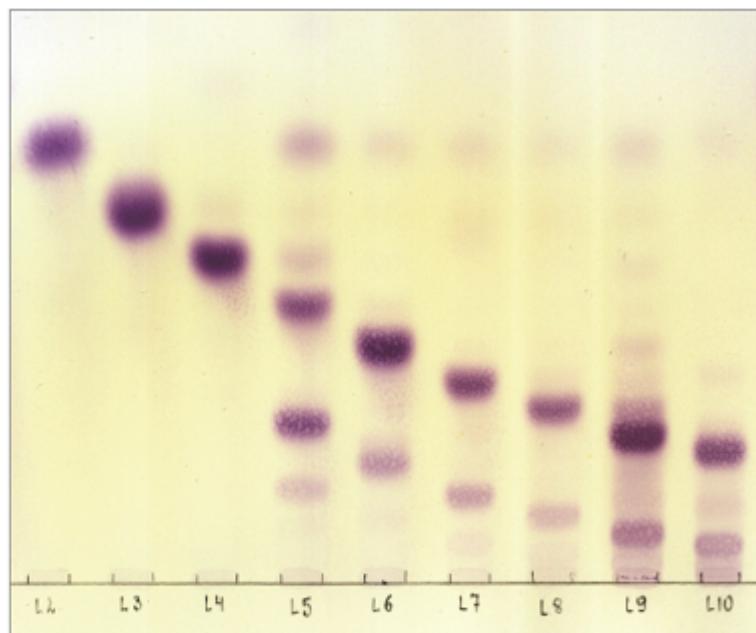
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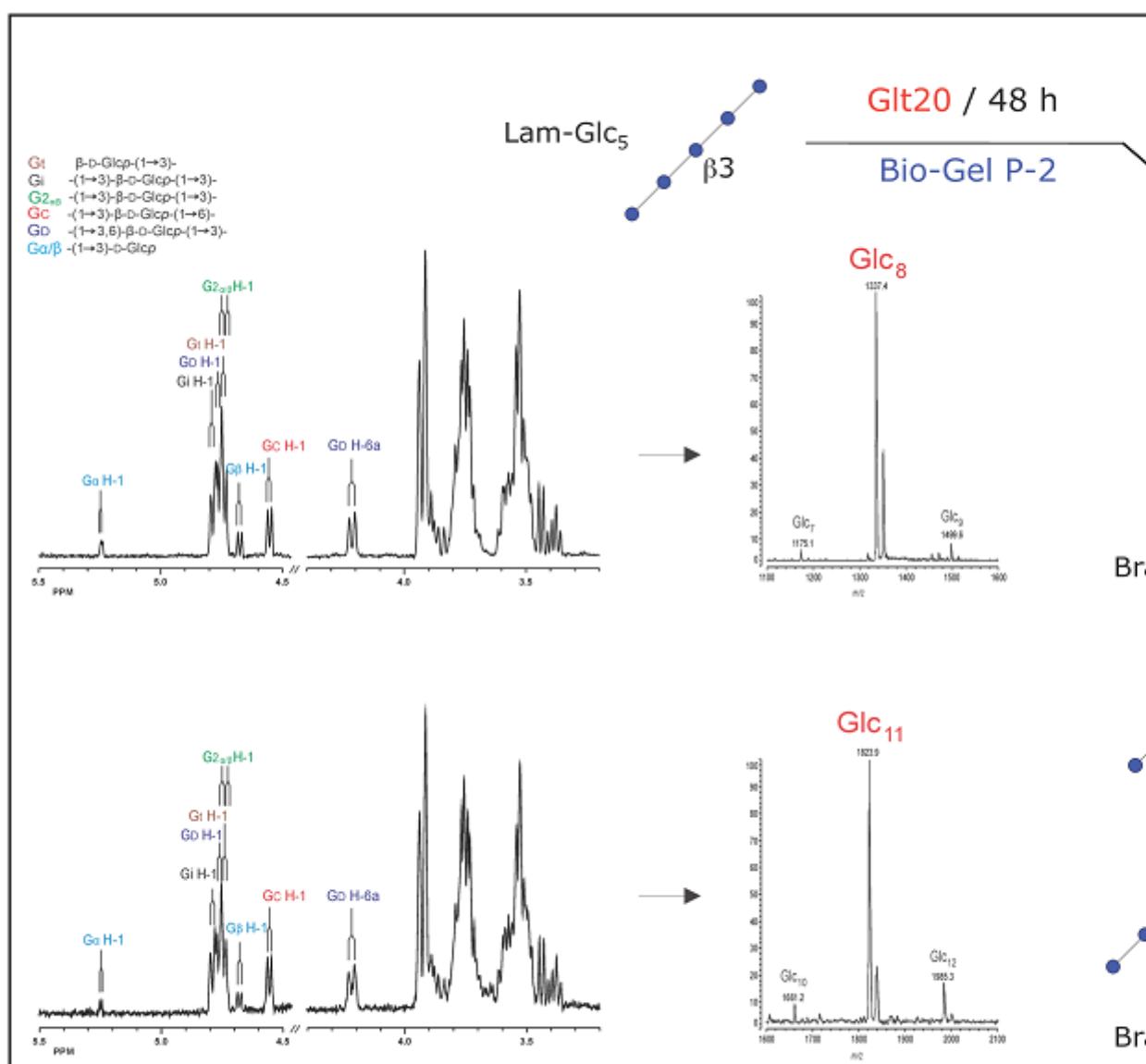
Incubation of ( $\beta$ 1 $\rightarrow$ 3)  
oligosaccharides, Lam  
with Glt20 at pH 6.5

