JOURNAL OF BACTERIOLOGY, July 1988, p. 3217–3222
0021-9193/88/073217-06$02.00/0

Vol. 170, No. 7

Lipopolysaccharides of *Thiocystis violacea*, *Thiocapsa pfennigii*, and *Chromatium tepidum*, Species of the Family *Chromatiaceae*

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Received 10 July 1987/Accepted 23 February 1988

The lipopolysaccharides (LPS) of three species of purple sulfur bacteria (*Chromatiaceae*), *Thiocystis violacea*, *Thiocapsa pfennigii*, and the moderately thermophilic bacterium *Chromatium tepidum*, were isolated. The LPS of *Thiocystis violacea* and *Chromatium tepidum* contained typical O-specific sugars, indicating O-chains. Long O-chains were confirmed for these species by sodium deoxycholate gel electrophoresis of their LPS. *Thiocapsa pfennigii*, however, had short or no O-chains. The core region of the LPS of all three species comprised D-glycero-D-mannoheptose as the only heptose and 2-keto-3-deoxyoctonate. The lipid A, obtained from the LPS by mild acid hydrolysis, contained glucosamine as the main amino sugar. Amide-bound 3-hydroxymyristic acid was the only hydroxy fatty acid. The main ester-bound fatty acid in all lipid A fractions was 12:0. Mannose and small amounts of 2,3-diamino-2,3-dideoxy-D-glucose were common constituents of the lipid A of the three *Chromatiaceae* species investigated. All lipid A fractions were essentially free of phosphate.

Lipopolysaccharide (LPS) analyses are useful for taxonomic considerations (20). The conservative structures of the lipid A and the somewhat less conservative core regions have also confirmed their value for questions of the genetic relationships among bacteria (18, 30). The phototrophic bacteria have gained special importance because many genera of gram-negative chemotrophic bacteria are more closely related to certain groups of purple non-sulfur bacteria than are the groups of purple non-sulfur bacteria among themselves (5, 7, 33). The species of non-sulfur purple bacteria seem to be of particularly high divergence and are intermixed with many different genetically related lines of nonphototrophic species. The purple photosynthetic bacteria and their relatives (32) have been found to contain unusual lipid A types which in many cases correlate with the similarity coefficients obtained by comparison of the 16S rRNA oligonucleotide catalogs (18, 30).

In contrast to purple non-sulfur bacteria, purple sulfur bacteria (*Chromatiaceae*) are a genetically more closely related family of bacteria (4), in spite of the morphological diversity of their various species. LPS analyses of *Chromatiaceae* are restricted to only two species, *Chromatium vinosum* (8, 9) and *Thiocapsa roseopersicina* (10). The LPS of these two species share typical properties, such as D-mannose as a constituent of the phosphate-free lipid A and the occurrence of D-glycero-D-mannoheptose as the only heptose in the core region. The present study reveals that the suggested genetic relationship of *Chromatiaceae* species is further substantiated by the chemical composition of the O-antigens of additional *Chromatiaceae*, such as *Thiocystis violacea*, *Thiocapsa pfennigii*, and the moderately thermophilic bacterium *Chromatium tepidum*.

MATERIALS AND METHODS

Sources of organisms. *Chromatium tepidum* MC was kindly provided by M. T. Madigan, University of Southern Illinois at Carbondale. *Thiocystis violacea* 2711 and *Thiocapsa pfennigii* 9111 were taken from the stock collection in Konstanz, Federal Republic of Germany.

