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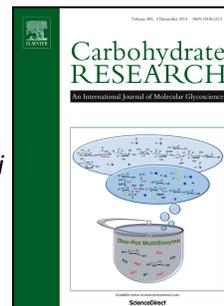
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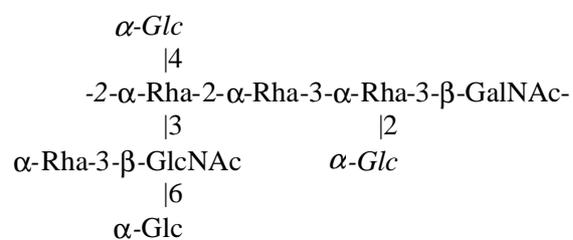
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Repeating unit of the *L. casei* BL23 cell wall polysaccharide.
 Components in italics are non-stoichiometrical.

ACCEPTED MANUSCRIPT

1 **Structural studies of the rhamnose-rich cell wall polysaccharide of *Lactobacillus casei* BL23**

2

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16

17

1 **Abstract**

2

3 *Lactobacillus casei* is a Gram positive lactic acid bacterium used in dairy fermentations
4 and present in the normal human gut microbiota. Certain strains are recognized as probiotics with
5 beneficial effects on human and animal health. *L. casei* BL23 is a potential probiotic strain
6 endowed with anti-inflammatory properties and a model strain widely used in genetic,
7 physiological and biochemical studies. A number of bacterial cell surface polysaccharides have
8 been shown to play a role in the immune modulation activities observed for probiotic lactic acid
9 bacteria. In the present work, we purified the most abundant carbohydrate polymer of *L. casei*
10 BL23 cell wall, a neutral wall polysaccharide (WPS) and established its chemical structure by
11 periodate oxidation, methylation analysis and 2D NMR spectroscopy. The WPS of *L. casei* BL23
12 was shown to contain α -Rha, α -Glc, β -GlcNAc and β -GalNAc forming a branched
13 heptasaccharide repeating unit (variant 1) with an additional partial substitution with α -Glc
14 (variant 2). A modified non-reducing end octasaccharide, corresponding to a terminal unit of the
15 WPS (variant 3), was also identified and allowed to define the biological repeating unit of the
16 WPS. To our knowledge, this is the first report of the identification of a biological repeating unit
17 based on a chemical evidence, in a cell wall polysaccharide of a Gram positive bacterial species.

18

19 **Keywords:** *Lactobacillus casei*; cell wall; polysaccharide; rhamnose; structure; NMR

20 spectroscopy

1 **1. Introduction**

2 *Lactobacillus casei* is a Gram positive lactic acid bacterium used in dairy fermentations. It
3 is commonly found in the normal gut and mouth microbiota of humans and animals. Certain *L.*
4 *casei* strains are typically categorized as probiotics, defined as live microorganisms which, when
5 administered in adequate amount, confer a health benefit on the host. *In vitro* studies and *in vivo*
6 studies in animal models have shown that certain *L. casei* strains have potential therapeutic
7 effects including anti-inflammatory, anti-allergic, anti-infectious or anti-cancer activities [1-3].
8 Also, clinical trials have shown that consumption of fermented milk containing probiotic *L. casei*
9 strains had a role in the prevention of various disorders such as infectious diarrhea, winter
10 infections in elderly subjects or abdominal dysfunction in healthy patients under stress [4, 5].
11 However, in most cases, the mode of action of these probiotic strains remains poorly understood.
12 Bacterial cell wall components constitute potential effectors of the immunomodulatory activity of
13 probiotics [6]. In *L. casei* strain YIT9018, antihypertensive and anti-infectious activities were
14 attributed to polysaccharide-glycopeptide or polysaccharide-peptidoglycan (PS-PG) complexes,
15 respectively [7, 8]. Also, immunomodulating properties were reported for the cell wall
16 polysaccharide (WPS) of *L. casei* strain Shirota (YIT 9029) [9].