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



MALDI-TOF-MS [M+K]<sup>+</sup>  
Lam-Glc<sub>5</sub> → Pro-Glc<sub>8</sub>, Pro  
Lam-Glc<sub>6</sub> → Pro-Glc<sub>10</sub>, Pro  
Lam-Glc<sub>7</sub> → Pro-Glc<sub>12</sub>, Pro  
Lam-Glc<sub>8</sub> → Pro-Glc<sub>14</sub>, Pro  
Lam-Glc<sub>9</sub> → Pro-Glc<sub>16</sub>, Pro  
Lam-Glc<sub>10</sub> → Pro-Glc<sub>18</sub>, Pro

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



## Bio-active (1 $\rightarrow$ 3,1 $\rightarrow$ 6)- $\beta$ -D-glucans

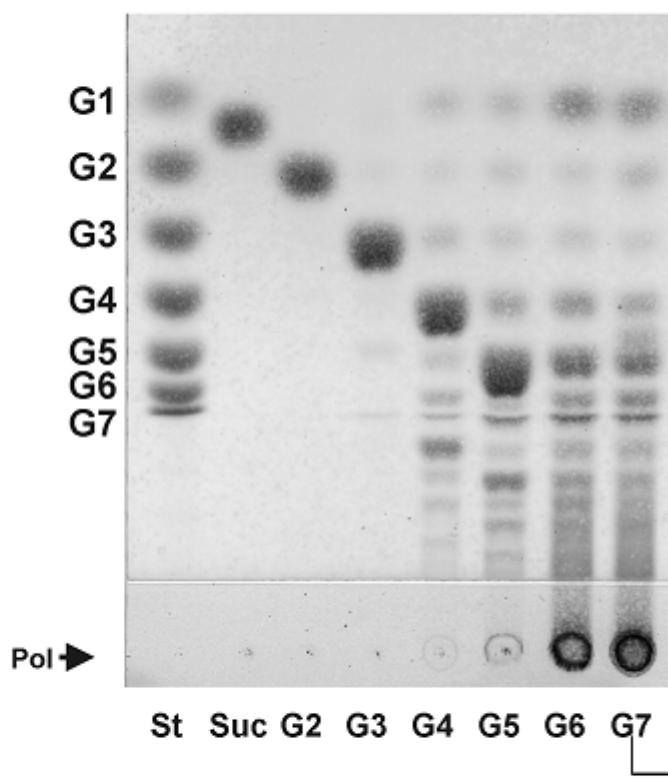
The immunostimulating properties of ( $\beta$ 1 $\rightarrow$ 3)-glucans with varying numbers of ( $\beta$ 1 $\rightarrow$ 6) branches have been recognized by decades.

The wide range of affinities appears to be due to the different sizes and numbers of branches in the ( $\beta$ 1 $\rightarrow$ 3, $\beta$ 1 $\rightarrow$ 6)-glucans.

Using Glt20, linear ( $\beta$ 1 $\rightarrow$ 3)-glucans can be converted to mixtures of ( $\beta$ 1 $\rightarrow$ 3, $\beta$ 1 $\rightarrow$ 6)-glucans, with major amounts of multiple branched structures.