Media and growth conditions. *Chromatium tepidum* MC was grown photoheterotrophically in the medium described by Madigan (17), modified as follows. A mixture containing (amounts are in grams per liter) NH_4COOCH_3 (0.5), NH_4Cl (0.4), $MgSO_4 \cdot 7H_2O$ (0.3), $CaCl_2 \cdot 2H_2O$ (0.05), $FeSO_4 \cdot 7H_2O$ (0.01), and yeast extract (1) was adjusted with 0.5 M NaOH to pH 7.0. After autoclaving the mixture, we added the following (per liter): (i) 5 ml of filter-sterilized KH_2PO_4 (10 g in 1 liter, adjusted to pH 7.0 with 1 M NaOH); (ii) 25 ml of sterile filtered $Na_2S \cdot 9H_2O$ (24 g in 1 liter) plus $NaHCO_3$ (40 g in 1 liter) adjusted to pH 7.0 with 2 M H_2SO_4; (iii) 1 ml of a separately autoclaved solution of (amounts are in milligrams per liter) $MnSO_4 \cdot 1H_2O$ (1,592), $Na_2MoO_4 \cdot 2H_2O$ (752), $ZnSO_4 \cdot 7H_2O$ (240), $CuNO_3 \cdot 3H_2O$ (40), NaCl (4,000), $(NH_4)_2SO_4$ (4,000), thiamine (4), and biotin (60). *Thiocystis violacea* 2711 and *Thiocapsa pfennigii* were grown in 5-liter bottle cultures as described in reference 4. Instead of SL10, the trace element solution SL12 was used; it had the following composition (milligrams per liter): EDTA disodium salt, (3,000), $FeSO_4 \cdot 7H_2O$ (1,100), $CoCl_2 \cdot 6H_2O$ (190), $MnCl_2$ (50), $ZnCl_2$ (42), $NiCl_2 \cdot 6H_2O$ (24); $Na_2MoO_4 \cdot 2H_2O$ (18), H_3BO_3 (300), $CuCl_2 \cdot 2H_2O$ (2). (The trace solution was adjusted to pH 6.0, and 1 ml per liter of medium was used.)

Mass cultures of all three strains were harvested at the late exponential growth phase and washed once with distilled water before lyophilization for storage at −20°C.

Isolation of LPS and capsule material. LPS was obtained from lyophilized cells (5 to 10 g [dry weight]) by the phenol-chloroform-petroleum ether method (6). Alternatively, bacteria were treated with 45% aqueous phenol-water at 68°C for 30 min as described in reference 31. After phase separation by centrifugation (2,500 × *g*, 10°C, 30 min) and dialysis against running tap water, the water and phenol phases were separately centrifuged at 105,000 × *g* (4°C, 4 h, three times) and the respective supernatants and sediments were lyophilized.

Capsule material was removed from *Thiocapsa pfennigii*

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9111. Cells (30 g [dry weight] of freshly harvested cells) were subjected to Ultra-Thurrax treatment with mild shear forces (5 times, 4°C, 1 min each time) to remove capsule material; the cells remained intact. After centrifugation (65,000 × *g*, 30 min), precooled acetone (4°C) was added to the supernatant. Precipitated material was sedimented at 4,000 × *g* (4°C, 30 min) and washed with distilled water before lyophilization.

Degradation of LPS. LPS (50 mg) was hydrolyzed under stirring in sealed glass tubes in 10 ml of 5% (*Thiocystis violacea* and *Chromatium tepidum*) and 10% (*Thiocapsa pfennigii*) acetic acid at 100°C for 1.5 h (acid strength depended on the strain). Degraded polysaccharide and lipid A were obtained by centrifugation at 2,500 × *g* (4°C, 30 min). Lipid A (pellet) was washed twice with 1 ml of warm (50°C) water and once with 1 ml of acetone. Lipid A and degraded polysaccharide were separately lyophilized.

Analytical chemical determinations. Neutral sugars and uronic acids were liberated by 0.5 M H_2SO_4 at 100°C for 4 h [neutralization was by $Ba(OH)_2$] or 0.1 M HCl at 100°C (48 h) for gas-liquid chromatography of neutral sugars. Amino sugars and amino acids were liberated in 4 M HCl at 105°C for 18 h. Neutral sugars were separated by thin-layer chromatography (the solvent system was acetic anhydride-pyridine-water, 12:5:4 [vol/vol]) and determined quantitatively as their alditol acetate derivatives (22) by gas-liquid chromatography (Varian Aerograph 1400-1; ECNSS-M; glass column [2 mm by 1.5 m]; 3% on Gas-Chrom Q; 100/200 mesh). Mass spectrometric fragmentation of the alditol acetates of neutral and amino sugars (reduced with $NaBD_4$) was performed in a Finnigan MAT combined gas-liquid chromatography–mass spectrometry automatic system, model 1020 B, with a CP-SIL-5 or Poly-A-103 column (11). Mass spectra were taken at 70 eV in the mass range of 43 to 400 *m/e* in 1 s.

