Chemical characterization of lipopolysaccharides from *Legionella feeleii*, *Legionella hackeliae* and *Legionella jordanis*

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Lipopolysaccharides (LPS) from *Legionella feeleii* serogroup 1, *L. hackeliae* serogroup 1 and *L. jordanis* were subjected to chemical analysis. All three LPS contained D-mannose, D-glucose, D-glucosamine, L-glycero-D-manno-heptose, 2-keto-3-deoxyoctonic acid and glycerol. In addition the LPS of *L. feeleii* was characterized by L-quinovose (tentatively identified) and L-fucosamine, *L. hackeliae* LPS by D-quinovosamine, D-galactosamine and D-galacturonic acid, and *L. jordanis* LPS by D-quinovosamine. Phosphorylated sugars were detected in all three LPS. The backbone sugar of the lipid A part was in each case 2,3-diamino-2,3-dideoxy-D-glucose substituted with a complex pattern of fatty acid, including 20–22 different amide-linked (non-branched and methyl-branched) 3-hydroxy fatty acids of chain-length ranging from 12 to 23 carbon atoms. The fatty acid patterns included also ester-linked nonhydroxylated entities and the uncommon 27-oxo-octacosanoic acid and 29-oxotriacontanoic acid. The LPS of *L. hackeliae* and *L. jordanis* also contained heptacosane-1,27-dioic and nonacosane-1,29-dioic acid, and their 2-hydroxy analogues were characteristic of *L. jordanis* LPS. SDS-PAGE patterns of the three LPS were distinctly different. Both *L. feeleii* and *L. jordanis* produced smooth-form LPS with characteristic ladder patterns, whereas *L. hackeliae* LPS were of more rough-type character.

Keywords: *Legionella*, lipid A, taxonomy, long-chain fatty acids

INTRODUCTION

Lipopolysaccharides (LPS) of species from genus *Legionella* appear to share a unique complex fatty acid pattern. Of the four *Legionella* LPS described all contain between 16 and 22 amide-linked 3-hydroxy fatty acids and several long-chain (ω -1)-oxo fatty acids (Sonesson *et al.*, 1989b, 1994; Moll *et al.*, 1992). This unique LPS composition correlates well with the distinct well-separated phylogenetic position (γ -2) of genus *Legionella* among Gram-negative taxa, as revealed by 16S rRNA sequence analysis (Woese *et al.*, 1985; Fry *et al.*, 1991).

As part of a chemotaxonomic study of *Legionella*, com-

parative analyses of LPS isolated from *L. feeleii*, *L. hackeliae* and *L. jordanis* were performed. These three LPS share high complexity in their fatty acid pattern but with distinct differences of practical potential for diagnostic application.

METHODS

Bacterial strains. *L. feeleii* serogroup 1 (ATCC 35072), *L. hackeliae* serogroup 1 (ATCC 35250) and *L. jordanis* (ATCC 33623) were grown (2 d, 37 °C) on buffered charcoal yeast extract (BCYE agar, Oxoid) and harvested as described previously (Sonesson *et al.*, 1989b). The cells of *Yersinia enterocolitica* Ye 128 and O:6,31 were grown and harvested as described by Jantzen & Lassen (1980).

Isolation of LPS. LPS was extracted from lyophilized bacterial cells using a combination of the hot phenol-water method (Westphal *et al.*, 1952) as modified by Johnson & Perry (1976), the phenol/chloroform/petroleum ether (PCP) method of Galanos *et al.* (1979) and finally by repeated washings with diethyl ether followed by chloroform/methanol (2:1, v/v) as described (Sonesson *et al.*, 1994).

The LPS was cleaved into lipid A and polysaccharide (PS) with

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Abbreviations: ASA, amino acid analyser; GlcN3N, 2,3-diamino-2,3-dideoxy-D-glucose; HPAEC, high-pH anion-exchange chromatography; HVPE, high-voltage paper electrophoresis; Kdo, 2-keto-3-deoxyoctonic acid; PED, pulsed electrochemical detector; PCP, phenol/chloroform/petroleum ether solvent; PS, polysaccharide; TFA, trifluoroacetyl; TMS, trimethylsilyl-.