17 WPS are crucial components of the Gram positive bacteria cell wall. Because of their
18 localization at the bacterial surface, they are mediators of bacterial interactions with the
19 environment such as host cells, abiotic surfaces or infecting bacteriophages [6]. Early studies
20 attempted to correlate the serological grouping of *L. casei* and the composition of the
21 polysaccharide components of their cell wall preparations [10]. However, despite the biological
22 importance of the WPS polymers of *L. casei*, only limited knowledge is available regarding their
23 chemical structure. Detailed structural investigations were carried out for the PS-PG complex of
24 strain Shirota but only a tentative structure has been proposed [8]. Recently, the structures of two
25 TCA-extracted polysaccharides from *L. casei* LOCK 0919 were established [11] and appeared to
26 be different from the tentative structure proposed for Shirota strain. Moreover, previous
27 comparison of the cell surface glycomes of 16 different *L. casei* strains with a lectin microarray
28 profiling revealed a large diversity of the cell surface carbohydrate structures between *L. casei*
29 strains [12].

30 *L. casei* BL23 is a potential probiotic strain which displays protective anti-inflammatory
31 effects in mice [1, 13], and is widely used as model strain in genetic, biochemical and

1 physiological studies. Its genome has been completely sequenced [14]. Except lipoteichoic acids
2 (LTA) [15], the cell wall carbohydrates of this strain have not been studied until now.

3 In this work, we fully characterized the chemical structure of the major WPS of *L. casei*
4 BL23 and showed that it is a complex rhamnose-containing polysaccharide essentially composed
5 of heptasaccharide repeating units (variant 1) with an additional partial substitution with α -Glc
6 (variant 2). We also identified an octasaccharide with an additional α -Glc at the non-reducing end
7 (variant 3), which corresponds, most probably, to the terminal unit of the WPS.

2. Results and Discussion

2.1. Extraction and preliminary analysis of the WPS

L. casei BL23 cells were pre-extracted with aqueous butanol in order to remove the LTA. Successive extractions with TCA and hot diluted HCl (section 3.1) afforded polysaccharide preparations with identical monosaccharide composition, containing Rha, Glc, GlcN and GalN in an approximate molar ratio of 2.6 : 1.5 : 1 : 1. Methylation analysis showed the presence of seven major components: terminal Rha, terminal Glc, 2-, 3-, and 2, 3-linked Rha, 3,6-linked GlcNAc and 3-linked GalNAc, in approximate ratio 1 : 2.5 : 1.2 : 0.3 : 0.8 : 0.6 : 0.5, indicating a heterogeneous and branched structure. The absolute configuration of the monosaccharides was determined by GC/MS analysis of acetylated 2-octyl glycoside derivatives, which indicated the presence of L-Rha, D-Glc, D-GlcNAc and D-GalNAc.

The WPS was purified on an anion-exchange column, where it was eluted in the neutral fraction. All acidic fractions contained proteins, nucleic acids and some minor polysaccharides, and were not investigated further.

¹H NMR spectrum of the WPS (Fig. 1) indicated the presence of *N*-acetyl aminogroups and several deoxysugars. It showed that the polymer was heterogeneous; anomeric signals at 4.7-5.4 ppm were of different intensity within one sample, probably due to the presence of structural variants and sugars with limited mobility. Thus it was not possible to identify any regular repeating unit. 2D NMR spectra (Fig. 2) contained many spin systems of the monosaccharides, most of them belonging to α -Rhap. Several variants of α -Glc_p, β -Glc_pNAc, and β -Gal_pNAc were identified. In order to obtain an oligosaccharide suitable for NMR analysis, WPS was subjected to Smith degradation.

2.2. Smith degradation of the WPS and structural analysis of OS1

Periodate oxidation of the polysaccharide followed by reduction with NaBD₄ and mild acid hydrolysis (Section 3.2) afforded an oligosaccharide OS1, isolated by gel chromatography on a Bio-Gel P2 column. It contained Rha, GlcNAc and GalNAc in an approximate molar ratio 0.8:1:1. Its methylation analysis showed the presence of terminal Rha, 2,3-linked Rha, terminal GlcNAc and 3-linked GalNAc, in an approximate ratio 0.8 : 1 : 1.4: 1.2. The preparation looked heterogeneous on NMR spectra and was further fractionated by reverse-phase HPLC, giving three main compounds. NMR analysis showed that they all had the same structure, differing only by the reducing-end oxidized fragment of the monosaccharide. One (OS1) had glyceraldehyde at

1 the reducing end, two others contained unsaturated fragments. Variants with non-standard
2 components at the reducing end, which are not expected products of normal oxidation and
3 hydrolysis, provided no important information and will not be further discussed.