Gas-liquid chromatographic determination of amino sugars as alditol acetate derivatives (19, 22) was performed on a fused-silica capillary SE-54 column (length, 50 m; inner diameter, 0.25 mm; column temperature, 250 to 280°C in a 1°C/min program; injector temperature, 300°C; carrier gas, nitrogen).

Fatty acids were liberated in methanolic HCl (30 ml of concentrated HCl in 150 ml of methanol; 100°C) and determined as methyl esters by gas-liquid chromatography on an EGSS-X column (15% on Gas-Chrom P; 100/200 mesh; 165°C, isothermal; carrier gas, nitrogen) and on an SE-54 column (length, 50 m; inner diameter, 0.25 mm; column temperature, 200 to 285°C in a 4°C/min program; injector temperature, 300°C; carrier gas, nitrogen). The same column was used for combined gas-liquid chromatography–mass spectrometry of the fatty acid methyl esters. Amide or ester binding of fatty acids was examined by hydroxylaminolysis (24) or alkaline transesterification (treatment of LPS with sodium methylate) (35). In the latter method, liberated fatty acids were identified by gas-liquid chromatography before and after methyl ester derivatization with diazomethane (12) to differentiate between acyloxyacyl residues and fatty acids bound to OH groups of the amino sugar backbone.

2-Keto-3-deoxyoctonate was liberated by 0.5 M H_2SO_4 at 100°C for 10 min (1 h in the case of *Thiocapsa pfennigii* 9111) and determined as described in reference 28. Staining of 2-keto-3-deoxyoctonate on high-voltage electropherograms was as described in reference 2. Uronic acids or amino sugars were separated by high-voltage paper electrophoresis (13) in pyridine-formic acid-acetic acid-water buffer (2:3:20:180 [vol/vol]) at pH 2.8 and in pyridine-acetic acid-water buffer (10:4:86 [vol/vol]) at pH 5.3 and stained with alkaline silver nitrate (26) or with ninhydrin (0.2% in acetone). Total uronic acids were colorimetrically determined (1). Quantitative determination of amino sugars (except 2,3-diamino-2,3-dideoxy-D-glucose) and amino acids was performed in an automatic amino acid analyzer equipped with a BT 7040 sample injector (Biotronik, Munich, Federal Republic of Germany) as described elsewhere (3). Organic phosphorus was determined by the method of Lowry et al. (15).

DOC-PAGE. Polyacrylamide gel electrophoresis (PAGE) was performed with sodium deoxycholate (DOC) as the detergent (14). The running gel consisted of 13% acrylamide, 0.35% bisacrylamide, 0.5% DOC, and 375 mM Tris hydrochloride buffer at pH 8.8, the stacking gel was 4% acrylamide, 0.1% bisacrylamide, 0.5% DOC, and 125 mM Tris hydrochloride at pH 6.8. The sample buffer contained 0.25% DOC, 10% glycerol, and 175 mM Tris hydrochloride at pH 6.8 and the electrode buffer contained 0.25% DOC, 192 mM glycine, and 26 mM Tris hydrochloride at pH 8.4. After pre-electrophoresis at 25 mA, the samples (0.1% in sample buffer) were separated at 18 mA (stacking gel) and 24 mA (running gel) with fresh buffer. Silver staining was done as described in reference 27.

