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The structure of the polysaccharide of the lipopolysaccharide produced by *Taylorella equigenitalis* type strain (ATCC 35865)

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ABSTRACT

Taylorella equigenitalis is a Gram-negative bacterium that causes venereally transmitted contagious equine metritis (CEM), and its identification and differentiation from other bacteria and *Taylorella* species is an important requirement for the control of CEM infection. Based on the results of NMR and MS analysis, the antigenic O-polysaccharide (O-PS) component of the lipopolysaccharide (LPS) produced by the type strain *T. equigenitalis* (ATCC 35865) was found to be a linear polymer composed of a repeating disaccharide unit, containing partially amidated 2,3-diacetamido-2,3-dideoxy-α-L-guluronic and 2,3-diacetamido-2,3-dideoxy-β-D-mannuronic acids, terminated with a 4-O-methylated non-reducing Gul*p*-NAc3NAcA residue, and has the structure.

(Terminal 4-O-Me)→ [→4)-α-L-Gul*p*NAc3NAcA-(1→4)-β-D-Man*p*NAc3NAcA-(1→]$_n$

The O-PS of the type strain *T. equigenitalis* LPS provides a specific antigenic marker for the discrimination of the pathogen from the related type strain of *T. asinigenitalis* sp. nov, a phenotypically indistinguishable non-pathogenic bacterium having a serologically and structurally unrelated LPS O-antigen. The analysis of a structurally unusual core oligosaccharide of the LPS is also reported.

1. Introduction

Contagious equine metritis (CEM), first described in the United Kingdom in 1977,[1,2] is a highly communicable venereal disease of horses caused by *Taylorella equigenitalis*,[3] a fastidious microaerophilic Gram-negative coccobacillus. Infected mares develop an acute purulent metritis and fail to conceive, resulting in economic losses and the requirement of pre-breeding tests and surveillance screening before importation into CEM-free countries. Horses are the only species infected naturally by *T. equigenitalis* and thoroughbreds appear to be particularly susceptible. The recognition of CEM among thoroughbred mares has had widespread repercussions in the breeding industry and as a result has stimulated interest in the development of diagnostic procedures for the specific identification of *T. equigenitalis*.

Recently, another *Taylorella* species, *T. asinigenitalis*, was isolated from male donkeys in the USA.[4] The newly described bacterium is phenotypically indistinguishable from *T. equigenitalis* and does not produce disease, but resides in the genital tract of male donkeys and can be passed to other donkeys and horses during mating.

The current diagnosis of CEM is based on the isolation of *T. equigenitalis* by conventional bacteriological culture from field genital swabs, the employment of PCR tests[5–10] and serology using both polyclonal and monoclonal antibody methodologies[11–14] directed to protein rather than to specific glycosyl derivatives.

The World Organization for Animal Health (http://www.oie.int/) has stated that no serological test described to date will by itself reliably detect infection for diagnosis and control purposes. However, serological tests have been used as an adjunct to the culture of *T. equigenitalis* in screening mares recently bred to a carrier stallion, but serology is not used as a substitute for culture.

The present investigation was undertaken to examine the possibility that the LPS O-antigens of Taylorella may be specific targets for species differentiation. Herein, we report that a unique O-PS antigen is produced by clinical isolates of *T. equigenitalis* and that antisera to this macromolecule provide diagnostics needed for the identification and discrimination of the bacterium, as well as a possible polysaccharide-based conjugate vaccine.

2. Experimental

2.1. Growth of *T. equigenitalis*

Starter growth of *T. equigenitalis* type strain (ATCC 35865; NRCC 6368) was made on two plates of enriched dried Chocolate Agar

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(Oxoid MP0330) supplemented with surface covering of sterile L-cystine (Sigma C8755, 0.1 mL, 45 mg/mL) and was incubated at 37 °C in a CO_2 incubator for 44 h. The combined plate growth was used to inoculate 60 L-cystine-treated plates that were grown under the same conditions, and their harvest was used to finally inoculate 18 sterile baffle flasks each containing 1.5 L of filter sterilized (0.22 μ) Brain Heart Infusion broth (Difco) supplemented with Hemin (Sigma, 10 mg/L) and adjusted to pH 6.8 with 1.5 M sulfuric acid. Inoculated flasks were each supplemented with L-cystine (0.45 g) and filter sterilized β-NAD (Sigma N-7004, 7.5 mg), and the flasks were then incubated at 37 °C and at 200 RPM for 42 h. The cooled (4 °C) flasks and cells were killed by treatment with phenol (3 g, 2 h) prior to the collection of cells by centrifugation.