4 The structure of the OS1 was elucidated by homo- and heteronuclear 2D NMR
5 spectroscopy. 2D gCOSY, TOCSY, ROESY, ^1H - ^{13}C HSQC and ^1H - ^{13}C HMBC spectra were
6 recorded. Spectra contained sets of signals belonging to $[3\text{-}^2\text{H}_1]$ glyceraldehyde and 4
7 hexopyranoses, identified as two α -Rha, β -GlcNAc, and β -GalNAc. The sequence of the
8 monosaccharides was determined using interresidual NOE: B1:A2, C1:D3, D1:B2, and E1:B3
9 (Table 1).

10 2-Substituted $[3\text{-}^2\text{H}_1]$ glyceraldehyde at the reducing end of the tetrasaccharide OS1 must
11 be derived from 2-substituted Rha, which leads to the structure of the OS1 oligosaccharide shown
12 in Fig. 3.

14 2.3. Structural analysis of the WPS

15 Analysis of 2D NMR spectra of the purified WPS suggested that the polysaccharide
16 contained three main variants of the repeating units (Fig. 3), although it cannot be excluded that
17 there are more variants of smaller abundance. To start the assignment of spectra of the WPS, we
18 selected well visible β -GalNAc D and D' spin systems, which showed intense NOE correlations
19 D1:B1,2, D'1:B'1,2, C1:D3, and C'1:D'3 (Fig. 2). Starting from C-3D-2B trisaccharides it was
20 possible to identify variants 1 and 2 (Fig. 2). Compared to the OS1, the WPS additionally
21 contained α -Glc G at O-6 of β -GlcNAc E, α -Rha K at O-3 of β -GlcNAc E, and non-
22 stoichiometric α -Glc F at O-2 of the α -Rha C'. The chain was connected by the linkage of α -Rha
23 A to O-3 of the α -Rha C. Thus, periodate oxidation destroys Rha A of the backbone chain and
24 removes terminal monosaccharides K, G and F. The following NOE connectivities were
25 observed: A1:C3; A1:B5; A'1:C'3; A'1:B'5; B1:A1,2; B'1:A'1,2; B''1:A''1,2; C1:D3; C'1:D'3;
26 D1:B1,2; D'1:B'1,2; E1:B3; E'1:B'3; F1:C'1,2; G1:E6; K1:E3; K'1:E'3; K''1E''3; J1:B''4 (Fig. 2),
27 leading to the presented structures (Fig. 3). They agreed with the observed transglycoside HMBC
28 correlations (Supplementary data).

29 Certain ^1H or ^{13}C signals, belonging to the side-chain terminal monosaccharides, were of
30 high intensity (like α -Rha K) whereas other monosaccharides showed signals of unexpectedly
31 low intensity relative to their actual content, probably due to their limited mobility.

1 All assignments were quite straightforward, but there were additional signals of α -Rha K" and B", and α -Glc J. Rha K" was linked to O-3 of monosaccharide E", which showed poorly visible signals. E" is proposed to be β -GlcNAc for consistency with the other variants, although it had H-3,4,5 signals overlapped and thus its identification is only tentative. It definitely had an amino group at C-2, which resonates at 56.8 ppm. Other signals of E" were also found in the HSQC spectrum at the expected locations and confirmed substitution at O-3 rather than O-4 (Table 2, Supplementary data). GlcNAc E" showed weak NOEs to H-3 (most intense, but still low and not visible on Fig. 2), H-2 and H-4 of the α -Rha B". α -Rha B" was not substituted at O-2 (C-2 at 70.1 ppm), and substituted by α -Glc J at O-4 (by NOE data only as no HMBC correlations were observed for variant 3 monosaccharides). All these data taken together allowed us to propose three variants of a branched repeating unit of the WPS of *L. casei* BL23 (Fig. 3).

12 The results of methylation analysis of the WPS preparations (Section 2.1) were in agreement with the established structures.

14 It is interesting to point out that variant 3 has a structure similar to variant 1 but with an additional terminal α -Glc J at the non-reducing end. Therefore, we propose that, for *L. casei* BL23 WPS, variant 3 represents the terminal non-reducing unit of the polymeric chain. Also, from the structure of variant 3 with the terminal α -Glc J at the non-reducing end, we could deduce the actual biological repeating unit (variants 1 and 2) that is the properly ordered oligosaccharide, which, after having been most probably preassembled on an undecaprenyl-phosphate carrier, is polymerized into the WPS [16, 17].