Fig. 1. (a) Silver-stained glycine SDS-PAGE of LPS from *S. abortusequi* S-type (lane 1, 0.8 µg), *L. feeleii* (lanes 2, 2 µg, and 3, 5 µg), *L. jordanis* (lanes 4, 3 µg, and 5, 6 µg), *S. minnesota* Ra-type (lane 6, 1 µg), *S. helsinki* SR-type (lane 7, 1 µg) applied to the 16.5% acrylamide gel. (b) *S. abortusequi* S-type (lane 1, 0.8 µg), *L. feeleii* (lanes 2, 2 µg, and 3, 5 µg) and *L. hackeliae* (lanes 4, 1 µg, and 5, 4 µg) applied to a 18% acrylamide gel.

1% acetic acid at 100 °C for 2 h (Wilkinson *et al.*, 1973), and the lipid A part was isolated as a pellet after centrifugation (1000 g). The PS part was recovered from the supernatant as described (Sonesson *et al.*, 1994).

A smooth (S)-type reference LPS of *Salmonella abortusequi* was a gift from Dr O. Lüderitz, Max-Planck-Institut für Immunbiologie, Freiburg, Germany, and *S. helsinki* SR LPS as well as *S. minnesota* Ra LPS, were supplied by Dr I. Helander, National Public Health Institute, Helsinki, Finland.

Analytical methods. The analytical methods and instrumental conditions applied in this study have been described in detail recently (Moll *et al.*, 1992; Sonesson & Jantzen, 1992; Sonesson *et al.*, 1989b, 1994).

Neutral and amino sugars were quantified both as alditol acetate derivatives and as trifluoroacetyl (TFA) methyl glycosides by GC, and as free sugars by high-pH anion-exchange chromatography (HPAEC) with pulsed electrochemical detection (PED). Amino sugars were also analysed by high-voltage paper electrophoresis (HVPE), TLC and amino acid analyser (ASA). Total hexosamine content was determined by the Morgan-Elson reaction as modified by Strominger *et al.* (1959). 2-Keto-3-deoxyoctonic acid (Kdo) was quantified after methanolysis (2 M HCl in methanol, 60 °C, 2 h) and TFA derivatization by GC (Bryn & Jantzen, 1986), and also colorimetrically by the thiobarbituric acid reaction (Waravdekar & Saslaw, 1959) after hydrolysis with 0.1 M sodium acetate buffer pH 4.4 (100 °C, 1 h) (Brade *et al.*, 1983). Uronic acids were determined by HPAEC-PED, GC and by the modified carbazole method (Bitter & Muir, 1962) and their identification was also supported by GC-MS of their 6,6-dideuterio-substituted alditol acetate derivatives (Dudman *et al.*, 1983). Phosphorylated sugars were determined indirectly by recording the increase of GC peak area after dephosphorylation of samples with aqueous 48% (w/w) hydrofluoric acid at 4 °C for 72 h (Sonesson *et al.*, 1989a). Phosphorylated sugars were also detected by HPAEC-PED, ASA and by GC analysis of trimethylsilyl (TMS) derivatized methyl glycosides (Zamze *et al.*, 1987). Absolute configurations

of the sugars were determined by GC analysis of 2-(R)-butyl and 2-(S)-butyl glycoside diastereomeric derivatives (Gerwig *et al.*, 1978) after TFA derivatization and retention time comparison to authentic standards.

Fatty acids were determined as methyl esters by GC. Hydroxyl groups were acetylated, TFA- or TMS-derivatized prior to GC analyses.

Analyses by GC were performed using fused-silica columns and flame-ionization detection. Besides retention time comparison, the identification of sugars and fatty acids was verified by GC-MS.

PAGE. SDS-PAGE was carried out as described by Laemmli (1970) using a 4% (w/v) stacking gel and a 16% or 18% separating gel. Gels were either silver stained for LPS (Tsai & Frasch, 1982), or with Coomassie brilliant blue for detection of proteins (Reisner *et al.*, 1975).