RESULTS

Thiocapsa pfennigii. The LPS of *Thiocapsa pfennigii* 9111 was extractable by the phenol-chloroform-petroleum ether method (yield, 0.7% of cell dry mass). On application of the hot phenol-water method, it was preferentially extracted into the water phase (yield, about 2% of cell dry weight). This fraction contained a large glucan moiety, despite α-amylase treatment (Table 1). With the exception of this glucan, the chemical compositions of the LPS isolated by the two methods were comparable. They included a heptose. The alditol acetate derivative of the heptose had a retention time of 4.7 (relative to xylitol acetate on the ECNSS-M column), identical to authentic D-glycero-D-mannoheptose. Heptose was also confirmed by mass spectrometric fragmentation of its $NaBD_4$-reduced alditol acetate, yielding characteristic primary fragments at *m/e* 145, 146, 217, 218, 289, 290, 361, and 362 and secondary fragments at *m/e* 331 [amn 433-(CH_3COOH + $H_2C{=}C{=}O$)] and 332 [amn 443-(CH_3COOH + $H_2C{=}C{=}O$)]. The presence of 2-keto-3-deoxyoctonate was confirmed by periodate-thiobarbituric acid assay (28) and by high-voltage paper electrophoresis. The latter technique indicated (tentatively) the presence of glucuronic acid. Glucosamine and glycine were observed in the amino acid analyzer (Table 1).

An additional amino sugar was detectable on high-voltage paper electropherograms on silver-nitrate staining. It comigrated with 2,3-diamino-2,3-dideoxy-D-glucose from *Rhodopseudomonas viridis* (21) and showed characteristic yellow-brown staining with ninhydrin. Identity with 2,3-diamino-2,3-dideoxy-D-glucose was obtained on separation of the respective amino sugar alditol acetate derivatives on an SE-54 fused silica capillary column (retention time, [t_R] with inositol acetate, 1.63), while glucosamine eluted with a t_R with inositol acetate of 1.36. Gas chromatographic-mass spectrometric analysis of the deuterium-reduced alditol acetate yielded fragments also described for the 2,3-diamino-2,3-dideoxy-D-glucose from *R. viridis* (21). Mass spectrometric fragmentation yielded primary fragments at *m/e* 145 and 288 in addition to corresponding secondary fragments at *m/e* 85, 103, 126, 127, 144, 168, 169, 186, and 228. Secondary fragments derived from the characteristic primary fragment at *m/e* 216 were observed at *m/e* 156 (*m/e* 216-CH_3COOH) and *m/e* 114 [*m/e* 216-(CH_3COOH + $H_2C{=}C{=}O$)].

TABLE 1. Chemical composition of LPS from *Thiocystis violacea* 2711, *Thiocapsa pfennigii* 9111, and *Chromatium tepidum* MC[a]

Constituent	nmol/mg (dry wt) of fraction in[b]:								
	Thiocystis violacea			*Thiocapsa pfennigii*			*Chromatium tepidum*		
	LPS	Degraded polysaccharide	Lipid A fraction	LPS	Degraded polysaccharide	Lipid A fraction	LPS	Degraded polysaccharide	Lipid A fraction
2-*O*-Methyl-6-deoxyhexose	241	342	5	—	—	—	—	—	—
Rhamnose	786	1,156	13	52	56	—	727	1,047	82
Ribose	23	28	—	—	—	—	57	68	17
Mannose	1,299	1,769	408	16	—	71	1,434	1,636	505
Galactose	189	215	19	—	—	—	103	143	14
Glucose	133	84	103	923[c]	1,049[c]	658[c]	23	27	8
D-Glycero-D-mannoheptose	86	95	—	35	42	—	168	226	Trace
2-Keto-3-deoxyoctonate	38	+	—	31	ND	ND	79	ND	ND
Glucuronic acid	139	183	ND	322	459	—	—	—	—
Galacturonic acid	—	—	—	—	—	—	68	87	ND
Glucosamine	105	15	401	58	—	236	113	20	346
2,3-Diamino-2,3-dideoxy-D-glucose	+	—	+	+	—	+	+	—	+
Quinovosamine[d]	71	35	22	—	—	—	92	95	—
3-OH-14:0	78	—	364	93	—	228	195	—	840
12:0	185	—	872	78	—	173	48	1	291
14:0	Trace	—	17	Trace	—	10	3	1	6
16:0	16	Trace	54	14	Trace	46	16	7	47
18:0	Trace	—	54	Trace	Trace	14	10	6	23
Phosphate	—	—	—	101	151	Trace	42	83	6
Glycine	—	—	—	41	52	—	—	—	—

[a] Extraction from whole cells was by hot phenol-water. Degradation into lipid A and degraded polysaccharide was performed by 5% acetic acid (10% for *Thiocapsa pfennigii*) at 100°C for 1.5 h.
[b] —, Absent; +, present but not quantified; ND, not determined.
[c] Mainly due to contaminating glucan.
[d] Small amounts of an additional amino sugar (t_R with glucosamine, 1.11 on the amino acid analyzer) were observed.

