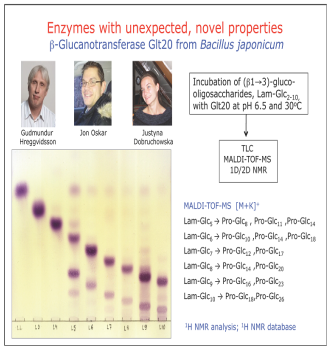



# Enzymes with Novel Properties

## Description

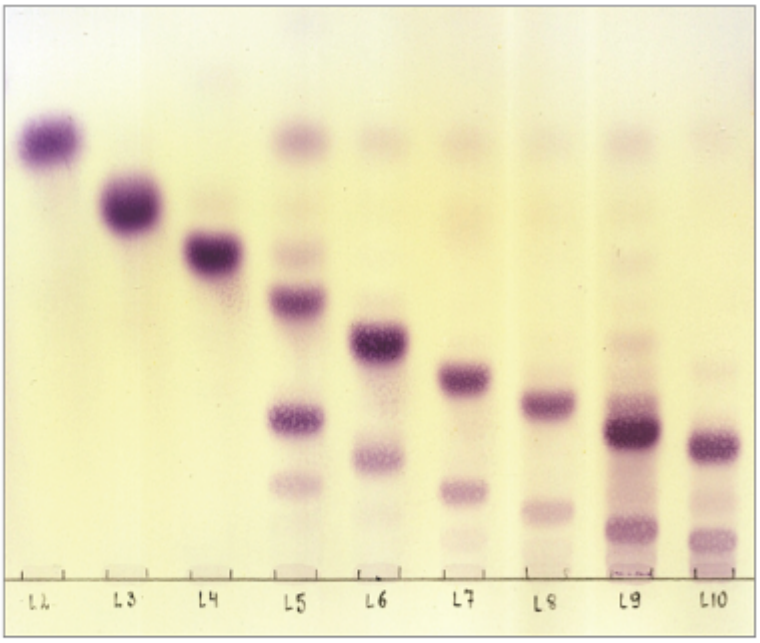
# Enzymes with unexpected, novel propert