21 Moreover, it is tempting to speculate that the biosynthesis of the polysaccharide is terminated by the addition of α -Glc J. Thus, the variant 3 would correspond to a glycoform of the repeating unit which cannot accept the addition of further repeating units, similar to two isomeric glycoforms of the core oligosaccharide in the lipopolysaccharide of *Pseudomonas aeruginosa*, only one of which can accept the addition of the O-antigen [17]. To our knowledge, such a phenomenon in polysaccharides of Gram positive bacteria has not been reported to date.

27 In conclusion, we report in this work a first detailed chemical structure of the WPS of *L. casei* BL23. Most previous investigations of *L. casei* carbohydrates, as for the majority of lactic acid bacteria, were focused on exopolysaccharides (EPS) [18-20], which are released in the surrounding medium and confer texture to fermented milk products manufactured with the producing strains. The chemical nature of these EPS was only partially characterized.

1 A structure was tentatively established by chemical methods for the PS-PG complexes of
2 the probiotic strain *L. casei* YIT9018 [7, 8]. Interestingly, the probable structure suggested for
3 PS-PG2 contains certain motives of the structure established in this study for *L. casei* BL23: - α -
4 Rha-3-(α -Glc-6) β -GlcNAc- [K-(G)E-] or -3- α -Rha-3- β -GalNAc-2- α -Rha- [-C-D-B-] ([8] and
5 Fig. 3). However, the proposed structure for the entire repeating unit differs from the structure
6 established in this work for *L. casei* BL23 WPS. Also, the structures recently established for two
7 polysaccharides isolated from *L. casei* strain LOCK019 with probiotic properties, differ
8 significantly from the BL23 WPS, one being devoid of Rha and the other containing only one
9 Rha residue in its hexasaccharide repeating unit [11]. These results are in agreement with the
10 diversity of WPS structures predicted among *L. casei* strains by lectin profiling of the cell surface
11 [12].

12 Knowing the *L. casei* WPS structure will help further evaluation of the mechanisms
13 involved in the immunomodulatory properties of *L. casei* BL23. Furthermore, previous studies
14 indicate that certain bacteriophages infecting *L. casei* strains recognize saccharide-containing
15 receptors at the bacterial surface during the adsorption step of phage infection [21, 22]. The
16 structure established here provides valuable information to understand the molecular interactions
17 between phages and the target bacterial surface.

3. Experimental

3.1. Bacteria growth and extraction of WPS

L. casei BL23 was grown in Man, Rogosa, Sharpe (MRS) broth at 37 °C for 24 h in closed bottles without shaking. Cells were collected by centrifugation and washed 3× with water (approx. 9 g cell pellet /liter of culture). Cells (20 g) were suspended in water (100 ml). 1-Butanol (100 ml) was added and the suspension stirred for 40 min at room temperature. The suspension was then transferred into teflon centrifuge tubes and centrifuged (10 000 × g, 10 min). After extraction with aqueous butanol, the cell pellet was washed with water, suspended in 5 % TCA, stirred at 5 °C for 48 h and centrifuged (12 000 × g, 10 min). Supernatant was dialyzed and lyophilized, to give crude TCA extract (extract 1, 600 mg). Cell debris were resuspended in 0.01N HCl and heated in a water bath at 100 °C with intensive stirring for 20 min. The mixture was cooled and centrifuged, supernatant deproteinated by addition of TCA (5 %), dialyzed and lyophilized to give crude 0.01 HCl extract (extract 2, 400 mg), and the cell debris were finally treated in the same manner with 0.1 N HCl to afford crude 0.1 N HCl extract (extract 3, 300 mg). Extracts 1, 2 and 3 were first fractionated on G-50 to give high molecular weight (HMW) and low molecular weight (LMW) fractions. Judging on the monosaccharide composition and behavior on an anion-exchange Q-Sepharose column, LMW fractions contained fragments of nucleic acids and were not investigated further. All HMW fractions had identical monosaccharide compositions, profiles of methylation analysis and ¹H-NMR spectra, and were designated as WPS.

3.2. Smith degradation of the WPS

The WPS was subjected to Smith degradation by standard methods [23], essentially as described earlier [24]. Briefly, WPS (100 mg) was dissolved in 35 mL of 0.05 M NaIO₄ and kept for 72 h in the dark at 20 °C. To stop the reaction, 1,2-ethanediol (1 mL) was added, the solution was dialyzed and the resulting product reduced with NaBD₄ overnight. The excess of NaBD₄ was destroyed with AcOH, the solution was dialyzed and concentrated. The material was partially hydrolyzed by AcOH (2%) at 100 °C for 2 h. The mixture was cooled, concentrated, and fractionated on a Bio Gel P-2 column. Oligosaccharide fractions were further separated by reverse-phase HPLC.

