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# Determination of the cell wall polysaccharide and teichoic acid structures from *Lactococcus lactis* IL1403

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ABSTRACT

In the lactic acid bacterium *Lactococcus lactis*, a cell wall polysaccharide (CWPS) is the bacterial receptor of the majority of infecting bacteriophages. The diversity of CWPS structures between strains explains, at least partially, the narrow host range of lactococcal phages. In the present work, we studied the polysaccharide components of the cell wall of the prototype *L. lactis* subsp. *lactis* strain IL1403. We identified a rhamnose-rich complex polysaccharide, carrying a glycerophosphate substitution, as the major component. Its structure was analyzed by 2D NMR spectroscopy, methylation analysis and MALDI-TOF MS and shown to be distinctly different from currently known lactococcal CWPS structures. It contains a linear backbone of repeated α-L-Rha disaccharide subunits, which is irregularly substituted with a trisaccharide occasionally bearing a glycerophosphate group. A poly (glycerol phosphate) teichoic acid, another important carbohydrate component of the IL1403 cell wall, was also isolated and structurally characterized.

## 1. Introduction

*Lactococcus lactis*, a species subdivided into two genetically distinct subspecies *cremoris* and *lactis*, is a member of the lactic acid bacteria as it produces lactic acid as the main by-product of hexose fermentation [1,2]. The production of lactic acid is crucial for their application in commercial dairy fermentations, in particular cheese production. This high intensity commercial exploitation has, however, supported the proliferation of bacteriophages in the dairy processing environment. Lactococcal phages are currently classified into ten taxonomic groups based on morphology and DNA sequence relatedness, and three of these groups, namely the 936, P335 and c2 groups, are most frequently encountered in the commercial dairy processing environment [3].

*L. lactis* strains and their infecting phages have recently become a useful Gram-positive model to delineate the intricacies of phage-host interactions [4–6]. The majority of lactococcal phages are predicted to recognise a carbohydrate moiety on the cell surface of their host [5,7–10]. Therefore, the biochemical structure of this saccharidic receptor, which constitutes a so-called cell wall polysaccharide (CWPS), has become a subject of growing interest [11–14]. In parallel with the study of lactococcal CWPS, 3D structures of lactococcal phage tail components that recognise these saccharidic receptor moieties have been elucidated [9,15–20]. In fact, one of the first lactococcal phage protein structures to be resolved was that of the receptor binding protein of the 936 group phage bIL170 [15].

The genome sequence of bIL170 was elucidated in 2002 [21], while that of its host, *L. lactis* IL1403, a plasmid-free strain derived from *L. lactis* IL594 [22], was released a year earlier [23]. This phage-host combination was employed as part of a study in which the genetic basis of lactococcal 936 phage adsorption was defined [8]. The genetic requirements associated with 936 phage adsorption were identified as a gene cluster of 25–30 kb predicted to be involved in the biosynthesis of CWPS [8]. As the number of complete lactococcal genomes increased in ensuing years, CWPS-specifying gene clusters were compared and three genetic groups (designated here as CWPS genotypes A, B and C) were revealed, while additional genotypes were also predicted to exist [5]. The chemical structures of the CWPS of three C-type lactococcal strains (MG1363, SMQ-388 and 3107) have been resolved. The CWPS are composed of repeating penta-/hexa-saccharide subunits linked by a phosphodiester bond [11–13], while a rhamnose-containing CWPS

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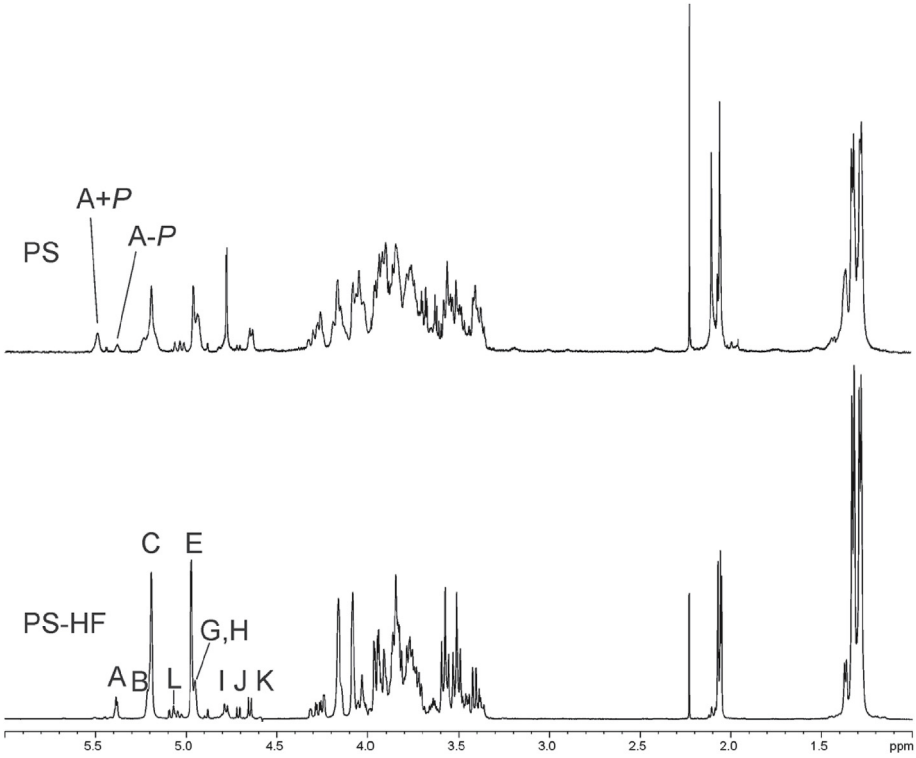


