Structural studies of the exopolysaccharide produced by *Lactobacillus paracasei* 34-1

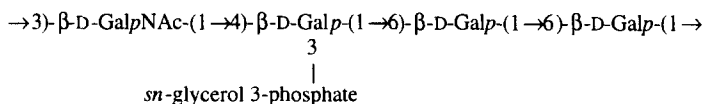Gerard W. Robijn [a], Hans L.J. Wienk [a], Dick J.C. van den Berg [b], Han Haas [b], Johannis P. Kamerling [a,*], Johannes F.G. Vliegenthart [a]

[a] *Bijvoet Center, Department of Bio-Organic Chemistry, Utrecht University, P.O. Box 80.075, NL-3508 TB Utrecht, The Netherlands*

[b] *Unilever Research Laboratory Vlaardingen, P.O. Box 114, NL-3130 AC Vlaardingen, The Netherlands*

Abstract

The exopolysaccharide produced by *Lactobacillus paracasei* 34-1 in a semi-defined medium was found to be a heteropolymer, composed of D-galactose, 2-acetamido-2-deoxy-D-galactose, and *sn*-glycerol 3-phosphate in molar ratios of 3:1:1. By means of deglycerophosphorylation, methylation analysis, and 1D/2D NMR studies (^{1}H, ^{13}C, and ^{31}P) the polysaccharide was shown to consist of repeating units with the following structure:

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow$$

$$\underset{\begin{array}{c}|\\ sn\text{-glycerol 3-phosphate}\end{array}}{3}$$

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1. Introduction

In the food industry, microbial exopolysaccharides are widely applied as thickening, gelling, and stabilizing agents [1]. At the moment, a growing interest can be observed in

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* Corresponding author.

the use of exopolysaccharides (EPSs) from lactic acid bacteria for these purposes. Because these microorganisms are considered GRAS (generally recognized as safe) for food applications, the excreted EPSs can form a new generation of food thickeners. In order to obtain insight into the relation between the rheological properties and the three-dimensional (3D) structure of a polysaccharide, knowledge of its primary structure is a prerequisite.

In this framework, structural studies have been performed on EPSs produced by *Streptococcus thermophilus* [2], *Lactococcus lactis* subsp. *cremoris* SBT 0495 [3], *L. lactis* subsp. *cremoris* H414 [4], *Lactobacillus delbrückii* subsp. *bulgaricus* rr [5], *Lb. helveticus* TY1-2 [6], *Lb. sake* 0-1 [7], and *Lb. helveticus* 766 [8]. Here we report the structure determination of the exopolysaccharide produced by *Lb. paracasei* 34-1 in a semi-defined medium.

2. Experimental

Production, isolation, and purification of the exopolysaccharide.—*Lb. paracasei* 34-1 was grown in a semi-defined medium [9] for 2 days at 20 °C (non-shaken, non-aerated), then trichloroacetic acid was added (final concentration 4% w/v), and the culture was stirred for 2 h. Cells and precipitated proteins were removed by centrifugation (27,000 g, 30 min, 4 °C). The supernatant solution was collected and the EPS was precipitated with two volumes of cold EtOH. An aqueous solution of the precipitated material was extensively dialyzed against twice-distilled water and, after removal of insoluble material by centrifugation, two volumes of EtOH were again added. The precipitated material was dissolved in water and purified by gel filtration on Sephacryl S-500 as described [7,8]. A small amount of the purified polysaccharide (250 μg) was fractionated on Superose-6 according to ref. [8]. The protein content of the purified sample was determined with the Pierce Protein Assay Reagent, using bovine serum albumin as a standard. The phosphorus content was determined colorimetrically [10].

Dephosphorylation of the polysaccharide.—A solution of lyophilized EPS (28 mg) in cold aq 48% HF (2.1 mL) was kept for 5 days at 0 °C. Then, the mixture was poured onto crushed ice (14 g), followed by immediate neutralization with cold 1 M NH$_4$OH. The crude product was purified by gel filtration on a Bio-Gel P-2 column (200–400 mesh, 90 × 1.5 cm), irrigated with twice-distilled water, using refractive index monitoring (Bischoff 8100 RI detector), which afforded the dephosphorylated polysaccharide in the void volume peak. A small amount (250 μg) of the dephosphorylated polysaccharide was subjected to fractionation on Superose-6, according to ref. [8].