3.3. General and analytical methods

3.3.1 Chromatographic methods

1 Gel-permeation chromatography was performed on Sephadex G-50 (GE Health Care,
2 2.6 x 1000 cm and 1 x 40 cm) and BioGel P-2 (Biorad, 2.6 x 80 cm) columns, eluted with 0.1
3 % AcOH. Fractions were assayed for total [25] and aminosugars [26].

4 Anion exchange chromatography was done on Hitrap Q column (5 mL size, GE-
5 Healthcare) with UV monitoring at 220 nm in a linear gradient of NaCl (10 min water, then
6 gradient to 1 M NaCl over 1 h, 3 mL/min). Fractions of 1 min were collected and tested for
7 the presence of eluted compounds by spotting on SiO₂ TLC plate, dipping in 5% H₂SO₄ in
8 EtOH and heating with heat-gun. All fractions of interest were dried in Savant drying
9 centrifuge and ¹H NMR spectra were recorded for each fraction without desalting. For 2D
10 NMR desalting was performed on Sephadex G15 column.

11 HPLC was carried out on a Phenomenex ONYX C18 column (150 x 4.6 mm), eluted
12 at 1 mL/min with water-MeOH gradient (0-50% in 30 min) with an UV detector at 220 nm.

14 3.3.2 Monosaccharide and methylation analysis

15 Monosaccharide and methylation analysis were performed as described previously
16 [24], by the Ciucanu & Kerek method [27], as modified by Read *et al.* [28]. Methylated
17 derivatives were identified using the Complex Carbohydrate Research Center partially
18 methylated alditol acetates (PMAA) database
19 (www.ccruc.uga.edu/specdb/ms/pmaa/pframe.html), and by comparison with the authentic
20 standards of methylation analysis of polysaccharides of various *L. lactis* strains [29, 30].

22 3.3.3 Determination of absolute configuration

23 A WPS sample (0.5 mg) was hydrolyzed with TFA as for monosaccharide analysis.
24 Acid was evaporated, and (R)-2-octanol (0.2 mL) and acetyl chloride (0.02 mL) were added at
25 room temperature to the dry sample. The reaction mixture was heated at 100 °C for 2 h, dried
26 with toluene by a stream of nitrogen, acetylated, and analyzed by GC-MS as described [31].
27 Standards were prepared from L-Rha, D-Glc, D-GlcNAc and D-GalNAc with (R)- and (R, S)-
28 2-octanol .

30 3.3.4 NMR spectroscopy

31 NMR experiments were carried out on a Varian INOVA 500 MHz (¹H) spectrometer with
32 3 mm Z-gradient probe at 25 °C with acetone internal reference (2.225 ppm for ¹H and 31.45
33 ppm for ¹³C) using standard pulse sequences gCOSY, TOCSY (mixing time 120 ms), NOESY
34 (for polysaccharide) and ROESY (for oligosaccharide) (mixing time 500 ms), HSQC and

1 HMBC (100 ms long range transfer delay). Acquisition time (AQ) was kept at 0.8-1 s for H-H
2 correlations and 0.25 s for HSQC, 256 increments was acquired for t1. The spectra were
3 processed and analyzed using the Bruker Topspin 2.1 program. Assignment of spectra was
4 performed using Topspin 2 (Bruker Biospin) program for spectra visualization and overlap.
5

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ACCEPTED MANUSCRIPT

1 **Figure legends**

2

3 **Figure 1:** ^1H NMR spectrum of WPS from *L. casei* BL23 (50 °C, 500 MHz).

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5 **Figure 2:** Fragment of the COSY (green), TOCSY (red) and NOESY (blue) spectra of WPS from
6 *L. casei* BL23. Horizontal lines connect signals belonging to the spin-systems as labeled.

7

8 **Figure 3:** Structure of OS1 derived by Smith degradation of WPS from *L. casei* BL23 and
9 structures of the three variants of the WPS repeating unit. Gal3d, [3- $^2\text{H}_1$]glyceraldehyde.