2.2. Preparation of LPS, O-PS, and core oligosaccharide

Cells of *T. equigenitalis* (199 g, paste) were extracted with vigorously stirred 50% (w/v) aqueous phenol (700 mL) at 60 °C for 10 min, and on cooling to 4 °C, the mixture was centrifuged (6000*g*) and the separated top aqueous and lower phenol phases were aspirated and dialyzed against running tap-water until free of phenol. The lyophilized retentates were each dissolved in 0.02 M sodium acetate (80 mL, pH 7.0) and treated sequentially for 2 h each at 37 °C with DNase, RNase, and Proteinase K, and after removal of insoluble material, the solutions were subjected to ultracentrifugation (105,000*g*, 4 °C, 12 h). The precipitated gels were dissolved in distilled water and lyophilized to yield 760 mg of aqueous phase LPS and 330 mg of phenol phase LPS.

Aqueous phase LPS (260 mg) was subjected to mild hydrolysis with 1.5% (v/v) acetic acid (100 mL, 100 °C, 2 h), and following the removal of precipitated lipid A, the solution was lyophilized, the residue dissolved in 0.05 M pyridinium acetate buffer (8 mL, pH 5.2) and fractionated by Sephadex G-50 column gel filtration with eluate monitoring by refractive index measurement using a Waters 403 refractometer. Fractions were collected and lyophilized to yield O-PS (K_{av} 0.01–0.13) and core oligosaccharide (K_{av} 0.66). Polysaccharide and core fractions were further purified by anion-exchange chromatography on a Hitrap Q column (Pharmacia) in water for 10 min, and then in a linear gradient of 0–1 M NaCl over 60 min with UV detection at 220 nm. Fractions were desalted by Sephadex G-15 column chromatography.

2.3. NMR spectroscopy

^1H and ^{13}C NMR spectra were recorded using a Varian Inova 500 and 600 MHz spectrometers with sample dissolved in 99% D_2O at 40 °C and internal acetone standard (2.225 ppm for ^1H and 31.07 ppm for ^{13}C) employing standard COSY, TOCSY (mixing time 120 ms), NOESY (mixing time 200 ms) and heteronuclear multiple-bond correlation (*g*HMBC, for 8 Hz long-range coupling constants).

2.4. Gel electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel-electrophoresis (SDS–PAGE) was performed on separating gels of 12% acrylamide and detection was done by silver staining after oxidation with periodate.[15]

2.5. Mass spectrometry

A Prince CE system (Prince Technologies, The Netherlands) was coupled to a 4000 QTRAP mass spectrometer (Applied Biosystems/MDS Sciex, Canada). A sheath solution (2:1 2-propanol–MeOH) was delivered at a flow rate of 1.0 μL/min. Separations were obtained on about 90 cm long bare fused-silica capillary using 15 mM ammonium acetate in deionized water, pH 9.0. The 5 kV or −5 kV of electrospray ionization voltage was used for positive ion and negative ion detection modes, respectively. For pseudo MS/MS analysis, the precursor ions were generated with an orifice voltage of +400 V and mass spectra were acquired with nitrogen in the RF-only quadrupole collision cell.

2.6. Monosaccharide analysis

Samples (0.5 mg) were hydrolyzed (0.2 mL of 3 M trifluoroacetic acid, 120 °C, 2 h), followed by evaporation to dryness under a stream of nitrogen. The residues were dissolved in water (0.5 mL), reduced with $NaBH_4$ (~5 mg, 1 h), neutralized with AcOH (0.3 mL), and dried, and MeOH (1 mL) was added. The residues were dried twice from MeOH solution, acetylated with Ac_2O (0.5 mL, 100 °C, 30 min), dried, and analyzed by GLC using a HP5 capillary column (30 m × 0.25 mm) with a flame ionization detector (Agilent 6850 chromatograph) in a temperature gradient from 170 to 260 °C at 4 °C/min, and by GLC–MS using a Varian Saturn 2000 ion-trap instrument with the same column specifications. Retention times of derivatives are quoted relative to hexa-*O*-acetylglucitol (T_{GA} = 1.0).