## β-Glucanotransferase Glt20 from *Bacillus japoni*

Gudmundur Hreggvidsson

Jon Oskar

Justyna Dobruchowska




Incubation of (β1→3
oligosaccharides, La
with Glt20 at pH 6.5

↓

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

MALDI-TOF-MS [M+K]⁺

Lam-$Glc_5$ → Pro-$Glc_8$ , Pro

Lam-$Glc_6$ → Pro-$Glc_{10}$ ,Pro

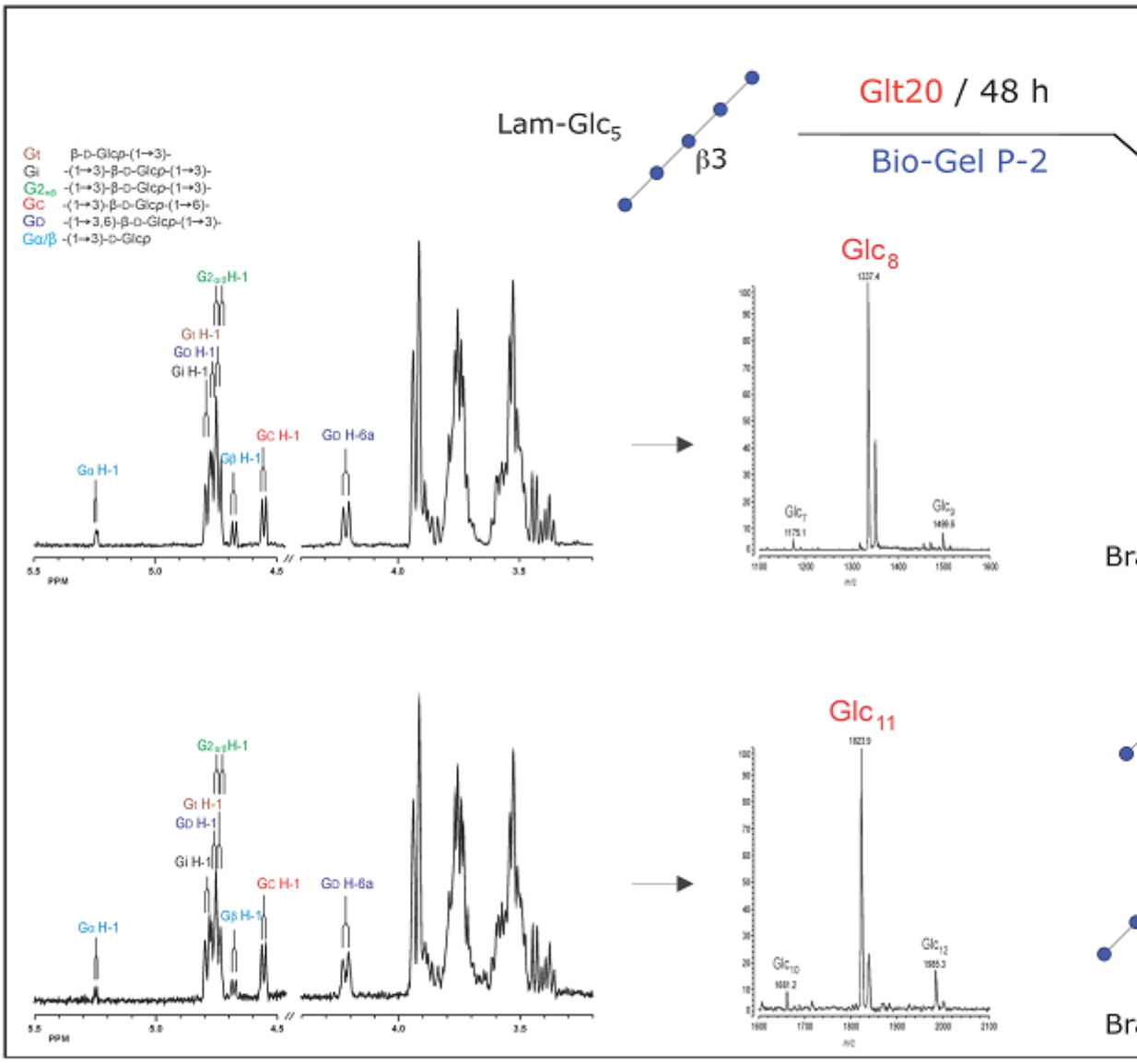
Lam-$Glc_7$ → Pro-$Glc_{12}$ ,Pro

Lam-$Glc_8$ → Pro-$Glc_{14}$ ,Pro

Lam-$Glc_9$ → Pro-$Glc_{16}$ ,Pro

Lam-$Glc_{10}$ → Pro-$Glc_{18}$,Pro

$^1$H NMR analysis; $^1$H NMR


Lam-Glc5
β3
Glt20 / 48 h
Bio-Gel P-2
Gt β-D-Glcp-(1→3)-
Gi -(1→3)-β-D-Glcp-(1→3)-
G2α/β -(1→3)-β-D-Glcp-(1→3)-
Gc -(1→3)-β-D-Glcp-(1→6)-
GD -(1→3,6)-β-D-Glcp-(1→3)-
Gα/β -(1→3)-D-Glcp
G2α/βH-1
Gt H-1
GD H-1
Gi H-1
Gc H-1
GD H-6a
Gβ H-1
Gα H-1
PPM
Glc8
Glc7
Glc9
Glc11
Glc10
Glc12