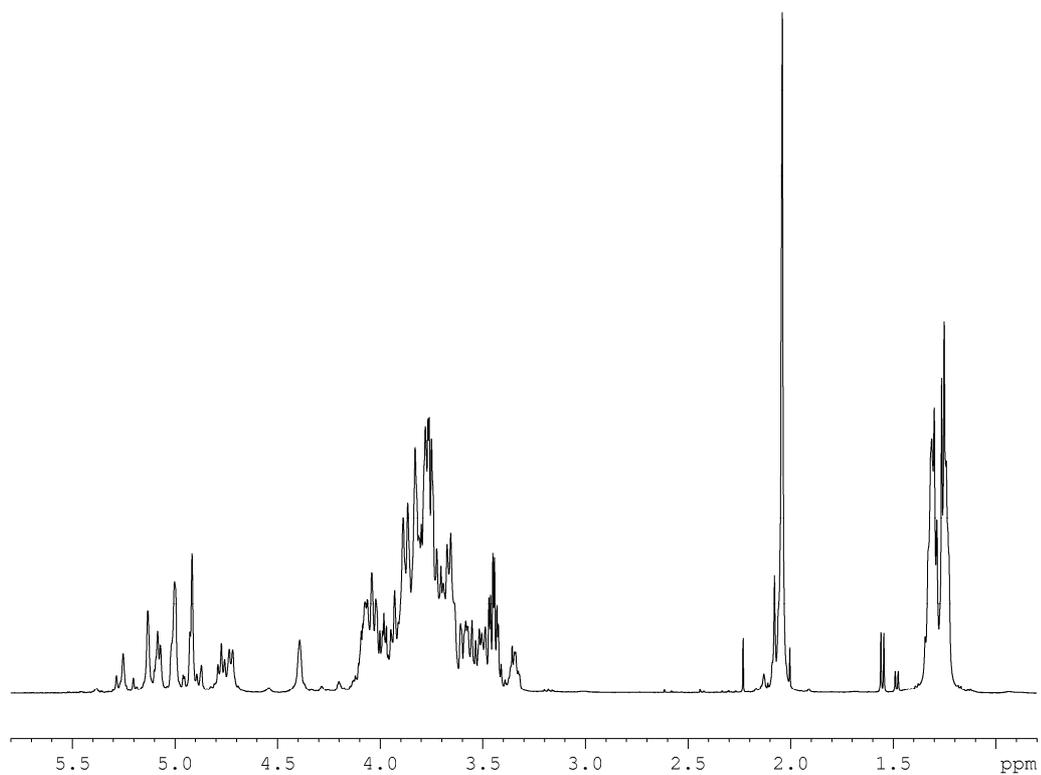
- 1 **Table 1.** NMR data for the OS1 from *L. casei* BL23 (δ , ppm; Varian INOVA 500 MHz 25 °C).
- 2 NAc: 2.03/23.5; 2.09/23.7 ppm; Gral3d, [3-²H₁]glyceraldehyde.

Sugar		H/C 1	H/C 2	H/C 3	H/C 4	H/C 5	H/C 6
α -Rha C, OS1	H	4.93	3.87	3.81	3.44	3.81	1.28
	C	103.5	71.6	71.1	73.1	70.4	17.9
α -Rha B, OS1	H	5.11	4.40	3.86	3.35	3.91	1.25
	C	99.9	78.5	82.1	72.0	70.4	18.0
β -GalNAc D, OS1	H	4.93	4.03	3.78	4.00	3.66	3.74; 3.77
	C	103.5	51.8	79.8	68.8	76.2	62.1
β -GlcNAc E, OS1	H	4.64	3.82	3.56	3.52	3.47	3.85; 3.94
	C	104.3	57.0	75.1	70.8	76.7	61.6
Gral3d A, OS1	H	5.10	3.63	3.70; 3.84			
	C	90.3	81.1	60.2			

1 **Table 2.** NMR data for the WPS from *L. casei* BL23 (δ , ppm; Varian INOVA 500 MHz 50 °C). NAc:
 2 2.04/23.6 ppm.