For the identification and determination of configuration of the 2,3-diacetamido-2,3-dideoxyhexuronic acid components of the O-PS, the GLC–MS of their derived 2-(*S*)-butyl 4,6-di-*O*-acety1-2,3-diacetamido-2,3-dideoxy-hexopyranoside derivatives were prepared using the following sequence procedure: (1) methanolysis of O-PS (2–3 mg, 0.5 mL 10:1 MeOH–AcCl 90 °C, 2 h); (2) evaporation to dryness and acetylation (1:1 pyridine–Ac_2O 90 °C, 30 min); (3) evaporation to dryness, dissolution in EtOH (0.5 mL), and reduction with $NaBH_4$ (15 mg in 0.5 mL of water at 70 °C, 2 h); (4) neutralization (AcOH), concentration to dryness, and distillation from MeOH (3 × 2 mL); (5) butanolysis with 2-(*S*) butanol (0.5 mL) + AcCl (0.06 mL), at 90 °C, 2 h; (6) evaporation to dryness and acetylation (1:1 pyridine–Ac_2O 90 °C, 30 min), evaporation; (7) O-deacylation with 25% NH_4OH (60 °C, 30 min); (8) trimethylsilylation with BSTFA-TMSCl (0.5 and 0.2 mL in 0.5 mL of pyridine, 90 °C, 30 min), evaporation, GLC–MS analysis. The standards for this procedure were synthesized as previously described by Knirel et al.[16]

2.7. Results and discussion

Conventional phenol–water extraction[17] of flask grown *T. equigenitalis* type strain followed by collection of precipitated gels on ultracentrifugation yielded identical material from both phenol and water phases in ~6% total yield (based on dry weight cells), in which the water phase LPS was contaminated with ~10% of an amylopectin-like D-glucan. Hydrolysis of the LPS with hot dilute acetic acid afforded an insoluble lipid A, and Sephadex G-50 column chromatography of the water soluble products yielded O-PS having $[\alpha]_D$ −108.7 (*c* 0.3, water), and a core oligosaccharide having $[\alpha]_D$ −24.2 (*c* 0.2, water). Each fraction was further purified by anion-exchange chromatography.

Consideration of the PAGE–SDS analysis of the LPS together with the Sephadex G-50 chromatographic mobility (K_{av} 0.01–0.13) of the O-PS fraction indicates that it has an average molecular mass of ~20,000 Da corresponding to a composition of ~70 subsequently identified glycose residues.

Trial monosaccharide analyses of O-PS and core oligosaccharide showed the presence of only D-*glycero*-D-*manno*-heptose however, the expected major components, revealed from NMR data, were not detected in acid- or anhydrous HF-treated O-PS due to their polymeric stability.

^1H NMR spectra of the O-PS contained two major overlapping anomeric resonances (Fig. 1, Table 1). One signal centered at