Gas–liquid chromatography and mass spectrometry.—GLC and GLC–MS analyses were performed with a Varian 3700 gas chromatograph and a Fisons MD800/8060 system, respectively, using conditions as described previously [8].

Monosaccharide and methylation analysis.—For monosaccharide analysis, two procedures were followed: (*A*) Polysaccharides were methanolyzed, followed by trimethylsilylation and GLC analysis as described [7,11,12]. The absolute configurations of the monosaccharides were determined according to refs. [13,14]. (*B*) Phosphoglycerol was detected by GLC–MS as described [7]. For methylation analyses, samples (native EPS

or deglycerophosphorylated EPS) were permethylated according to ref. [15]. The permethylated polysaccharide was obtained either by extraction with CH_2Cl_2 (deglycerophosphorylated EPS), or by dialysis of the reaction mixture, after dilution with water, followed by lyophilization (native EPS). In the case of native EPS, it should be noted that attempts to isolate the permethylated polysaccharide by extraction with CH_2Cl_2 or $CHCl_3$ did not succeed, probably because of the presence of non-methylated phosphate groups. After methylation, samples were hydrolyzed with CF_3CO_2H, reduced with $NaBD_4$, and acetylated with Ac_2O [7]. The partially methylated alditol acetates were analyzed by GLC on CPSil43 and GLC–MS on DB-1 [12,16].

Determination of the absolute configuration of 1-phosphoglycerol.—The absolute configuration of the 1-phosphoglycerol side chain was established essentially according to ref. [7]. A sample of native EPS (1 mg) was permethylated according to ref. [17], except that the permethylated EPS was not obtained from the reaction mixture via extraction with $CHCl_3$ or CH_2Cl_2, but by dialysis of the mixture, after dilution with water and neutralization with 2 M NH_4OH. After lyophilization, the residue was treated with aq 48% HF, and after removal of HF in vacuo, the sample was treated with $(1S,4R)$-$(-)$-camphanic acid chloride as described [18]. The resulting product was analyzed by GLC–MS on DB-1, using selected ion monitoring at m/z 153 and 255.

NMR spectroscopy.—Proton-decoupled 75.469-MHz ^{13}C NMR spectra and 121.496-MHz ^{31}P NMR spectra were recorded in D_2O on a Bruker AC-300 spectrometer, at probe temperatures of 27, 67, or 80 °C. Chemical shifts were referenced to external MeOH (δ 49.00) for ^{13}C or to external 85% H_3PO_4 (δ 0.00) for [31]P. 1D ^{1}H and 2D NMR spectra were recorded on a Bruker AMX-500 or AMX-600 spectrometer at probe temperatures of 27 or 80 °C. Prior to analysis, samples were exchanged twice in D_2O (99.9 atom% D, Isotec) with intermediate lyophilization, and then dissolved in 99.96 atom% D_2O (Isotec). [1]H Chemical shifts are referenced to internal acetone (δ 2.225). Suppression of the HOD signal was achieved either by applying a WEFT pulse sequence [19] in 1D ^{1}H NMR experiments, or by presaturation for 1 s in the 2D experiments.

2D HOHAHA spectra were recorded using MLEV-17 mixing sequences with an effective spin-lock time of 25–100 ms. 2D NOESY experiments were performed with a mixing time of 200 ms. A homospoil pulse of 10 ms followed by a recovery of 30 ms was applied during the mixing time. DQF–COSY spectra were collected according to ref. [20]. Phase-sensitive [13]C–[1]H 2D HMQC experiments with inverse detection [21] were carried out at a ^{1}H frequency of 500.139 MHz (125.769 MHz for ^{13}C). ^{12}C-Bound protons were suppressed using a TANGO pulse sequence. [13]C Decoupling was not applied during the acquisition of the ^{1}H FID. All 2D NMR data were processed on Silicon Graphics IRIS workstations (Indigo, Indigo 2 or 4D/35), using TRITON software (Bijvoet Center, Department of NMR Spectroscopy).

3. Results and discussion

Isolation, purification, and composition of the polysaccharide.—The exopolysaccharide produced by *Lb. paracasei* 34-1 in a semi-defined medium [9,22], isolated via ethanol precipitation of the cell-free medium, was further purified by gel filtration

chromatography on Sephacryl S-500, affording the polysaccharide in a broad void volume peak. Additional analysis of the purified material by high-resolution FPLC on Superose-6 provided the polysaccharide as a sharp void volume peak, suggesting a polymer of high molecular mass. The protein content of the purified material was less than 3%.