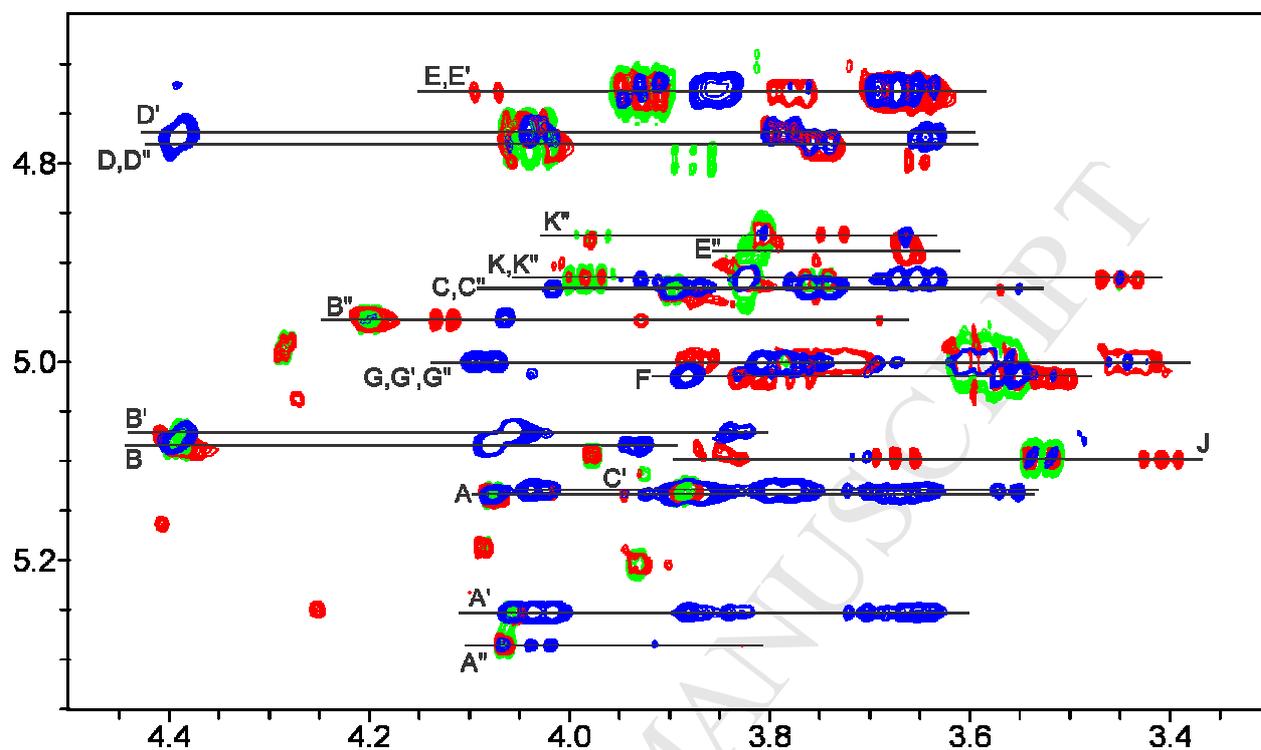
Sugar		H/C 1	H/C 2	H/C 3	H/C 4	H/C 5	H/C 6
α -Rha A	H	5.14	4.08	3.94	3.49	3.71	1.32
	C	102.2	78.8	71.3	73.5	70.3	18.0
α -Rha A'	H	5.25	4.07	3.83	3.49	3.71	1.32
	C	101.5	78.7	71.3	73.5	70.3	18.0
α -Rha A''	H	5.29	4.07	3.84	3.50		1.32
	C	101.5	78.7	71.3	73.5		18.0
α -Rha B	H	5.08	4.39	3.86	3.35	3.67	1.24
	C	102.2	78.2	82.3	72.4	71.0	18.0
α -Rha B'	H	5.07	4.39	3.86	3.35	3.67	1.24
	C	102.2	78.2	82.3	72.4	71.0	18.0
α -Rha B''	H	4.96	4.20	4.12	3.71	3.92	1.34
	C	102.4	70.1	80.3	79.7	70.2	18.0
α -Rha C,C''	H	4.93	3.90	3.88	3.56	3.87	1.30
	C	102.5	71.5	78.5	72.7	70.6	18.0
α -Rha C'	H	5.13	3.89	4.03	3.56	3.77	1.32
	C	100.1	77.0	75.8	72.7	70.3	18.0
β -GalNAc D,D''	H	4.78	4.05	3.79	4.03	3.65	3.76
	C	103.4	52.8	80.4	69.0	76.2	62.0
β -GalNAc D'	H	4.76	4.05	3.75	4.03	3.65	3.76
	C	103.4	52.8	80.4	69.0	76.2	62.0
β -GlcNAc E	H	4.72	3.93	3.65	3.78	3.68	3.80; 4.09
	C	104.5	56.8	83.0	69.4	75.5	66.7
β -GlcNAc E'	H	4.71	4.01	3.65	3.75	3.68	3.80; 3.99
	C	104.5	56.8	83.0	69.4	75.5	66.9
β -GlcNAc E''	H	4.88	3.84	3.67	3.67	3.67	3.80; 3.96
	C	104.5	56.8	83.0	69.6	75.5	66.7
α -Rha K, K'	H	4.92	3.82	3.75	3.45	3.98	1.26
	C	102.5	72.0	71.5	73.2	70.3	18.0
α -Rha K''	H	4.87	3.81	3.74	3.44	3.98	1.26
	C	102.5	72.0	71.5	73.2	70.3	18.0
α -Glc G,G',G''	H	5.00	3.60	3.77	3.44	3.73	3.80; 3.87
	C	99.3	72.7	74.6	70.9	73.2	62.0
α -Glc F	H	5.01	3.57	3.78	3.52	3.82	3.80; 3.87
	C	98.6	72.7	73.9	70.7	73.5	62.0
α -Glc J	H	5.10	3.53	3.68	3.42	3.85	3.80; 3.87
	C	100.2	72.3	73.9	70.9	73.6	62.0