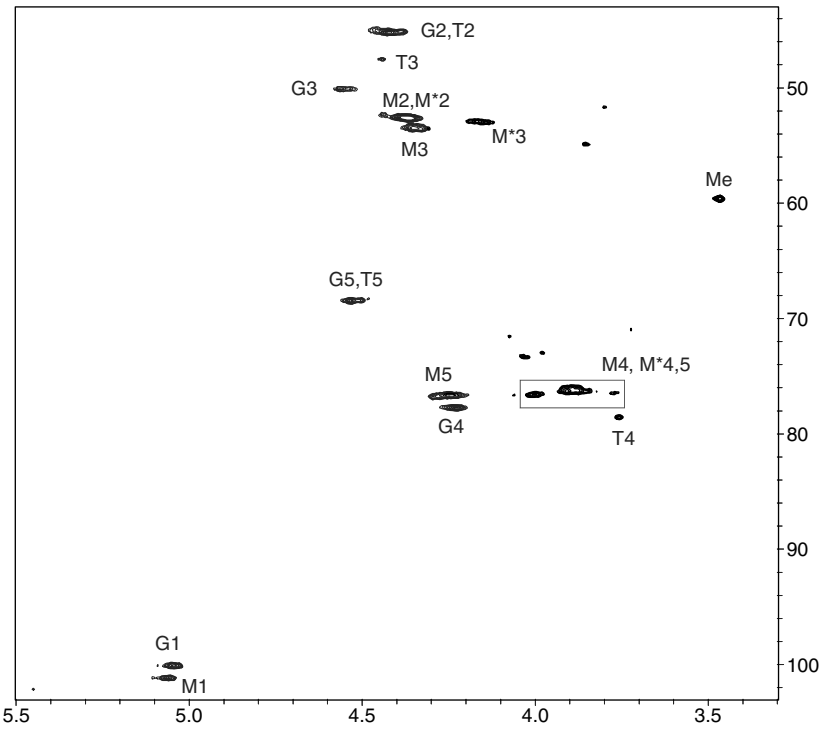

Figure 1. $^{1}H–^{13}C$ HSQC correlation of the *Taylorella equigenitalis* (ATCC 35865) LPS O-chain. **M*** denotes signals of the minor mannose residues $\mathbf{M}^1$, $\mathbf{M}^2$, $\mathbf{M}^3$.

Table 1
NMR data for *Taylorella equigenitalis* (ATCC 35865) LPS O-polysaccharide (D_2O, 40 °C)

Residue	H/C 1	H/C 2	H/C 3	H/C 4	H/C 5	H/C 6
M	5.03	4.37	4.34	3.90	4.22	
	101.1	52.5	53.5	76.1	76.7	173.1
G	5.05	4.42	4.51	4.21	4.45	
	100.1	45.1	50.1	77.6	68.7	172.8
T (4-OMeG)	5.04	4.38	4.44	3.76	4.50	
	100.1	45.1	47.4	78.5	68.7	
FucNAc4*N* **X**	4.60	3.79	4.16	3.85	3.98	1.33
		51.6		54.8	68.4	16.9
$\mathbf{M}^1$	5.08	4.44	4.17	3.84	4.07	
$\mathbf{M}^2$	4.82	4.39	4.14	3.77	4.00	
$\mathbf{M}^3$	4.72	4.37	4.14	3.77	4.00	

5.03 ppm belonged to the spin system of 2,3-diacetamido-2,3-dideoxymannopyranosyluronic acid (**M**), with the presence of amino groups at positions 2 and 3 being revealed by the chemical shifts of C-2 and C-3 (52.5, 53.5 ppm). The H-5 correlation in the HMBC with a carbonyl group (C-6) at 173.1 ppm confirmed that the residue is a hexuronic acid. Its *manno*-configuration followed from observed small couplings between protons H-1, H-2, and H-3 (<3 Hz) , and large couplings $J_{3,4}$ and $J_{4,5}$ ~10 Hz. The β-configuration of this residue followed from the low-field carbon chemical shifts of C-5 and from observed intraresidue NOEs of H-1:H-3 and H-1:H-5. The spectra additionally contained several minor non-overlapped spin-systems of the same monosaccharide (residues $\mathbf{M}^1$, $\mathbf{M}^2$, $\mathbf{M}^3$).

The second major anomeric proton signal at 5.05 ppm (residue **G**) also belonged to a 2,3-diacetamido-2,3-dideoxy-hexopyranosyluronic acid residue, similarly identified as described above. The spin system of **G** had small interproton coupling constants and hence could belong to either an α-*gulo*- or α-*talo*-hexopyranose. The carbon chemical shifts were in agreement with an α-*gulo*-configuration, and were in accord with data previously recorded for *Pseudomonas aeruginosa* serogroup O2 O-PSs.[18] The most characteristic signal for a 2-acetamido-2-deoxy-α-gulopyranoside is the high-field chemical shift of C-2 located at ~45 ppm that is not encountered in any other 2-acetamido-2-deoxyhexose. Residue **G** was therefore assigned the α-*gulo* configuration.