The major fatty acids were 3-OH-14:0 and 12:0, aside from traces of other fatty acids (Table 1). Hydroxylaminolysis revealed 3-OH-14:0 as amide bound and 12:0 as ester bound. 3-Acyloxyacyl residues were not detectable. 3-OH-14:0 was confirmed by mass spectrometric fragmentation of its methyl ester [base peak at *m/e* 103 and characteristic fragment at *m/e* 208; *m/e* 258 (M^+)-$(CH_3OH + H_2O)$].

Degradation of the LPS of *Thiocapsa pfennigii* required 10% acetic acid (100°C, 1.5 h). The lipid A and degraded polysaccharide fractions were both obtained in approximately 15% yields (of LPS dry weight). Enrichment of mannose (relative to the neutral sugars) was obtained in the lipid A fraction (Table 1). Lipid A contained essentially all of the glucosamine, all 2,3-diamino-2,3-dideoxy-D-glucose, and all fatty acids of the LPS but was nearly free of phosphate. The degraded polysaccharide contained the heptose, 2-keto-3-deoxyoctonate and glucuronic acid and little of other compounds.

It should be mentioned that galacturonic acid was found in large amounts in the material shed from freshly harvested whole cells by Ultra-Thurrax treatment (yield, about 2% of bacterial dry weight). In addition, this fraction contained the following (amounts are in nanomoles per fraction [dry weight]): xylose (82), rhamnose (35), mannose (331), galactose (1,009), glucose (1,298), and phosphate (90). The fraction was essentially free of glucosamine and thus of LPS.

The DOC-PAGE pattern of the *Thiocapsa pfennigii* LPS, obtained by phenol-chloroform-petroleum ether and phenol-water-extractions, showed only one major core band in the size range of R-type LPS (Fig. 1). O-chains were either lacking or present in only small amounts.

Thiocystis violacea. The LPS from *Thiocystis violacea* 2711 was preferentially extracted into the water phase of hot

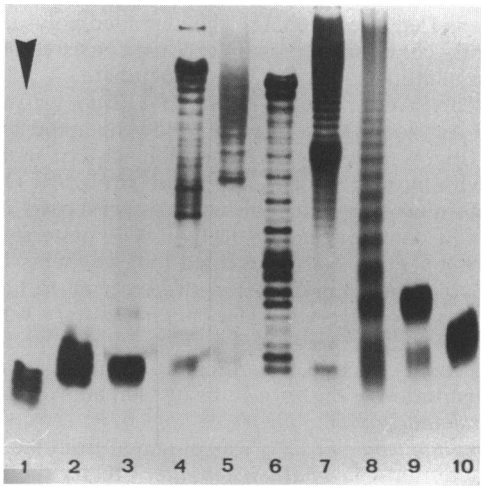

FIG. 1. DOC-PAGE (13%) and silver staining of the LPS from *Chromatiaceae* and *Salmonella* species (as molecular weight markers). Lanes: 3, *Thiocapsa pfennigii* 9111; 4, *Thiocystis violacea* 2711; 5, *Thiocapsa roseopersicina* (10); 6, *Chromatium tepidum* MC; 7, *Chromatium vinosum* D (9); 1, Re-mutant *Salmonella minnesota* R595; 2, Rc-mutant *Salmonella minnesota* R5; 8, S-form *S. typhimurium* var. *Copenhagen*; (9), SR-mutant *Salmonella typhimurium* SH777; 10, Ra-mutant *Salmonella typhimurium* his386. For the structure of the marker LPS, see reference 16. The following amounts of material were applied (lanes): 1, 2, 9, and 10, 1 μg; 8, 3 μg; 3 and 5, 5 μg; 4, 6, and 7, 7.5 μg. The arrow shows the direction of migration.