Miscellaneous estimations. Phosphate was determined by the method of Lowry *et al.* (1954); nucleic acids were determined by ultraviolet absorption at 260 nm. RNA contamination was also indicated by GC analyses of ribose. Protein was measured by the Lowry method using serum albumin as standard. Presence of ornithine-containing lipids was examined by GC analysis of ornithine (Sonesson *et al.*, 1988).

RESULTS

General properties of the LPS

LPS of *L. feeleii* and *L. hackeliae* were recovered from the waterphase after the hot phenol-water extraction whereas *L. jordanis* LPS was isolated from the phenolic phase. All three LPS preparations were further purified by the PCP extraction method. Addition of water to the phenolic PCP extracts failed to induce precipitation of LPS. Instead, LPS of *L. feeleii* and *L. hackeliae* precipitated upon addition of ethanol, and addition of diethyl ether (Moll *et al.*, 1992).

Table 1. Constituents of the polysaccharide part of *Legionella* LPS

LPS results are expressed as nmol per mg LPS dry weight. For comparison the PS values are given relative to the LPS values by setting the D-mannose values equal. Corresponding absolute values (nmol per mg dry weight of PS) can be obtained by using a factor of 2.38 for *L. feeleii*; 2.48 for *L. hackeliae*; and 2.03 for *L. jordanis*.

Component	<i>L. feeleii</i>		<i>L. hackeliae</i>		<i>L. jordanis</i>	
	PS	LPS	PS	LPS	PS	LPS
Glycerol	ND	8	14	14	19	50
Glycerol phosphate	5	7	ND	ND	37	49
L-Rhamnose	ND	ND	1	3	14	18
L-Quinovose*	215	231	ND	ND	ND	ND
D-Mannose	369	369	99	99	107	107
D-Glucose	18	14	4	2	36	45
D-Glucose phosphate	ND	ND	63	57	31	34
D-Glycero-D-manno-heptose	28	25	ND	ND	14	13
L-Glycero-D-manno-heptose	44	49	2	1	33	31
L-Glycero-D-manno-heptose phosphate	ND	ND	4	4	ND	ND
Kdo	35	42	78	91	38	46
Galacturonic acid†	ND	ND	131	125	ND	ND
D-Glucosamine	165	196	208	241	123	157
D-Glucosamine-phosphate	386	352	ND	ND	168	179
D-Galactosamine	ND	ND	219	235	ND	ND
D-Galactosamine-phosphate	ND	ND	205	219	ND	ND
D-Quinovosamine‡	ND	ND	930	1042	1442	1614
L-Fucosamine‡	559	593	ND	ND	ND	ND
GlcN3N	ND	246	ND	210	ND	197
Total hexosamine§	NP	422	NP	1191	NP	1250
Kdo colorimetric	NP	56	NP	58	NP	41
Total phosphate	423	514	314	636	196	432

ND, Not detected; NP, not performed.

* Tentatively identified.

† Measured by the modified carbazole method.

‡ Quantified as alditol acetate derivative by GC using the same response factor as for glucosamine.

§ Total hexosamine content obtained by the Morgan-Elson reaction.

|| Measured by the thiobarbituric reaction test after hydrolysis in 0.1 M acetate (pH 4.4) at 100 °C for 60 min.

induced precipitation of *L. jordanis* LPS. The final LPS preparations of *L. feeleii* and *L. hackeliae* were soluble in water. In contrast, *L. jordanis* LPS was insoluble in water but completely soluble in chloroform/methanol (2:1, v/v). The yields of LPS (% bacterial dry weight) were 0.7% for *L. feeleii*, 1.8% for *L. hackeliae* and 2.1% for *L. jordanis*, respectively.

The preparation of *L. jordanis* LPS was essentially free from nucleic acids (< 0.1% by weight) and protein (< 0.1% by weight). Corresponding values for *L. feeleii* LPS were 2% and 3% and for *L. hackeliae* LPS 3% and 2%, respectively. Ornithine (marker for ornithine-containing lipids) and palmitoleic acid (phospholipid marker) were undetectable by GC in all three LPS preparations.