By comparison of the ^{13}C NMR chemical shifts of *T. equigenitalis* O-PS with those of *P. aeruginosa* polysaccharides data,[19] it could be concluded that gulo and manno-2,3-diacetamido-2,3-dideoxyhexuronic acids have different absolute configurations. We found that these monosaccharides could be easily converted into 2,3-diacetamido-2,3-dideoxyhexoses by $NaBH_4$ reduction of their methyl esters. Glycosidic linkages of 2,3-diacetamido-2,3-dideoxyhexoses were not as stable as those of the parent uronic acids and could be cleaved by treatment with 1 M HCl in optically pure 2-butanol, converting the monosaccharides into 2-butyl glycosides. The products were then acetylated or trimethylsilylated and analyzed by GLC–MS. In this way, the monosaccharides were for the first time identified chemically. Acetylated glycosides of the *manno*-sugar were also inseparable, but could be separated by GC as their TMS-derivatives, although even in this case separation was very close and for the identification of the components co-injection of the sample with a standard was necessary (interestingly, acetates of 2-butyl glycosides of *gluco*- and *galacto*-2,3-diacetamido-2,3-dideoxyhexoses are easily separated in GLC). Unfortunately, both acetylated and TMS (*R*)- and (*S*)-2-butyl derivatives of the *gulo*-sugar were not resolved by GLC. Thus, we established that 2,3-diacetamido-2,3-dideoxymannuronic acid has the D-configuration, and consequently 2,3-diacetamido-2,3-dideoxyguluronic acid has the L-configuration. This agrees well with the relatively high negative specific optical rotation of the O-PS ([α] −108.7).

The glycose 2,3-diacetamido-2,3-dideoxy-β-D-mannopyranosyluronic acid has been found in *P. aeruginosa* polysaccharides, and in several cases, like the components now reported in the O-PS of *T. equigenitalis,* are also accompanied by 2,3-diacetamido-2,3-dideoxy-α-L-gulopyranosyluronic acid residues.[20]

Mass spectrometric analysis of the O-PS under high ionization voltage, as described in Section 2.5, led to the observation of mono- and oligosaccharide fragments (up to pentasaccharide) of the polymer. The spectra showed that a significant percentage of the uronic acid residues were amidated. For the monosaccharide fragments, the signal of the amide of 2,3-diacetamido-2,3-dideoxyhexuronic acid at *m/z* 258 was twice the height of the signal of the non-amidated acid at *m/z* 259 (Fig. 2). For the disaccharide fragment, the signal of the acid-amide combination (*m/z* 516) was the most intense, and the observed amide-amide and acid-acid signals (*m/z* 515 and 517) were twice as small. All possible combinations of acid and amide functions were also observed in higher oligosaccharide fragments. The signal of the methylated monosaccharide (**T**) observed at *m/z* 273.6 indicated that it was present as a free acid. Since the intensity of peaks in mass spectra cannot be used for quantification because of the different ionization properties of varying fragments, however, the presence of a large amount of amidated uronic acid residues was clearly visible. The distribution of amidated residues within the chain remains undetermined.

NOE and HMBC correlations indicated that residues **M** and **G** substitute each other at position 4, thus forming the repeating unit [→4-**M**→4-**G**-]. Components $\mathbf{M}^1$, $\mathbf{M}^2$, and $\mathbf{M}^3$ as evidence for the sequence $\mathbf{M}^3 \rightarrow \mathbf{M}^2 \rightarrow \mathbf{M}^1$, ending with a residue of β-FucNAc4N. The latter residue was a component of the core region, and was identified as described below. Weak correlations of the fragment $\mathbf{M}^3 \rightarrow \mathbf{M}^2 \rightarrow \mathbf{M}^1$ did not allow tracking of the sequence to the repeating unit polymer.

One special residue of the 2,3-diacetamido-2,3-dideoxy-α-gulopyranosyluronic acid (residue **T**) was present at the non-reducing end of the O-PS polymer. This residue was methylated at *O*-4, leading to a downfield shift of its NMR C-4 signal and was consistent with the methyl signal showing NOE and HMBC correlations with H/C-4 of residue **G**.

The heterogenous nature of the O-PS and its resistance to periodate oxidation due to absence of 1,2-diol features are consistent with its observed weak silver staining and diffuse band appearance (Fig. 3) on PAGE analysis. The lane track of the *T. equigenitalis* LPS showed a close similarity with phylogenic related *Bordetella bronchiseptica* LPS[21] that reflects an O-PS diffuse structural similarity. The PAGE profiles of the LPSs of the pathogenically disease related bacteria *Oligella urethralis*[22] and *Alcaligenes faecalis*[23] suggest their essential rough LPS character lacking an O-PS component.