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

## Enzymes with unexpected, novel properties 4,6- $\alpha$ -Glucanotransferase GTFB of *Lactobacillus reuteri*



Incubation of 90 min with 25 mM sucrose and 1 mM malto-oligosaccharides  
TLC analysis of the products.



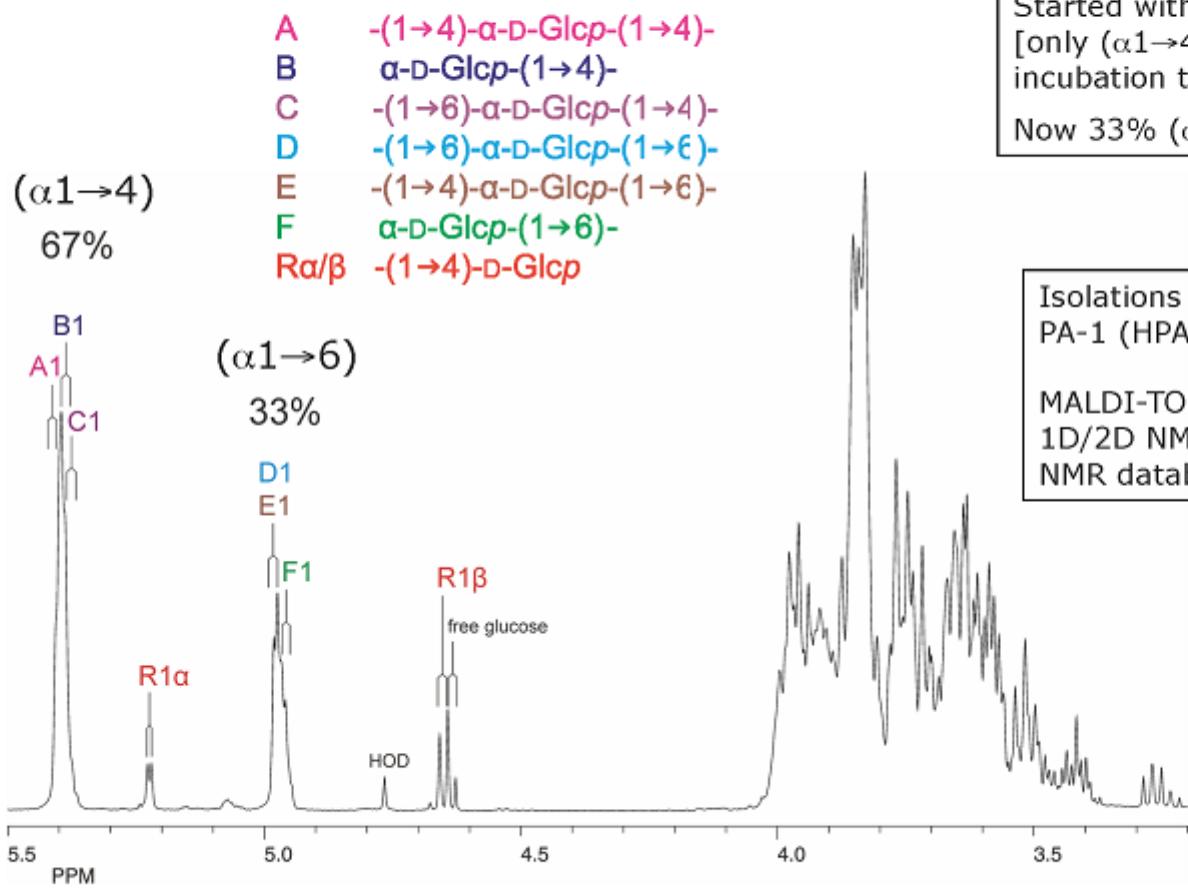
Lubbert Dijkhuizen

$^1\text{H}$  NMR analysis

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

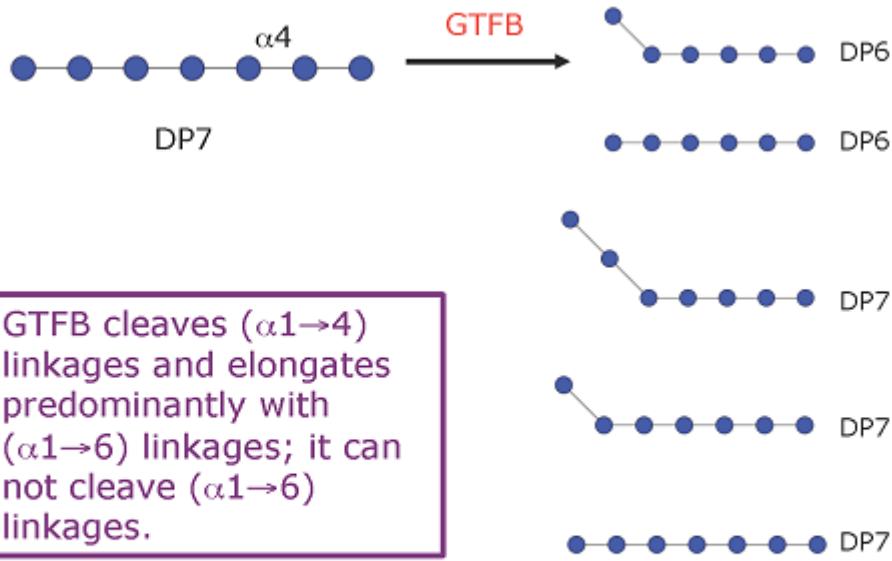
St = standards; Suc = sucrose; G1 = glucose; G2 = maltose; G3 = raffinose; G4 = maltotetraose; etc.

## <sup>1</sup>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



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

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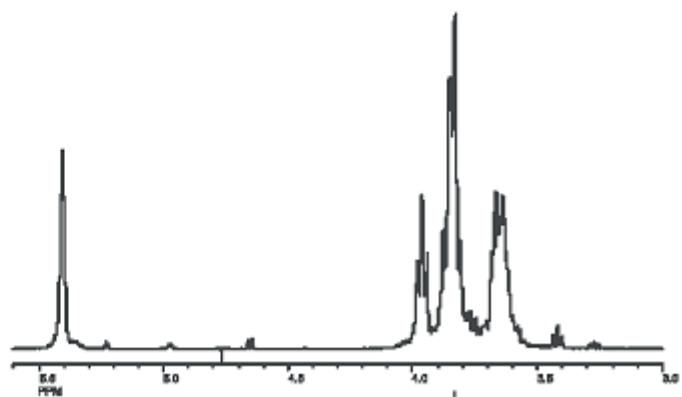
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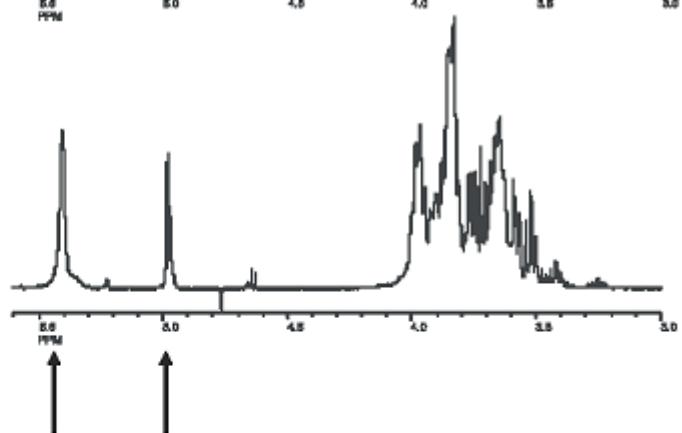
emulsifying, sweetening, gelling or water-binding properties, as well as their prebiotic properties. LAB contain multiple enzymes, such as glucansucrases (GSs)/glucosyltransferases (GTs) and α-D-glucosidases, which can convert their natural substrate sucrose into various oligosaccharides (OSs). EPSs, being complex α-D-glucose polymers, are produced by many LAB strains. These strains possess multiple GTF enzymes.

When searching for novel carbohydrate-active enzymes, it is important to take into account that some of them may be used in industrial applications. For example, the *L. reuteri* 121 strain contains a cluster of genes (*lre121\_g0001*) encoding a GTFB 4 $\alpha$ -glucanotransferase (GTFB).

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



<sup>1</sup>H-NMR spectrum of starch substrate



<sup>1</sup>H-NMR spectrum of GTFB-treated starch

( $\alpha$ 1 $\rightarrow$ 4)    ( $\alpha$ 1 $\rightarrow$ 6)    →    GTFB creates ( $\alpha$ 1 $\rightarrow$ 6) glycans

Potential food application



Soluble dietary fiber

### Category

1. News