1 **Fig. 1.** ^1H NMR spectrum of WPS from *L. casei* BL23 (50 °C, 500 MHz).



2

- 1 **Fig. 2.** Fragment of the COSY (green), TOCSY (red) and NOESY (blue) spectra of WPS from *L. casei*
- 2 BL23. Horizontal lines connect signals belonging to the spin-systems as labeled.

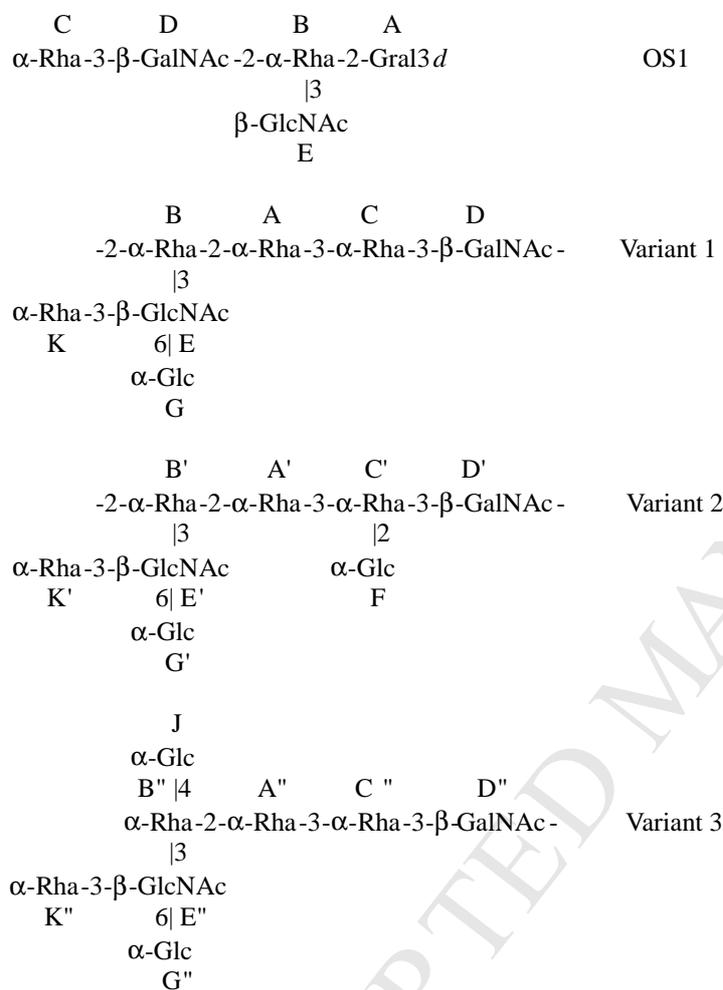


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1 **Figure 3:** Structure of OS1, product of Smith degradation of WPS from *L. casei* BL23 and
 2 structures of the three variants of the WPS repeating unit. Gal3d, 3-deutero-glyceraldehyde.

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Highlights

- The structure of a cell wall polysaccharide from probiotic *Lactobacillus casei* BL23 was elucidated.
- Three main variants of the repeating units were identified by NMR spectroscopy.
- The repeating unit consists in a branched heptasaccharide with a partial substitution.
- A terminal unit with an additional α -Glc at the non-reducing end was also identified.