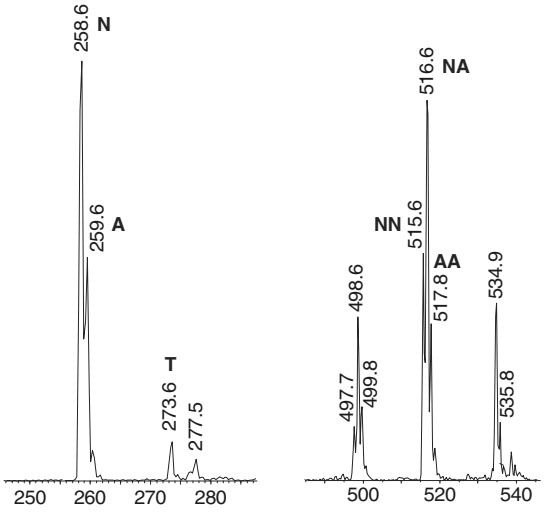

Figure 2. Positive mode high orifice voltage electrospray mass spectrum of the *T. equigenitalis* strain (ATCC 35865) O-polysaccharide. Major peaks correspond to $[M+H]^+$ glycosyloxonium ions. **N** indicates the amide of 2,3-diacetamido-2,3-dideoxy-hexuronic acid, **A** is the free acid, and **T** is derived from 4-*O*-methyl-2,3-diacetamido-2,3-dideoxy-hexuronic acid (terminal residue of the polysaccharide chain).

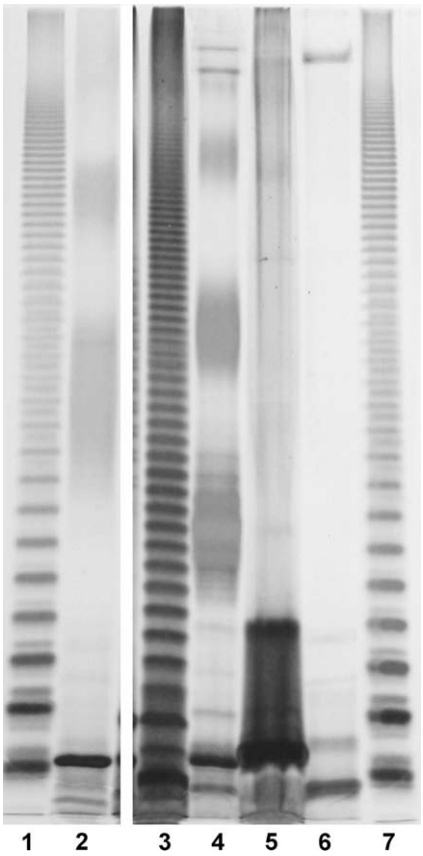

Figure 3. SDS–PAGE of lipopolysaccharides. Lanes 1 and 7, *Salmonella typhimurium*; Lane 2, *T. equigenitalis* (ATCC 35865); Lane 3, *T. asinigenitalis* (ATCC 700933); Lane 4, *Bordetella brochiseptica*; Lane 5, *Oligella urethalis*; and Lane 6, *Alicaligenes faecalis*.

A putative oligosaccharide product, isolated after AcOH hydrolysis of the LPS, represented the core region of the LPS, as deduced from the presence of characteristic aldoheptose residues. The core fraction was analyzed by NMR (Fig. 4, Table 2) and mass spectrometry. NMR spectra showed the presence of three D-*glycero*-α-D-*manno*-heptose residues, a 2-acetamido-4-amino-2,4,6-trideoxy-β-galactopyranose, and three 2,3-diacetamido-2,3-dideoxy-β-mannopyranosyluronic acids. Analysis of the linkages between these residues led to the conclusion that they are a mixture (2:1) differing only in the O-acetylation of the terminal 2,3-diacetamido-2,3-dideoxy-β-mannopyranosyluronic acid residue (Scheme 1). The signals of the Kdo residue, typically present at the reducing end of the oligosaccharide, were of very low intensity, and could not be reliably identified by NMR.