Monosaccharide analysis of the native EPS (1), including the determination of absolute configurations (procedure A), revealed the presence of D-Gal and D-GalNAc in a molar ratio of 2.5:1.0. A qualitative analysis (procedure B), focused on the detection of phosphorylated components, demonstrated the presence of phosphoglycerol, as determined by GLC–MS. The phosphorus content was colorimetrically determined to be 3.5%, which corresponds to 0.9 mol/mol tetrasaccharide repeating unit (vide infra). The 1D ^{31}P NMR spectrum of 1 (not shown) contains one signal at δ 0.88, indicative of a phosphodiester [23]. In terms of absolute configuration, the phosphoglycerol was shown to be sn-glycerol 3-phosphate via GLC–MS analysis of the mono-O-camphanoyl-di-O-methylglycerol derivative [7]. Methylation analysis of 1 showed the presence of 4-substituted galactose, 6-substituted galactose, and 3-substituted 2-acetamido-2-deoxygalactose in molar ratios of ca. 0.4:2.0:0.4. Furthermore, a small amount of 3,4-disubstituted galactose was observed (0.1). According to NMR experiments (vide infra) all residues are in the pyranoid ring form.

Treatment of 1 with aq 48% HF at 0 °C resulted in an almost complete deglycerophosphorylation of the polysaccharide as judged by ^{1}H NMR spectroscopy (vide infra). Since the product obtained was eluted in the void volume of Bio-Gel P-2, the phosphoglycerol moiety is not part of the backbone of the polysaccharide. On Superose-6 the product was eluted in a broad range beyond the void volume of the column, demonstrating that dephosphorylation was accompanied by partial degradation of the polymer. Quantitative monosaccharide analysis (procedure A) of the deglycerophosphorylated EPS (2) revealed a composition of D-Gal and D-GalNAc in a molar ratio of 3.1:1.0. Methylation analysis of 2 showed the presence of 4-substituted galactopyranose, 6-substituted galactopyranose, and 3-substituted 2-acetamido-2-deoxygalactopyranose in approximate molar ratios of 1.3:2.0:0.8, suggesting a linear polysaccharide. No 3,4-disubstituted galactopyranose was observed.

The 1D ^{1}H NMR spectra of 1 and 2 (Fig. 1) each show four doublets in the anomeric region (δ 4.9–4.4), indicating a tetrasaccharide repeating unit. The four monosaccharide residues in the native EPS were arbitrarily designated A–D, as indicated in the spectrum of 1 (Fig. 1A). The 1-phosphoglycerol moiety was labelled E. In the spectrum of 1, the coupling constants of the anomeric signals at δ 4.721 (residue A, $^{3}J_{1,2}$ 8.3 Hz), 4.487 (residue B, $^{3}J_{1,2}$ 7.7 Hz), 4.479 (residue C, $^{3}J_{1,2}$ 7.3 Hz), and 4.432 (residue D, $^{3}J_{1,2}$ 7.7 Hz) demonstrate the pyranoid ring form and β configuration for all the residues. The signal originating from the protons of the N-acetyl group of the GalNAc residue is present at δ 2.076. Comparison of the 1D ^{1}H NMR spectra of 1 and 2 indicates that the extent of deglycerophosphorylation in preparation 2 is about 90%.