phenol-water extracts. The yields (sediments of respective ultracentrifugations) were about 1% of the cell dry weight. LPS was not successfully extractable by the phenol-chloroform-petroleum-ether method. The main neutral sugars in the LPS were mannose and rhamnose, in addition to galactose and glucose. An unknown sugar, which eluted at a t_R with xylitol acetate of 0.37, showed a mass spectrum as the deuterium-reduced alditol acetate with primary fragments at *m/e* 275, 118, and 87 and the secondary fragment at *m/e* 173 (derived from *m/e* 275 by loss of CH_3COOH [amn 60] and $H_2C{=}C{=}O$ [amn 42]), thus revealing it to be a 2-0-methyl-6-deoxyhexose. In addition, a heptose with a t_R identical to that of the D-glycero-D-mannoheptose from *Thiocapsa pfennigii* (see above) was observed. 2-Keto-3-deoxyoctonate and glucuronic acid were (tentatively) identified by the same methods used for *Thiocapsa pfennigii*. Glucosamine, quinovosamine, and traces of an unknown amino sugar (Table 1) were observed in the amino acid analyzer. The presence of small amounts of 2,3-diamino-2,3-dideoxy-D-glucose were confirmed as described for *Thiocapsa pfennigii* (see above).

Aside from small amounts of other fatty acids, amide-bound 3-OH-14:0 and ester-bound 12:0 were the main fatty acids. There was no indication of glucosamine-bound 3-acyloxyacyl residues. The phosphate content of the LPS of *Thiocystis violacea* was negligible.

The ultracentrifugation (105,000 × *g*, 4°C, 4 h) supernatant of the water phase of phenol-water extracts (see above) was free of non-LPS compounds, except for RNA and glucans. Uronic acids were found only in trace amounts. Thus, there was no hint of a capsular polysaccharide.

Degradation of the LPS to obtain free lipid A and degraded polysaccharide required 5% acetic acid (100°C, 1.5 h). The yields were 10% (lipid A) and 70% (degraded polysaccharide) of the LPS dry weight. The lipid A fraction contained nearly all of the glucosamine and 2,3-diamino-2,3-dideoxy-D-glucose, as well as all of the fatty acids, of the LPS. Mannose was highly enriched in the lipid A fraction. The lipid A was free of phosphate. The degraded polysaccharide was slightly contaminated by lipid A and consisted of neutral sugars in addition to 2-keto-3-deoxyoctonate.

The DOC-PAGE pattern of the LPS of *Thiocystis violacea* revealed very long O-chains with a wide gap in the ladderlike pattern (Fig. 1 and 2). The migration pattern indicated a R-core with most of the core stubs substituted by O-chains. The pattern was multimodal, with at least two intensity maxima. In comparison, the DOC-PAGE pattern of *Thiocapsa roseopersicina* also revealed long O-chains but with no distinct limitation in chain length. A gap in the ladderlike pattern was also observed. The banding pattern was multimodal, with one major and probably two minor banding sets. The only faintly stained R-core band indicated almost complete substitution of the core stubs by O-chains in *Thiocapsa roseopersicina* as well.

Chromatium tepidum. LPS was obtained from the thermophilic bacterium *Chromatium tepidum* MC by the hot phenol-water procedure. It was preferentially extracted into the water phase. The yield in the sediment after ultracentrifugation was low, about 0.6% (after removal of contaminating glucan and nucleic acids by enzymatic treatments). The LPS contained a number of hexoses (Table 1), mainly rhamnose and mannose. The alditol acetate of the heptose eluted identically to D-glycero-D-mannoheptose on gas-liquid chromatography (see above). Galacturonic acid was the only uronic acid detected on high-voltage electropherograms. Quinovosamine and an unknown amino sugar like that detected in *Thiocystis violacea* (t_R with glucosamine, 1.11)