## Bio-active (1→3,1→6)-β-D-glucans

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

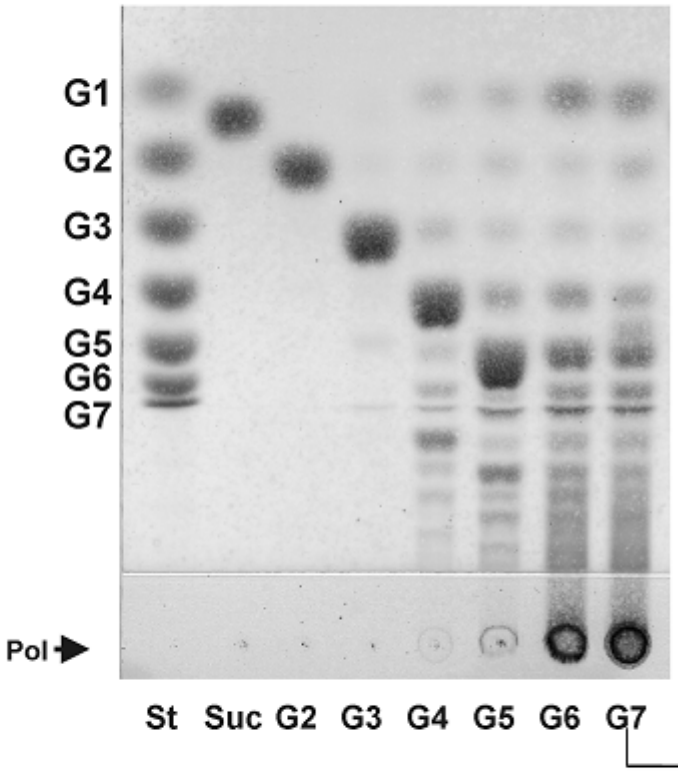
The wide of affinities appears to be due to the diffe
sizes and numbers of branches in the (β1→3,β1→6
glucans.