Cleavage of LPS yielded 22% of lipid A and 51% of PS for *L. feeleii* (of LPS dry weight); corresponding values

for *L. hackeliae* were 15% and 63%, and for *L. jordanis* 27% and 47%, respectively.

The SDS-PAGE profiles of the three LPS preparations were distinctly different (Fig. 1). *L. feeleii* LPS showed a characteristic S-type form LPS profile with distinct ladder-like bands covering both low and high molecular mass regions (Fig. 1). *L. jordanis* LPS also exhibited a ladder pattern but the spaces between the bands were somewhat larger and the range smaller (Fig. 1a). *L. hackeliae* LPS gave a more rough (R)-form type profile with a major band in the low molecular mass region (Fig. 1b).

Chemical composition of the three LPS

The chemical compositions of the LPS isolates are presented in Tables 1 and 2. The analyses accounted for 69% of *L. feeleii* LPS, 73% of *L. hackeliae* LPS and 76%

Table 2. Constituents of the lipid A part of *Legionella* LPS

LPS results are expressed as nmol per mg LPS dry weight. For comparison the lipid A values are given relative to the LPS values by setting the 3-OH-i14:0 values equal for *L. feeleii*; the 3-OH-n20:0 values equal for *L. hackeliae*; and the 3-OH-a15:0 values equal for *L. jordanis*. Corresponding absolute values (nmol per mg dry weight of lipid A) can be obtained by using a factor of 2.24 for *L. feeleii*; 2.14 for *L. hackeliae*; and 2.30 for *L. jordanis*.

Component	<i>L. feeleii</i>		<i>L. hackeliae</i>		<i>L. jordanis</i>	
	Lipid A	LPS	Lipid A	LPS	Lipid A	LPS
Glycerol	8	8	12	14	4	50
Glycerol-phosphate	ND	7	9	14	ND	49
D-Glucosamine	24	196	21	241	38	157
Glc3N3	236	246	229	210	207	197
Total phosphate	104	431	220	636	155	432
Amide-linked FA*						
3-OH-n12:0	3.9	4.1	2.7	3.0	2.1	2.3
3-OH-n13:0	3.7	3.5	3.8	4.1	4.6	4.9
3-OH-i14:0	73	73	72	75	57	59
3-OH-n14:0	54	50	33	36	21	19
3-OH-a15:0	31	29	74	76	103	103
3-OH-n15:0	2.3	2.2	1.9	2.0	2.4	2.5
3-OH-i16:0	ND	ND	3.1	3.0	4.5	4.3
3-OH-n16:0	5.9	5.8	5.9	6.1	7.7	7.6
3-OH-a17:0	4.0	3.8	3.1	2.9	1.8	1.9
3-OH-n17:0	0.8	0.7	2.9	2.7	1.9	1.7
3-OH-i18:0	ND	ND	2.2	2.0	7.6	7.4
3-OH-n18:0	39	42	12	10	19	20
3-OH-a19:0	1.8	1.9	1.6	1.5	30	29
3-OH-n19:0	5.1	5.4	4.1	3.8	7.3	7.2
3-OH-i20:0	6.2	5.9	0.5	0.5	5.6	5.4
3-OH-n20:0	69	71	87	87	48	45
3-OH-i21:0	1.8	2.0	3.4	3.1	ND	ND
3-OH-a21:0	4.7	5.1	12	10	31	29
3-OH-n21:0	22	20	9.1	8.8	6.3	5.9
3-OH-i22:0	2.3	2.4	2.3	2.1	5.2	5.1
3-OH-n22:0	48	45	39	37	2.8	2.6
3-OH-a23:0	2.6	2.7	3.0	2.8	ND	ND
Sum amide-linked FA	381	376	379	379	369	363
Ester-linked FA normal chain length						
i14:0	2.2	2.8	ND	ND	0.9	1.3
a15:0	5.6	5.8	13	17	34	39
n15:0	3.3	3.4	1.8	2.0	ND	ND
i16:0	26	30	22	27	18	21
n16:0	55	61	32	37	1.2	1.4
a17:0	15	17	23	27	19	23
n17:0	ND	ND	1.0	0.9	ND	ND
n18:0	3.1	3.5	1.8	1.9	1.0	1.1
n20:0	5.8	6.0	1.5	1.8	1.1	1.2
Sum	116	130	96	115	75	88
Long chain length†						
27-OH-n28:0	14	14	trace	trace	3.7	5.1
27-oxo-n28:0	214	238	113	122	145	164
29-oxo-n30:0	31	37	55	58	46	50
n27:0-dioic	ND	ND	17	15	7.8	10

Table 2 (cont.)