The positive mode ESI mass spectrum of the core fraction contained peaks of single and doubly charged ions, corresponding to masses of 1498, 1516, 1558, 1576 D ($Hep_3HexNNA_2dHexNN_1$-Kdo_1Ac_5 calculated mass 1517.3; with 6 acetyl groups with calculated mass 1559.4). The estimated number of the acetyl groups suggests that one amino group in the oligosaccharide is free. NMR of the O-deacylated core also showed five signals from *N*-acetyl groups. Furthermore, analysis of the HSQC spectra at pH 12 showed that the signal of H-4 of the 2-acetamido-4-amino-2,4,6-trideoxy-β-galactopyranose (residue **X**) moved 0.4 ppm upfield, whereas the other signals remained essentially unshifted. Thus, it is concluded that the amino group at C-4 of this monosaccharide is non-acylated. The structure of the core oligosaccharide was determined and is shown in Scheme 1.

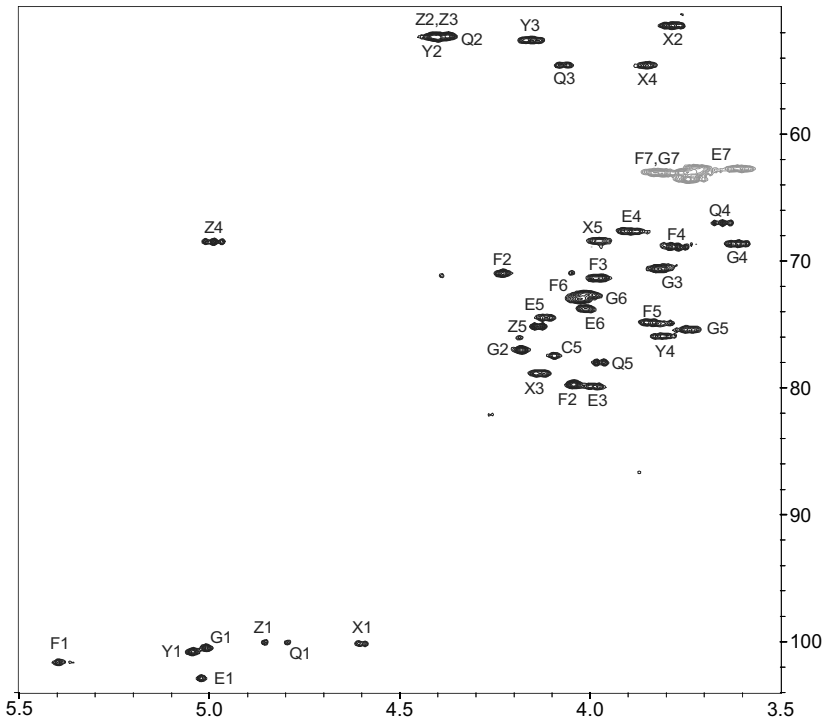

Figure 4. ^{1}H–^{13}C HSQC correlation of the *Taylorella equigenitalis* (ATCC 35865) LPS core.

Table 2
NMR data for *Taylorella equigenitalis* (ATCC 35865) LPS core oligosaccharide (D_2O, 35 °C). Signal of Kdo H/C-5: 4.09/77.4 ppm. Signals of *N*-acetates: 175.4–176.5 ppm; H-2/C-2: 1.91–2.06/22.9–23.3 ppm.

Residue	1	2	3	4	5	6	7
DDHep **E**	5.02	4.23	3.99	3.89	4.11	4.01	3.74/3.74
	102.8	71.0	79.8	67.6	74.4	73.7	63.5
DDHep **F**	5.39	4.04	3.98	3.77	3.84	4.02	3.74/3.82
	101.6	79.7	71.3	68.8	74.8	72.9	63.0
DDHep **G**	5.01	4.18	3.81	3.61	3.73	4.00	3.60/3.72
	100.4	77.0	70.5	68.6	75.3	72.6	62.7
FucNAc4N **X**	4.60	3.78	4.13	3.84	3.97	1.32	
	100.0	51.3	78.8	54.5	68.3	16.7	
ManNAc3NAcA **Y**	5.04	4.41	4.15	3.80	3.88		
	100.7	52.2	52.5	75.9		176.2	
ManNAc3NAcA **Q**	4.79	4.37	4.07	3.65	3.97		
	100.0	52.2	54.5	67.0	77.9	174.1	
4-*O*-Ac-ManNAc3NAcA **Z**	4.85	4.40	4.38	4.99	4.13		
	100.0	52.2	52.2	68.4	75.1	173.4	
Acetate on **Z4**		2.07					
	173.8	21.1					

In order to analyze the structure of the Kdo region of the LPS, an attempt was made to prepare completely deacylated LPS by strong alkaline treatment but this led to destruction of the sugar components.