The 1D ^{13}C NMR spectrum of 1 (not shown) contains four signals in the anomeric region (δ 110–100) at δ 104.68, 103.73, 103.02, and 102.63, confirming the proposed tetrasaccharide repeating unit. Typical signals for the GalNAc residue appear at δ 22.70 (NDCOCH_3) and 51.41 (C-2). The signals at δ 60.74 and 61.21 are attributed to
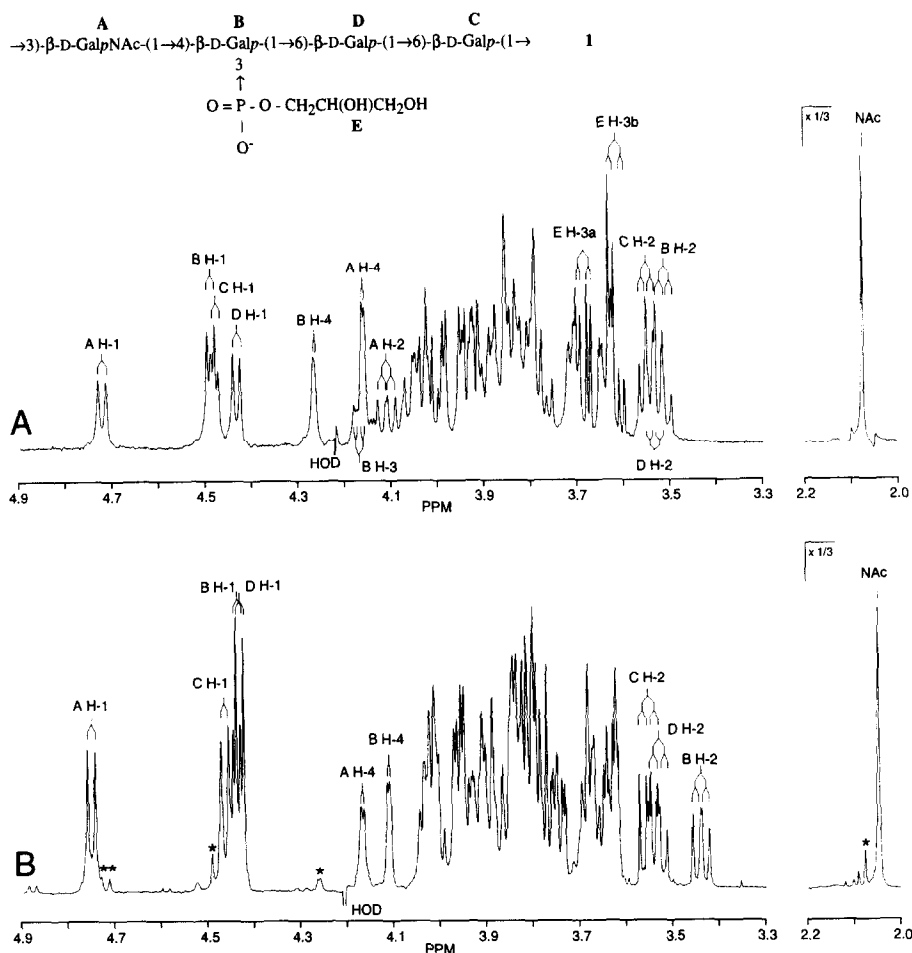
Fig. 1. 500-MHz ^{1}H NMR spectra of native EPS (1) (A), and deglycerophosphorylated EPS (2) (B), recorded in D$_2$O at 80 °C. Signals marked with an asterisk (∗) in B originate from protons of non-deglycerophosphorylated repeating units.

hydroxymethyl carbons (unsubstituted C-6). Furthermore, the signals at δ 66.80 (**E** C-1, d, $^2J_{P,C}$ −5.7 Hz) and 62.53 (**E** C-3, s) reflect the presence of a 1-phosphoglycerol moiety (*sn*-glycerol 3-phosphate, vide supra) in the EPS. The assignments of the signals of the 1-phosphoglycerol group are supported by the ^{13}C NMR parameters of reference 1-phosphoglycerol (δ 65.60, d, C-1, $^2J_{P,C}$ −4.9 Hz; δ 72.04, d, C-2, $^3J_{P,C}$ 7.5 Hz; δ 63.14, s, C-3 [23]). In the spectrum of **2** (not shown), the 1-phosphoglycerol resonances **E** C-1 and **E** C-3 have almost completely disappeared, confirming the high extent of deglycerophosphorylation.

Based on these results, the native EPS is composed of a linear tetrasaccharide repeating backbone with a composition of 3-substituted D-GalNAc, 6-substituted D-Gal, and 3,4-disubstituted D-Gal in molar ratios of 1:2:1, which is substituted by *sn*-glycerol

3-phosphate, probably via O-3 of the 3,4-disubstituted Gal residue. It should be noted that, for this conclusion, the methylation analysis data of **1** and **2** have only been used qualitatively. A poor stoichiometry has also been reported for other polysaccharides containing either phosphate [7,24] or HexNAc [25] residues. The complete primary structure of the EPS was unambiguously established by 2D NMR experiments carried out on the native (**1**) and the deglycerophosphorylated (**2**) EPSs.

2D NMR spectroscopy.—By means of 2D COSY, HOHAHA, NOESY, and ^{13}C–^{1}H HMQC experiments almost all ^{1}H and ^{13}C resonances in the 1D ^{1}H and ^{13}C NMR spectra of **1** and **2** have been assigned. The ^{1}H and ^{13}C NMR data are collected in Table 1.