Component	<i>L. feeleyi</i>		<i>L. hackeliae</i>		<i>L. jordanis</i>	
	Lipid A	LPS	Lipid A	LPS	Lipid A	LPS
n29:0-dioic	ND	ND	108	112	8.3	11
2-OH-27-oxo-n28:0	ND	ND	ND	ND	50	57
2-OH-29-oxo-n30:0	ND	ND	ND	ND	13	16
2-OH-n27:0-dioic	ND	ND	ND	ND	18	20
2-OH-n29:0-dioic	ND	ND	ND	ND	19	22
Sum	259	289	293	309	311	355
Sum ester-linked FA	375	419	389	422	386	443
Ratio amide/ester linked FA	1.0	0.9	1.0	0.9	1.0	0.8

ND, Not detected.

* Fatty acids are designated by the number of total carbon atoms with prefixes 'a' for anteiso-branching, 'i' for iso-branching, 'n' for normal chain and OH for hydroxyl substitution with the position indicated.

† Long-chain fatty acids were quantified using the response factor of the methyl ester derivative of n27:0, n28:0 and n30:0 relative to n22:0 (internal standard).

of *L. jordanis* LPS, dry weight respectively. All three LPS preparations were rich in phosphate and contained phosphorylated sugars (see below). Ethanolamine pyrophosphate was detected by ASA (not quantified) in LPS of *L. hackeliae* and *L. jordanis*.

Neutral sugars. All three LPS contained D-mannose, D-glucose and L-glycero-D-manno-heptose and glycerol (Table 1). The heptose isomer D-glycero-D-manno-heptose was also identified in LPS of *L. feeleyi* and *L. jordanis*, and minor amounts of L-rhamnose was found in LPS of *L. hackeliae* and *L. jordanis*. Phosphorylated neutral sugars detected included glycerol phosphate in LPS of *L. feeleyi* and *L. jordanis*, glucose phosphate in *L. hackeliae* and *L. jordanis*, and L-glycero-D-manno-heptose phosphate in *L. hackeliae* LPS. Due to phosphate migration during hydrolysis (both α - and β -glycerophosphate were detected) and the lack of standards, the phosphate position of the phosphorylated sugars could not be determined.

L. feeleyi LPS contained significant amounts of an unusual compound (X). GC-MS analysis indicated a 6-deoxyhexose structure but the retention time differed from that of commonly occurring 6-deoxyhexoses, L-rhamnose and L-fucose (Lindberg, 1990). LPS of *Y. enterocolitica* Ye 128, reported to contain 6-deoxy-L-altrose (Hoffman *et al.*, 1980), and LPS of *Y. enterocolitica* serovar 6,31, reported to contain 6-deoxygulose (Kalmykova *et al.*, 1988), were isolated. Three different derivatives were prepared from hydrolysates/methanolysates of these LPS preparations as well as for the commercial available 6-deoxy-D-glucose: alditol acetates, acetyl and TFA methyl glycosides. The retention times of the different 6-deoxyhexose derivatives were compared to that of compound X. The derivatives of 6-deoxyaltrose eluted clearly ahead of those from X, whereas derivatives of 6-deoxyglucose and 6-deoxygulose had approximately the

same retention as X. By co-injection with preparations of *L. feeleyi* LPS (using both nonpolar and polar columns) the derivatives of 6-deoxyglucose (quinovose) were shown to possess retention characteristics identical to those for X, whereas the derivatives of 6-deoxygulose did not fully coincide. Other 6-deoxyhexose configurations (i.e. *allo*, *ido* and *talo*) all have GC retention properties clearly distinct from the *gluco* configuration (Voiland & Michel, 1985) and were therefore not investigated. The TFA 2-(R)-butyl glycoside diastereomer derivative of D-quinovose and the TFA 2-(S)-butyl glycoside diastereomer derivative of X showed identical retention times, whereas corresponding derivatives of 2-(S)-butyl D-quinovosides and 2-(R)-butyl glycosides of X had divergent retention times. These results tentatively indicated L-quinovose as a major neutral sugar constituent of *L. feeleyi* LPS. NMR studies are needed to confirm the *gluco* configuration.