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

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

# Enzymes with unexpected, novel propert

## 4,6-α-Glucanotransferase GTFB of *Lactobacillus reuteri*




Incubation of 90
with 25 mM sucr
mM malto-oligosa

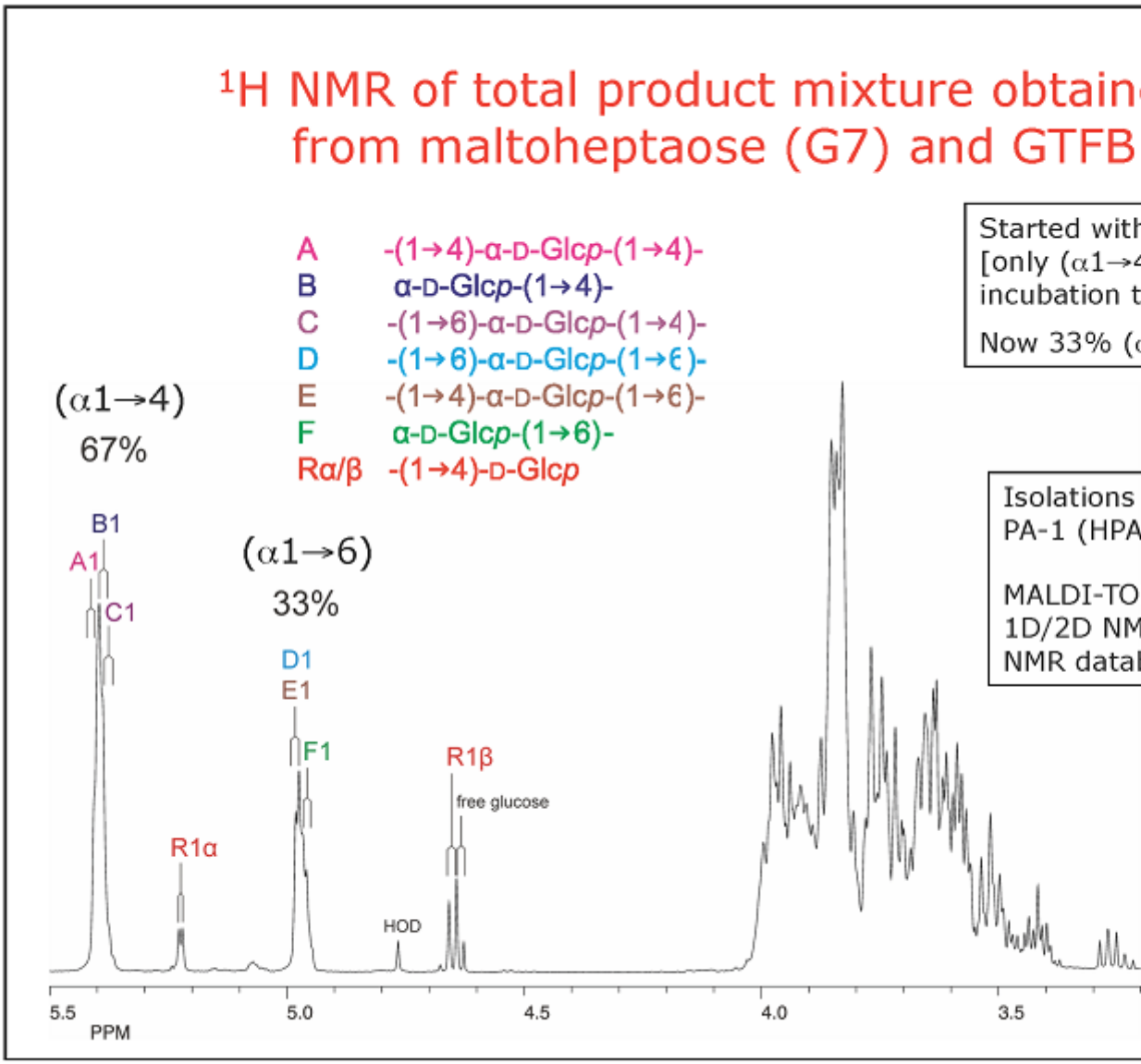
TLC analysis of th
products.