In the 1D ^{1}H NMR spectrum of **1** (Fig. 1A), the resonances of H-2,3,4 of residues **A**, **B**, **C**, and **D** were traced via connectivities with the correlative H-1 signals in the HOHAHA (Fig. 2) and COSY (not shown) spectra, and the corresponding carbon resonances were found via correlation to these protons in the HMQC spectrum (not shown). Intraresidual H-1,H-5 contacts in the NOESY spectrum of **1** (Fig. 3A) allowed the assignment of H-5 for each of the four residues **A–D**, and the corresponding carbon signals were traced via the HMQC spectrum. The chemical shifts of **A** H-6a,6b and **B** H-6a,6b were found via correlation with **A** C-6 (δ 61.21) and **B** C-6 (δ 60.74), respectively, in the HMQC spectrum. The assignments of the ^{13}C resonances at δ 61.21 to **A** C-6 and at δ 60.74 to **B** C-6 were made by comparison with the NMR data of the deglycerophosphorylated EPS **2** (vide infra). The assignments of the two sets of chemical shifts attributed to **C** C-6,H-6a/6b and **D** C-6,H-6a/6b, as determined from the HMQC spectrum from the ^{13}C resonances at δ 68.63 and 69.74, are interchangeable. Finally, the complete ^{1}H spin-coupling system of the 1-phosphoglycerol moiety **E** was established starting from the resonance of **E** H-3a at δ 3.686 in the HOHAHA spectrum and from correlation to the corresponding carbon signals in the HMQC spectrum, and agrees with literature data [7,23,25].

In conjunction with the methylation analysis data, the relatively downfield chemical shifts of **B** H-4 (δ 4.265) and **B** C-4 (δ 75.46) demonstrate that residue **B** is 4-substituted (Me-β-D-Gal$p$, δ_{H-4} 3.92 [26], δ_{C-4} 69.7 [27]). Likewise, the ^{13}C chemical shifts for **C** C-6 and **D** C-6 (δ 68.63, 69.74) clearly show that galactosyl residues **C** and **D** are 6-substituted (Me-β-D-Gal$p$, δ_{C-6} 62.0 [27]). Residue **A** was identified as GalNAc by the typical chemical shifts of **A** H-2 (δ 4.109) and **A** C-2 (δ 51.41), and the downfield chemical shift for **A** C-3 (δ 79.85) confirms that residue **A** is 3-substituted (β-D-Gal$p$NAc, δ_{C-3} 72.3 [27]). The $^1J_{C-1,H-1}$ values of 162 Hz for residue **A** and 161 Hz for residues **B**, **C**, and **D** confirm β configurations for all the residues.

Similarly, almost all resonances of the deglycerophosphorylated EPS (**2**) were assigned via 2D NMR analysis. In contrast to the NMR analysis of **1**, a straightforward assignment of H-5,6a,6b and C-5,6 of residues **A** and **B** was possible for **2**. The resonances of **A** H-5 and **B** H-5 were detected on the HOHAHA tracks (not shown) of **A** H-4 at δ 4.168 and **B** H-4 at δ 4.111, respectively, and by the intraresidual connectivities **A** H-1,H-5 and **B** H-1,H-5 in the NOESY spectrum of **2** (Fig. 3B). On the HOHAHA tracks of **A** H-5 and **B** H-5 the signals from **A** H-6a,6b and **B** H-6a,6b were found, respectively, and the ^{13}C resonances **A** C-5,6 and **B** C-5,6 were established via correlation to the corresponding protons in the HMQC spectrum (not shown).

Table 1

^{1}H NMR chemical shifts [a] of native EPS (**1**) and deglycerophosphorylated EPS (**2**), recorded at 80 °C. ^{13}C NMR chemical shifts [b] of **1** and **2**, as determined from 1D ^{13}C NMR spectra, recorded at 67 and 80 °C, respectively. The ^{13}C NMR assignments were deduced from 2D [13]C–[1]H HMQC experiments, recorded at 80 °C