Amino sugars. D-Glucosamine was present in all three LPS. Glucosamine phosphate was detected in LPS of *L. feeleyi* and *L. jordanis* and in 66 and 50 mol% of total glucosamine content, respectively. D-Galactosamine was only present in *L. hackeliae* LPS and about 50 mol% was phosphorylated (Table 1).

All three LPS contained a 2-amino-2,6-dideoxyhexose as a major constituent. *L. feeleyi* LPS contained L-fucosamine, identified by ASA, HPAEC-PED and GC whereas LPS of *L. hackeliae* and *L. jordanis* contained the isomer D-quinovosamine.

The presence of 2,3-diamino-2,3-dideoxy-D-glucose (GlcN3N) in all three LPS was verified by HVPE and ninhydrin staining. Identical retention and characteristic orange-brown staining (Mayer *et al.*, 1990) to the GlcN3N standard were obtained. Corresponding results were

obtained by TLC. Retention time and mass spectra obtained by GC-MS were identical to that of the GlcN3N standard, the electron impact (EI) mass spectrum in accordance with that reported by Roppel *et al.* (1975).

The total hexosamine values obtained by the Morgan-Elson reaction were lower than the total molar amounts of unphosphorylated hexosamine and 2-amino-2,6-dideoxyhexosamine obtained by GC.

Acidic sugars. Kdo was identified as the TFA methyl glycoside derivative by GC-MS in all three LPS investigated. Molar amounts of Kdo obtained by the thio-barbituric acid reaction were in approximately the same range (Table 1) and no increased amounts of Kdo were obtained when analysing dephosphorylated LPS. Attempts to detect a 2-keto-octonic acid-Kdo disaccharide, as found in LPS of *L. israelensis* and *L. micdadei* (Sonesson *et al.*, 1994), were negative. Galacturonic acid was detected in *L. hackeliae* LPS by HPAEC-PED and GC-MS, and was quantified by the modified carbazole method. No uronic acids were detected in LPS of *L. feeleii* and *L. jordanis*, either by chromatographic techniques or by the colorimetric assay performed.

Fatty acid composition

The fatty acids identified included fatty acids with methyl-branched (anteiso and iso) and linear carbon chains (Table 2). Both LPS of *L. feeleii* and *L. hackeliae* contained eight nonhydroxy fatty acids with palmitic acid as the major one, whereas *L. jordanis* LPS contained seven and with 12-methyltetradecanoic acid as the most abundant.

Twenty different 3-hydroxy fatty acids were identified in LPS of *L. feeleii* and *L. jordanis*; two more were detected in *L. hackeliae* LPS, in the range C₁₂ to C₂₃. The major 3-hydroxy acid in *L. feeleii* LPS was 3-hydroxy-12-methyltridecanoic acid, in *L. hackeliae* LPS 3-hydroxycosanoic acid and in *L. jordanis* LPS 3-hydroxy-12-methyltetradecanoic acid.

The long-chain oxo acids 27-oxo-octacosanoic acid and 29-oxotriacontanoic acid occurred in all three LPS. Dioic long-chain acids (i.e. heptacosane-1,27-dioic and nonacosane-1,29-dioic acid) were identified in LPS of *L. hackeliae* and *L. jordanis*. In addition, *L. jordanis* LPS contained the 2-hydroxy analogues of these four acids. Their identification has been published separately (Moll *et al.*, 1992; Sonesson *et al.*, 1993). LPS of *L. feeleii* and *L. jordanis* were also found to contain 27-hydroxyoctacosanoic acid. 27-Oxo-octacosanoic acid was the most prominent fatty acid in all three LPS (Table 2).