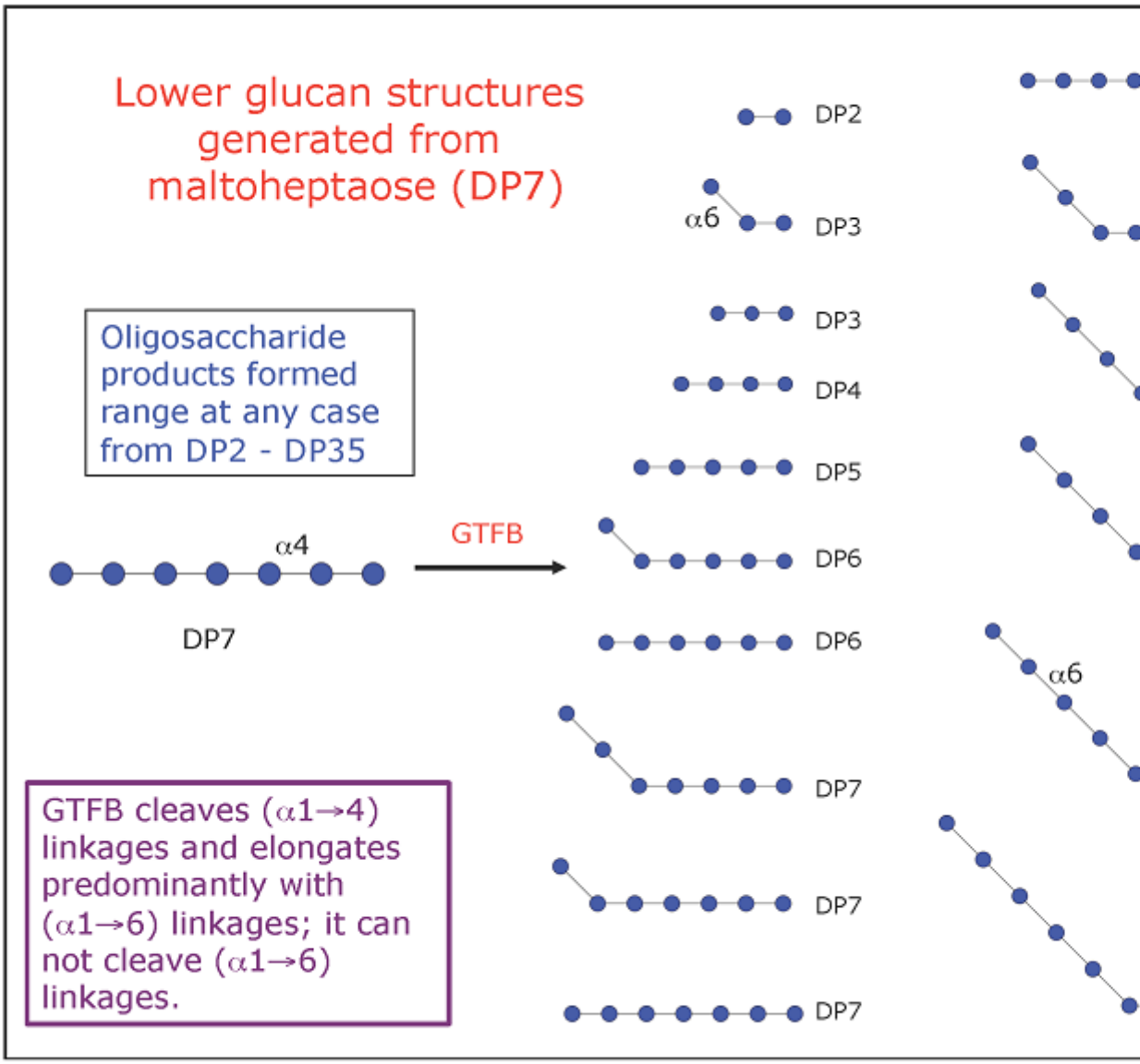
Lubbert Dijkhuizen

$^1$H NMR analysis

Reaction conditions: 13 h, 37°C / 50 mM NaOAc buffer, pH 4.7 / 1 mM $CaCl_2$

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


¹H NMR of total product mixture obtain
from maltoheptaose (G7) and GTFB
A -(1→4)-α-D-Glcp-(1→4)-
B α-D-Glcp-(1→4)-
C -(1→6)-α-D-Glcp-(1→4)-
D -(1→6)-α-D-Glcp-(1→6)-
E -(1→4)-α-D-Glcp-(1→6)-
F α-D-Glcp-(1→6)-
Rα/β -(1→4)-D-Glcp
Started with
[only (α1→4
incubation t
Now 33% (
(α1→4)
67%
(α1→6)
33%
Isolations
PA-1 (HPA
MALDI-TO
1D/2D NM
NMR datal
B1
A1
C1
D1
E1
F1
R1α
R1β
free glucose
HOD
5.5
PPM
5.0
4.5
4.0
3.5


Lower glucan structures generated from maltoheptaose (DP7)
Oligosaccharide products formed range at any case from DP2 - DP35
α4
DP7
GTFB
DP2
α6
DP3
DP3
DP4
DP5
DP6
DP6
α6
DP7
DP7
DP7
GTFB cleaves (α1→4) linkages and elongates predominantly with (α1→6) linkages; it can not cleave (α1→6) linkages.