Residue	Proton	**1** [c]	**2** [c]	Carbon	**1**	**2**
A	H-1	4.721 (8.3)	4.750 (8.3)	C-1	102.63 (162) [d]	102.26 (162) [d]
	H-2	4.109 (~ 10.5)	4.01	C-2	51.41	51.92
	H-3	3.89 (2.8)	3.92 (3.1)	C-3	79.85	79.85
	H-4	4.159	4.168	C-4	68.22	68.23
	H-5	3.70	3.67	C-5	74.64	74.76
	H-6a	3.79	3.80	C-6	61.21	61.26
	H-6b	3.79	3.80			
N-Acetyl	CH$_3$	2.076	2.047	COCH$_3$	22.70	22.69
				COCH$_3$	n.d. [e]	174.77
B	H-1	4.487 (7.7)	4.438 (7.8)	C-1	103.02 (161) [d]	103.38 (161) [d]
	H-2	3.515	3.440 (9.7)	C-2	70.41 (3.7) [f]	71.17
	H-3	4.16	3.744 (2.7)	C-3	77.53 (−6.5) [g]	73.20
	H-4	4.265	4.111	C-4	75.46	76.10
	H-5	3.70	3.68	C-5	74.10	74.52
	H-6a	3.82 [h]	3.825 [h]	C-6	60.74	60.86
	H-6b	3.76 [h]	3.765 [h]			
C	H-1	4.479 (7.3)	4.464 (7.6)	C-1	104.68 (161) [d]	104.51 (161) [d]
	H-2	3.545	3.554 (~ 9.8)	C-2	70.96	70.98
	H-3	3.63	3.630 (3.4)	C-3	72.65	72.72
	H-4	3.95	3.952	C-4	68.94	68.89
	H-5	3.815	3.84	C-5	74.10	73.95
	H-6a	4.02 [h,i]	4.01 [h,j]	C-6	68.63 [i]	68.89 [i]
	H-6b	3.865 [h,i]	3.88 [h,j]			
D	H-1	4.432 (7.7)	4.431 (7.8)	C-1	103.73 (161) [d]	103.66 (161) [d]
	H-2	3.530	3.531 (9.8)	C-2	70.96	70.98
	H-3	3.64	3.636 (3.4)	C-3	72.96	72.98
	H-4	3.98	3.968	C-4	68.74	68.89
	H-5	3.835	3.84	C-5	73.76	73.95
	H-6a	4.05 [h,i]	4.03 [h,j]	C-6	69.74 [i]	69.50 [j]
	H-6b	3.87 [h,i]	3.905 [h,j]			
E	H-1a	3.92 [k]	–	C-1	66.80 (−5.7) [g]	–
	H-1b	4.01 [k]	–			
	H-2	3.91	–	C-2	70.96 (n.d.) [f]	–
	H-3a	3.686 (4.2, −11.8)	–	C-3	62.53	–
	H-3b	3.615 (5.5, −11.8)	–			

[a] In ppm relative to the signal of internal acetone at δ 2.225. [b] In ppm relative to the signal of external methanol at δ 49.00. [c] [1]H–[1]H coupling constants (Hz). [d] [1]$J_{C-1,H-1}$ (Hz). [e] n.d. = not detected. [f] [3]$J_{P,C}$ (Hz). [g] [2]$J_{P,C}$ (Hz). [h] The assignments of H-6a and H-6b may have to be interchanged within one residue. [i,j] The assignments of the sets **C** C-6,H-6a/6b and **D** C-6,H-6a/6b may have to be interchanged for both **1** and **2**. [k] The assignments of H-1a and H-1b may have to be interchanged.
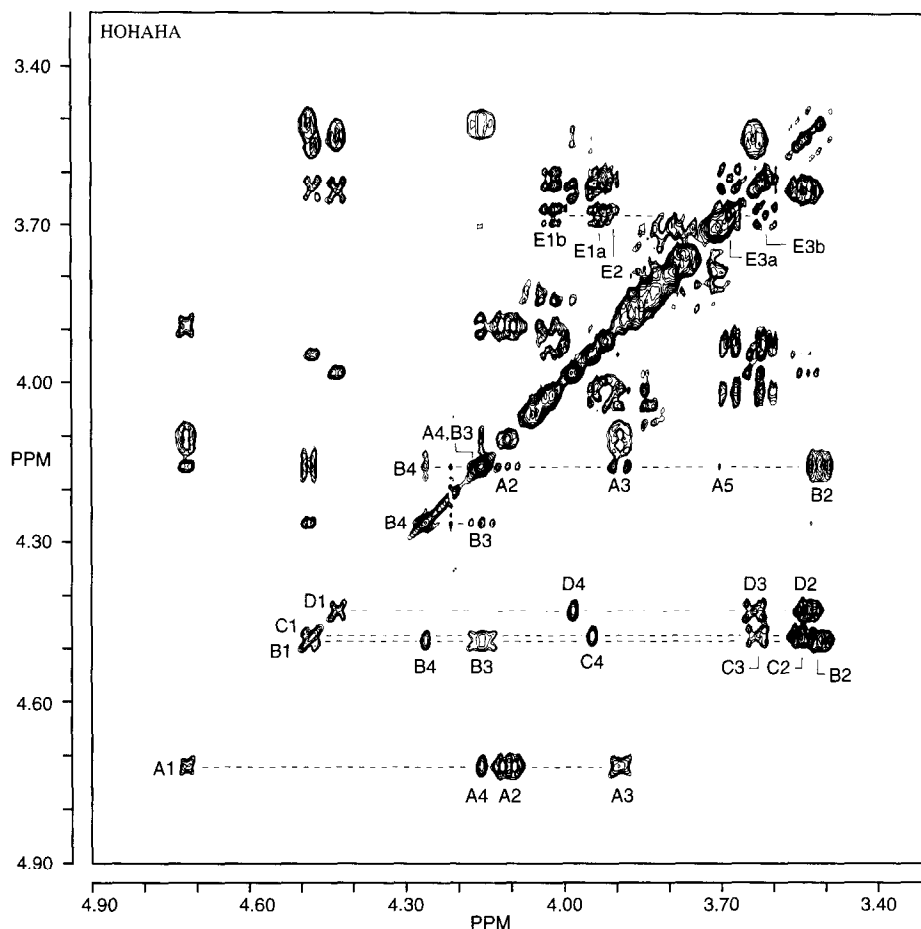
Fig. 2. 500-MHz 2D HOHAHA spectrum (mixing time 100 ms) of native EPS (1), recorded in D_2O at 80 °C. Diagonal peaks of the anomeric protons, of H-4 of residues **A** and **B**, of H-3 of residue **B**, and of H-3a of residue **E** are indicated. Cross-peaks belonging to the same scalar-coupling network are indicated near a dotted line starting from the corresponding diagonal peak.