The 3-hydroxy fatty acids were all found to be bound in stable (presumably amide) linkages whereas the nonhydroxy acids and the long-chain hydroxy, oxo and dioic acids were all ester-linked. The molar distribution of the fatty acid classes in LPS of *L. feeleii* were 17% nonhydroxy acids, 47% 3-hydroxy fatty acids and 36% long-chain acids. Corresponding molar fatty acid values in LPS of *L. hackeliae* were 14%, 47% and 39%, and in LPS of *L. jordanis* 11%, 45% and 44%, respectively.

Composition of lipid A preparations

Three sugars were identified in the lipid A preparations, mainly GlcN3N and minor amounts of glucosamine and glycerol (Table 2). The molar proportion between GlcN3N and glucosamine was approximately 10:1 in lipid A of *L. feeleii* and *L. hackeliae*, and 5:1 in lipid A of *L. jordanis*. Hydrofluoric acid treatment did not increase the amounts of GlcN3N and glucosamine, only glycerol in lipid A of *L. hackeliae* increased. Molar amounts of phosphate in the lipid A preparations were moderate.

The fatty acid profiles of native LPS were grossly recovered in the lipid A preparations. The analyses accounted for 69%, 68% and 72% of the lipid isolates (dry weight) of *L. feeleii*, *L. hackeliae* and *L. jordanis* LPS, respectively.

Composition of the polysaccharide parts

With the exception of GlcN3N, the sugars determined in the native LPS preparations were also found in the PS part preparations (Table 1). The molar proportions suggested that minor losses of neutral sugars and some degradation of Kdo occurred during cleavage of LPS. The phosphorylated sugars identified in the LPS preparations were components of the PS chains and the PS isolates were found to be rich in phosphate. No fatty acids were detected in the PS preparations.

The analyses accounted for (of PS dry weight) 85% of the *L. feeleii* PS, 91% of the *L. hackeliae* PS and 78% of the *L. jordanis* PS, respectively.

DISCUSSION

L. feeleii serogroup 1, *L. hackeliae* serogroup 1 and *L. jordanis* belong to the more frequently occurring human pathogenic species of *Legionella*. All three have been isolated from patients who contracted Legionnaires' disease or pontiac fever (Fang *et al.*, 1989). Only *L. feeleii* can be identified relatively easily, being oxidase and β -lactamase negative, and unable to liquefy gelatin (Brenner, 1987). The distinguishing phenotypic features of *L. hackeliae* and *L. jordanis*, on the other hand, are sparse. Also fatty acid profiles (Mayberry, 1992; Jantzen *et al.*, 1993) and ubiquinone patterns of profiles of *L. hackeliae* and *L. jordanis* are quite related and typical of several *Legionella* species. Those of *L. feeleii* are more specific, and especially its ubiquinone pattern appears to be a useful diagnostic feature (Lambert & Moss, 1989; Wilkinson *et al.*, 1990).

As found for other LPS of *Legionella* species (Moll *et al.*, 1992; Sonesson *et al.*, 1989b, 1994), the LPS of this study were characterized by a remarkably complex pattern of fatty acids including long-chain (ω -1)-oxo fatty acids (Table 2). The long-chain dioic acids found in *L. hackeliae* and *L. jordanis* appear not to be as general LPS constituents of *Legionella* as 27-oxo-octacosanoic acid, which so far has been found in LPS of 15 *Legionella* species (unpublished results). The 2-hydroxy-27-oxo-octacosanoic acid, 2-hydroxy-29-oxotriacontanoic acid, 2-hydroxyheptacosane-1,27-dioic acid and 2-

hydroxynonacosane-1,29-dioic acid found in *L. jordanis* LPS were also detected in LPS of *L. maceachernii* and *L. micdadei* (Sonesson *et al.*, 1994), whereas the 27-hydroxyoctacosanoic acid constituent of *L. feeleyi* and *L. jordanis* LPS was originally identified in LPS of *Rhizobium trifolii* (Hollingsworth & Lill-Elghanian, 1989). 27-Hydroxyoctacosanoic acid and other (ω -1)-hydroxy fatty acids are described as chemotaxonomic hallmarks of species belonging to the α -2 branch of *Proteobacteria* (Bhat *et al.*, 1991), but appear thus also to be characteristic of several *Legionella* species, of the γ -2 branch of *Proteobacteria*.