**Fig. 1.** $^{1}$H NMR spectrum of CWPS and CWPS-HF obtained from *L. lactis* IL1403. A + P refers to the GalNAc residue A with PGro at O-6; A-P is the same residue without PGro.

component has also been identified in MG1363 and 3107 [24]. The CWPS-encoding gene clusters are composed of a conserved region that is predicted to encode the rhamnan component, and a more divergent region, predicted to encode the so-called polysaccharide pellicle [12]. The genetic diversity observed among the CWPS-specifying gene clusters eludes to the likely biochemical diversity of their structures prompting an expansion of current knowledge of the biochemical structures of lactococcal CWPS.

*L. lactis* subsp. *lactis* IL1403 has been a focus of numerous genetic and biotechnological studies, and a strain that possesses a B-type CWPS genotype [5], for which no biochemical information is currently available. In the present study, we report the novel chemical structure of the CWPS of *L. lactis* subsp. *lactis* IL1403. The structure of a co-purified poly (glycerolphosphate) teichoic acid was also elucidated.

## 2. Results

### 2.1. Extraction and purification of cell wall carbohydrates

The standard method of extraction of CWPS with cold TCA, used previously for other *L. lactis* strains [12], resulted in a very poor yield of carbohydrate material in the case of *L. lactis* subsp. *lactis* IL1403. We released CW-associated carbohydrates from the cells by autoclaving, one of the conventional methods for extraction of capsular polysaccharides [25]. Fractionation of this preparation by ion-exchange chromatography revealed the presence of two major products, which according to further analysis corresponded to a polysaccharide and a teichoic acid. The teichoic acid preparation was used for further structural analysis (see 2.3).

Alternatively, cells were extracted with hot diluted HCl as described previously [24,26] (see Experimental procedures). Crude extracts were fractionated on a Sephadex G-50 column. The polysaccharide material, designated here as CWPS, eluted as a broad peak close to the 5000 Da dextran standard. CWPS-HF was obtained from the crude extract by treatment with HF followed by gel-filtration and had a similar elution profile. Both polysaccharides were subjected to NMR and chemical analyses.

### 2.2. Structure elucidation of the CWPS

Monosaccharide composition of CWPS-HF was established by GC-MS analysis of hydrolyzed samples after conversion into alditol acetates. Absolute configurations of monosaccharides were determined by GC-MS of their acetylated (*R*)-2-butyl-glycosides. CWPS-HF contained L-Rha, D-Glc, D-GlcN, D-GalN at approximate ratios of 4.6: 1: 0.8: 0.6. Methylation analysis showed the presence of terminal Glc, 2-, 3-, and 2,4-substituted Rha, 3-substituted GlcN, and 4-substituted GalN.

The chemical structure of the CWPS-HF was established by a combination of 1D and 2D NMR techniques, MALDI-TOF MS, and methylation analysis.

NMR spectra of the intact CWPS were complex (Fig. 1a), in part due to incomplete phosphorylation. NMR spectra of the dephosphorylated CWPS-HF still contained many signals of different intensity (Fig. 1b). There were two main anomeric signals of rhamnose residues C and E, and many smaller signals designated A-L (Figs. 1 and 2). Interpretation of 2D NMR spectra ($^{1}$H-$^{1}$H gCOSY, $^{1}$H-$^{1}$H TOCSY, $^{1}$H-$^{1}$H NOESY, $^{1}$H-$^{13}$C HSQC, $^{1}$H-$^{13}$C HMBC) enabled identification of spin systems of monosaccharides shown in Fig. 4. Monosaccharides were identified by characteristic TOCSY patterns and signal positions in the $^{13}$C NMR spectrum. The anomeric configuration of Rha (all α) followed from TOCSY correlations H-1:H-3 (which would be absent in case of β-Rha) and position of C-5 signals at ~70 ppm (~73 ppm was expected for β-Rha). Anomeric configuration of gluco- and galacto-sugars followed from $J_{1,2}$ coupling constants (~4 Hz for α-, ~8 Hz for β-anomers). Major sharp signals belonged to a polymer with a disaccharide repeating unit [-3-α-Rha*p*-2-α-Rha*p*-] (residues E-C). Their linkages were determined by NOE (E1:C1; E1:C2; C1:E3; C1:E5) and $^{13}$C NMR signal positions (Table 1, Figs. 2–4).

A minor tetrasaccharide fragment β-Glc-4-α-GalNAc-3-β-GlcNAc-4-α-Rha (K-A-I-D) was identified by NOE correlations (K1:A4; A1:I3;