Comparison of the ^1H and ^{13}C NMR data of **1** and **2** showed that most of the resonances of the constituent monosaccharides in the two ^1H NMR spectra are similar (within 0.1 ppm), but a major difference is found for **B** H-3 ($\Delta\delta$ +0.42), demonstrating phosphorylation at C-3 of residue **B** [23,28]. This is in agreement with the methylation analysis data and the $^{31}P,^{13}C$-coupling constants in the 1D ^{13}C NMR spectrum of **1** (δ 70.41, **B** C-2, $^3J_{P,C}$ 3.7 Hz; δ 77.53, **B** C-3, $^2J_{P,C}$ −6.5 Hz). Also in the ^{13}C NMR spectra of **1** and **2** no major differences are observed except for **B** C-3 ($\Delta\delta$ +4.33), confirming phosphorylation at **B** C-3.

The monosaccharide sequence in the EPS was established by 2D NOESY experiments. On the **B** H-1 track in the NOESY spectrum of **1** (Fig. 3A) a cross-peak with **D** H-6b (or **C** H-6b) was observed, suggesting a **B** → **D** (or **B** → **C**) sequence, whereas an
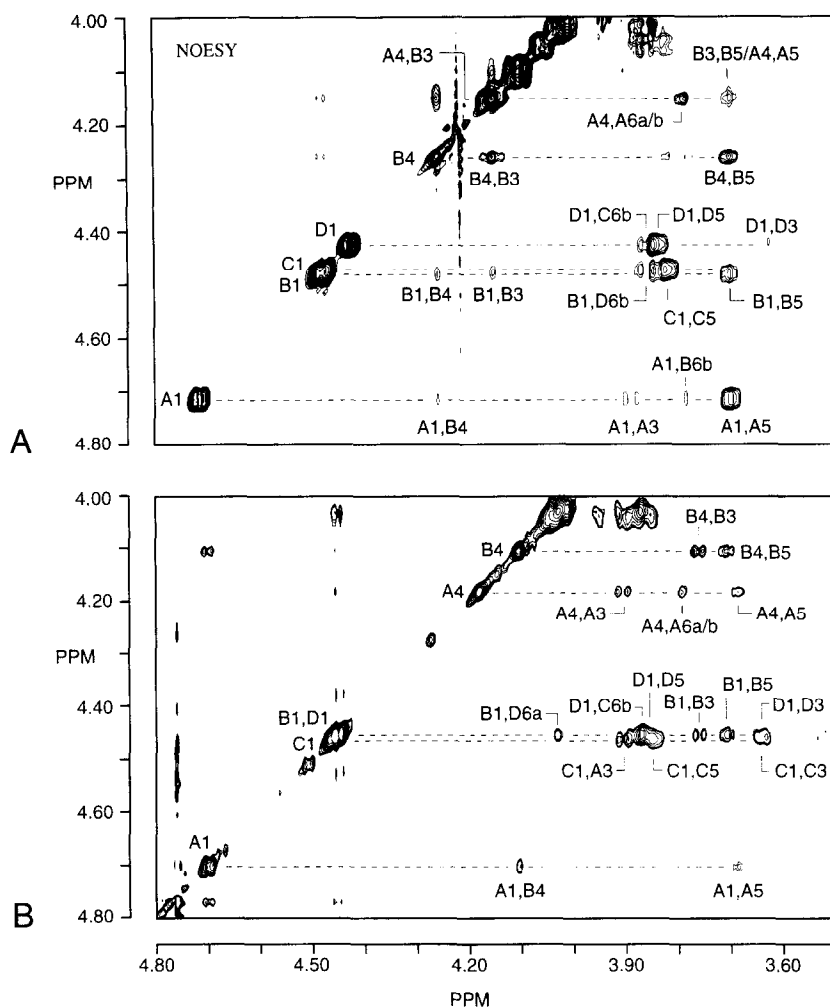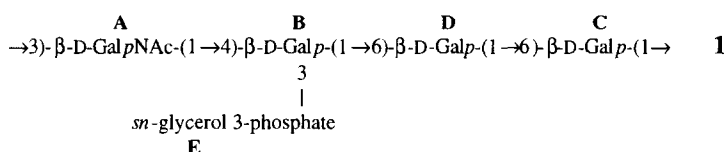
Fig. 3. Partial 500-MHz 2D NOESY spectra (mixing times 200 ms) of native EPS (**1**) (A), and deglycerophosphorylated EPS (**2**) (B), recorded in D$_2$O at 80 and 27 °C, respectively. A1 corresponds to the diagonal peak belonging to **A** H-1; A1,B4 refers to a connectivity between **A** H-1 and **B** H-4, etc.

interresidual connectivity **D** H-1,**C** H-6b demonstrated the **D** → **C** linkage. Furthermore, two weak interresidual connectivities **A** H-1,**B** H-4 and **A** H-1,**B** H-6b were observed, suggesting an **A**(1 → 4)**B** linkage, since residue **B** was established to be 4-substituted (vide supra). Hence, the combined NOE data of **1** suggest the **A** → **B** → **D** → **C** sequence for the repeating unit of the EPS, although the **C** → **A** linkage was not confirmed by the NOESY spectrum of **1**. The unexpected cross-peak **B** H-1,**B** H-4 may be attributed to a spin-diffusion effect.