The main lipid A backbone sugar was found to be GlcN3N for all three LPS. This sugar has so far only been encountered in nature as a lipid A backbone sugar and is a hallmark of *Nitrobacter* and *Rhodospseudomonas* as the sole lipid A sugar. Lipid A backbones consisting of GlcN3N and additional small amounts of glucosamine are characteristic of other genera such as *Chromatium* and *Bradyrhizobium* (Mayer *et al.*, 1990). The LPS of this study appear to belong to the latter group in this respect since minor, apparently nonstoichiometric, amounts of glucosamine were identified in the lipid A preparations. Low amounts of glycerol were also detected and its origin appears not to have been phospholipids since palmitoleic acid (phospholipid marker) was not detected in lipid A or LPS preparations.

The sugar compositions of the three LPS were quite different from one another. Generally, amino sugars dominated quantitatively, and the presence of a 2-amino-2,6-dideoxyhexose as well as D-glucosamine, in *L. hackeliae* also D-galactosamine, was noteworthy. Several rare LPS sugar constituents were identified. L-Quinovose, tentatively identified in *L. feeleyi* LPS, has so far not been reported as a LPS constituent (Lindberg, 1990). Galactosamine phosphate, found in PS of *L. hackeliae* LPS, is also uncommon. Glucose phosphate, present in LPS of *L. hackeliae* and *L. jordanis*, has also been found in LPS of other *Legionella* species (Sonesson *et al.*, 1989b, 1994), in *Acinetobacter calcoaceticus* LPS (Kawahara *et al.*, 1987) and in *Pseudomonas pavonacea* (Wilkinson *et al.*, 1973). Glycerol phosphate, recently reported in the O-chain of *Y. kristensenii* LPS (L'vov *et al.*, 1992), was found in all three LPS of this study and has previously also been detected in other *Legionella* LPS (Sonesson *et al.*, 1994). Galacturonic acid, a common component of bacterial extracellular PS and LPS (Lindberg, 1990), was identified in *L. hackeliae* LPS and represents the first uronic acid detected in LPS of *Legionella*.

In studies of whole-cell sugar composition of *L. jordanis* an O-methyl pentose and an unusual hexose were reported (Fox *et al.*, 1990); these components were not detected in the LPS preparation of *L. jordanis*.

In contrast to LPS of *L. feeleyi* and *L. hackeliae*, *L. jordanis* LPS partitioned exclusively into the phenolic phase during extraction and was only partially soluble in water but completely soluble in chloroform/methanol (2:1, v/v). Similar hydrophobic character also characterizes LPS of *L. pneumophila* (Conlan & Ashworth, 1986;

unpublished results), but was nevertheless fairly surprising due to the S-type character of *L. jordanis* LPS revealed by SDS-PAGE (Fig. 1). At least six bands were evident, separated by relatively large spatial distances indicating a large repeating O-chain unit, probably with D-quinovosamine as the main constituent. *L. feeleyi* LPS also showed a typical S-type ladder pattern with at least 24 bands, whereas *L. hackeliae* LPS revealed a more R-type character.

A relation between phylogenetic grouping based on 16S rRNA sequence data and LPS composition has been demonstrated for bacteria of the α -2 and α -3 subdivisions of *Proteobacteria* (Bhat *et al.*, 1991; Mayer *et al.*, 1990). The results obtained so far indicate that also the γ -2 branch of *Proteobacteria*, which includes the species of *Legionella*, can be described by a unique LPS composition, with the complex fatty acid substitution of lipid A as the most outstanding chemotaxonomic hallmark. However, both the fatty acid and the sugar patterns appear to contain several markers useful for species differentiation. Further chemical studies of *Legionella* LPS are in progress to extend this chemotaxonomic description of the genus.

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