The lipid component of the LPS, released by mild AcOH hydrolysis, was analyzed, and it appeared to be an unusual lipid A structure and will be reported in a separate communication.

The repeating unit of the O-chain of *T. equigenitalis* LPS has a strong similarity with the O-chains of several of *P. aeruginosa* serogroup O2 LPS,[20] differing only by the absence of a FucNAc residue and replacement of an acetimidoyl by an acetyl group at the 3 position of the parent diaminomannuronic acid. However, serologically these polysaccharides are probably unrelated because of the relatively short linear polymeric structures found in *T. equigenitalis* LPS. It is probable that only non-reducing terminal monosaccharides play an immunodominant role, and that antibodies directed to the inner residues of the polymer may not be significant epitopes. This hypothesis is based on a suggested analogy to the immunological findings of related structural features present in *B. bronchiseptica*, in which the non-reducing residues in their short O-PS were shown to play an immunodominant role.[21]

The O-PS containing repeating disaccharide units built of C-5 epimeric residues of β-D-Man*p*NAc3NAcA and α-L-Gul*p*NAc3NAcA have a structural relationship with alginates, in which the basic epimers, differing in configuration about C-5, are β-D-mannuronic acid and α-L-guluronic acid.[24] Biosynthetic pathways leading to the production of the *T. equigenitalis* O-PS have not been reported but it will be interesting to discover if the L-GulNAc3NAcA residues in the O-PS arise from precursor D-ManNAc3NAcA residues via the enzymic action of a C-5 epimerase in the way of alginate biosynthesis, and that of other bacterial polyuronic acid-containing polysaccharides.[25,26]

Polysaccharide:

(Terminal 4-O-Me)→ -[-4-α-L-GulNAc3NAcA-4-β-D-ManNAc3NAcA]-
G **M**

Minor components of the polysaccharide:

-4-β-D-ManNAc3NAcA-4-β-D-ManNAc3NAcA-4-β-D-ManNAc3NAcA-3-β-FucNAc4N-
M^3 **M^2** **M^1** **X**

Core oligosaccharide:

β-ManNAc3NAcA-4-β-ManNAc3NAcA-3-β-FucNAc4N-2-α-DDHep-2-α-DDHep-3-α-DDHep-
Kdo
Z/Q **Y** **X** **G** **F** **E**

Z is *O*-acetylated at O-4. Z:Q = 2:1.

Pseudomonas aeruginosa

2(a),2c

-4)-β-D-ManpNAc3NAmA-(1-4)-α-L-Gul*p*NAc3NAcA-(1-3)-β-D-Fuc*p*NAc-(1-

2(a),2d,2f

-4)-α-L-Gul*p*NAc3NAmA-(1-4)-β-D-Man*p*NAc3NAcA-(1-3)-α-D-Fuc*p*NAc4*O*Ac-(1-

Scheme 1. Structure of the O-specific polysaccharide and core region of the LPS from *T. equigenitalis* type strain (ATCC 35865) and repeating units of similar *P. aeruginosa* polysaccharides.

From the results of serological work, antisera directed toward the O-PS of *T. equigenitalis* (ATCC 35865) and clinical isolates have proved effective for the identification of the bacterial equine pathogen and for its discrimination from the related non-pathogenic *T. asinigenitalis* isolates.[27] This discrimination is not unexpected in view of our finding that the LPS O-antigen of *T. asinigenitalis* (ATCC 700933) is an unrelated polymer of a repeating disaccharide unit composed of (1→3)-linked residues of 2,4-diacetamido-2,4,6-trideoxy-β-D-glucose and 2-acetimidoylamino-2-deoxy-β-D-glucuronic acid.[27,28]

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