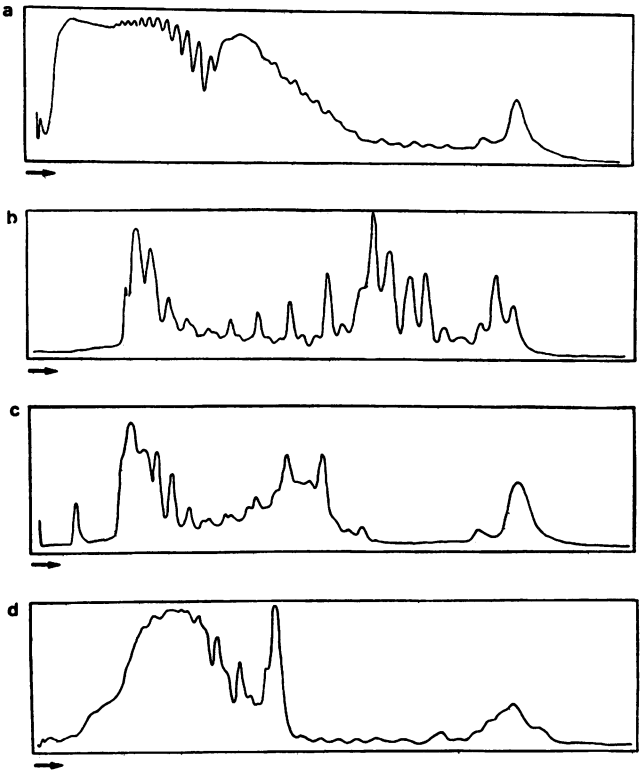

FIG. 2. Densitograms of electrophoretic migration patterns of some of the LPS separated by DOC-PAGE (Fig. 1). Panels: a, *Chromatium vinosum* D (9); b, *Chromatium tepidum* MC; c, *Thiocystis violacea* 2711; d, *Thiocapsa roseopersicina* (10). The arrows show the direction of migration.

were observed in the amino acid analyzer in addition to glucosamine. As with the other two species, 2,3-diamino-2,3-dideoxy-D-glucose) was observed on high-voltage paper electropherograms. The thiobarbituric acid assay with the LPS was positive, indicating the presence of 2-keto-3-deoxyoctonate.

The fatty acid composition showed 3-OH-14:0 as the dominant fatty acid, aside from 12:0 and small amounts of additional fatty acids. Liberation of fatty acids by alkali cleaved off all fatty acids except 3-OH-14:0, which thus is amide bound. Alkaline transesterification by treatment with sodium methylate gave no indication of 3-acyloxyacyl residues.

There was no indication of capsule polysaccharide in *Chromatium tepidum* MC, since the supernatant of ultracentrifugation of the water phase of the hot phenol-water extracts was free of non-LPS compounds except glucan and RNA. However, the supernatant contained a rather large protein moiety (total, about 500 nmol of amino acid residues per mg [dry weight]), with glutamic acid, glycine, alanine, serine, and aspartic acid dominating.

The LPS was split into degraded polysaccharide and lipid A upon application of 5% acetic acid at 100°C for 1.5 h. The yields were 10% (lipid A) and 60% (degraded polysaccharide) of the LPS dry weight. Glucosamine, together with the fatty acids, was highly enriched in the lipid A fraction. Significant enrichment, relative to the other neutral sugars, was also obtained for mannose, indicating that mannose is a lipid A constituent. The lipid A fraction contained only traces of phosphate. The degraded polysaccharide fraction

also contained mannose, in addition to the total of the residual neutral and acidic sugars, as well as quinovosamine. It contained no 2,3-diamino-2,3-dideoxy-D-glucose, which was only found in lipid A (Table 1).

Long O-chains with a distinct maximum chain length in the LPS were revealed by DOC-PAGE (Fig. 1 and 2). One major set of bands was observed in addition to two minor ones. Only a single prominent R-core band was detected, indicating an R-core in the size range of the *Salmonella* Rc chemotype. Long O-chains were attached to the core in the LPS of *Chromatium tepidum*. The DOC-PAGE of the LPS of *Chromatium vinosum* D, performed as a comparison, also showed very long O-chains. Almost complete substitution of the core by O-chains was indicated by the finding of only a faint band in the core region.