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

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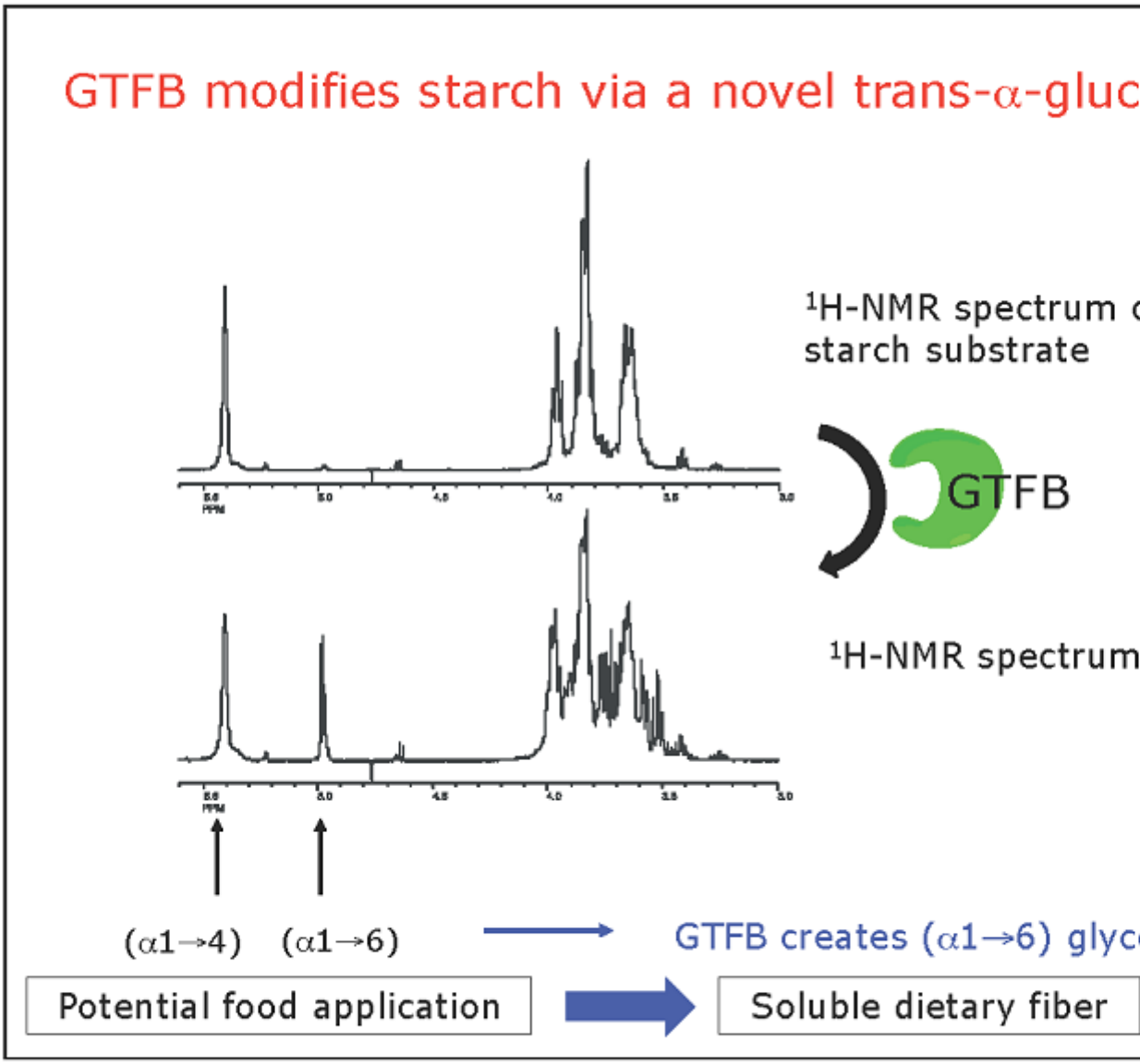
emulsifying, sweetening, gelling or wate
well as their prebiotic properties. LAB
glucansucrases (GSs)/glucosyltransferases
to convert their natural substrate sucrose
EPSs, being complex α-D-glucose poly
strains possess multiple GTF enzymes.

When searching for novel carbohydrate-
which may be used in industrial applicatio




**Category**

1. News