To improve resolution in the spectra, all 2D NMR experiments of native EPS (**1**) were recorded at 80 °C. However, for deglycerophosphorylated EPS (**2**) it was not

possible to obtain NOESY spectra at this temperature, due to the fact that the EPS had been partially depolymerized during treatment with aq 48% HF (vide supra), which resulted in a higher mobility of the polysaccharide and hence hampered NOE magnetization transfer. A good quality 2D NOESY spectrum of **2** could be obtained at 27 °C (Fig. 3B) showing a strong interresidual cross-peak between **A** H-1 and **B** H-4, confirming the **A**(1 → 4)**B** linkage. Furthermore, the connectivities **B** H-1,**D** H-6a and **D** H-1,**C** H-6b support the **B** → **D** → **C** sequence. Finally, a strong interresidual connectivity, which could be unambiguously assigned as **C** H-1,**A** H-3, established the **C** → **A** sequence, thus confirming the suggested **A** → **B** → **D** → **C** sequence.

The combined results, from chemical and NMR studies, have proved that the EPS produced by *Lb. paracasei* 34-1 is composed of tetrasaccharide repeating units with the following structure:

$$
\begin{array}{cccc}
\mathbf{A} & \mathbf{B} & \mathbf{D} & \mathbf{C} \\
\end{array}
$$

$$\rightarrow 3)\text{-}\beta\text{-D-Gal}p\text{NAc-}(1 \rightarrow 4)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow 6)\text{-}\beta\text{-D-Gal}p\text{-}(1 \rightarrow \qquad \mathbf{1}$$

$$
\begin{array}{c}
3 \\
| \\
sn\text{-glycerol 3-phosphate} \\
\mathbf{E}
\end{array}
$$

Acknowledgements

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