DISCUSSION

The LPS of *Thiocystis violacea* and the moderately thermophilic bacterium *Chromatium tepidum* contain very long O-chains, as indicated by the patterns of DOC-PAGE. The core stubs seem to be heavily substituted. Extensive banding on sodium dodecyl sulfate gels, characterizing O-chains with repeating units, have already been described for *Chromatium vinosum* and *Thiocapsa roseopersicina* in reference 8. An exception was *Thiocapsa pfennigii* in which rough-type LPS was indicated by the DOC-PAGE pattern. Large amounts of glucose, as revealed by chemical analysis, are assumed to be of high-molecular-weight glucan origin. It was difficult to remove glucan; therefore, the LPS fraction obtained remained contaminated to a certain degree.

Long O-chains are also expressed by other members of the gamma subgroup of the purple bacteria (34), for example, by the *Enterobacteriaceae* (16). In contrast, several purple non-sulfur bacteria of the alpha subgroup (33) have short O-chains or lack them completely (29). Gaps in the ladder-like pattern of DOC-PAGE in *Thiocystis violacea* and *Thiocapsa roseopersicina* may indicate S-layers, as published for *Thiocapsa floridana* (synonym, *Thiocapsa roseopersicina* [25]) (23) and as indicated by DOC-PAGE for some *Rhodopseudomonas palustris* strains (J. H. Krauss et al., unpublished data). The finding of long O-chains is in full agreement with the low or failing extractability of the LPS from most *Chromatiaceae* studied so far by the phenol-chloroform-petroleum ether method, which is known to extract R-type LPS preferentially (6). Accordingly, the LPS of *Thiocapsa pfennigii*, which lack O-chains, was extractable by this method. A capsular polysaccharide with a composition different from that of the LPS was detected in this study by Ultra-Thurrax treatment of the cells of this species.

The core regions of the LPS of the *Chromatiaceae* species studied so far, *Chromatium vinosum*, *Chromatium tepidum*, *Thiocystis violacea*, *Thiocapsa roseopersicina*, and *Thiocapsa pfennigii*, contain all D-glycero-D-mannoheptose as the only heptose and 2-keto-3-deoxyoctonate (8, 10; this study). Thus, a common core structure may be assumed for all of them. At least in the case of R-type LPS from *Thiocapsa pfennigii*, uronic acids are part of the core. This may be the reason for the need for 10% acetic acid to split off lipid A and the low amount of 2-keto-3-deoxyoctonate. This very acid-labile compound is partially destroyed under conditions of acid hydrolysis. Furthermore, it is well known that uronic acid-containing LPS are very resistant to complete hydrolysis.

There are also significant similarities among the lipid A compositions of the *Chromatiaceae* species studied so far. In accordance with previous findings on *Chromatium vinosum* and *Thiocapsa roseopersicina* LPS (9, 10), the lipid A from *Thiocystis violacea*, *Chromatium tepidum*, and *Thiocapsa pfennigii* contain glucosamine as the main amino sugar. The structural role of 2,3-diamino-2,3-dideoxy-D-glucose, present in small amounts in all three species examined in this study and also in *Chromatium vinosum* and *Thiocapsa roseopersicina* (H. Mayer, unpublished data), remains to be elucidated. The lipid A fractions are free of phosphate and contain mannose. Furthermore, there are similarities among all of the species, even in the fatty acid content in that 3-OH-14:0 is the only amide-bound fatty acid and most of them contain 12:0 as the predominant ester-bound fatty acid. Thus, a common lipid A structure may be likely, at least for the species of *Chromatiaceae* investigated so far.

The similarities in LPS composition, including those of the conservative lipid A and R-core structure, are in full agreement with the results of 16S rRNA cataloging (4). These data suggest that the *Chromatiaceae* genera represent a genetically coherent cluster of species. There is no further resolution of the genera or species on the basis of lipid A or core composition, since it was possible in many cases with the purple non-sulfur bacteria (18, 29, 30), although many of the genetically more distant *Chromatiaceae* species (34) remain to be studied.

ACKNOWLEDGMENTS

We gratefully acknowledge combined gas-liquid chromatography–mass spectrometry analysis by D. Borowiak, amino acid analysis by U. J. Jürgens, and technical help by E. Metz.

The work was supported by the Deutsche Forschungsgemeinschaft.

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