

Characterization of the Cell Wall and Outer Membrane of *Rhodopseudomonas capsulata*

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Received 17 January 1984/Accepted 28 March 1984

Sucrose density gradient centrifugation of cell envelopes of chemotrophically grown cells of *Rhodopseudomonas capsulata* St. Louis (= ATCC 23782) resulted in the separation of a cytoplasmic membrane from a cell wall fraction (buoyant densities, 1.139 and 1.215 g/cm³, respectively). The cell wall fractions (untreated or Triton extracted) contained peptidoglycan- and lipopolysaccharide-specific components. Their neutral sugar content, mainly rhamnose and galactose, was high (250 and 100 µg/mg [dry weight] of material) due to a non-lipopolysaccharide polymer. The fatty acid content was low (≤60 µg/mg [dry weight] of material), and half of it was contributed by lipopolysaccharide (3-OH-C_{10:0}, C_{12:1}, and 3-oxo-C_{14:0}). The predominant other fatty acid was C_{18:1}. An outer membrane fraction, obtained by lysozyme treatment of the Triton-extracted cell wall, showed essentially the same chemical composition except for almost complete removal of peptidoglycan. Saline extraction (0.9% NaCl, 37°C, 2 h) removed a lipopolysaccharide-protein(-phospholipid?) complex from whole cells of *R. capsulata* St. Louis. The polypeptide patterns of the cell wall and outer membrane as revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis comprised 20 to 25 different polypeptides (most of them very faint) and were dominated by a single, heat-modifiable major protein (M_r 69,000 after solubilization below 60°C; M_r 33,000 at temperatures above 70°C).

Cell walls of phototrophic bacteria are of the gram-negative type and, thus, are comprised of a peptidoglycan layer and an outer membrane. Isolated cell wall fractions have been obtained from *Rhodospirillaceae* species such as *Rhodospirillum rubrum* (3, 28) and *Rhodopseudomonas sphaeroides* (2, 7) as well as from the *Chromatiaceae* species *Chromatium vinosum* (15, 18, 19). Peptidoglycan-specific constituents, lipopolysaccharide, phospholipids, and proteins have been found in respective preparations (10, 43). An increasing number of reports are available on the characterization of the isolated major outer membrane proteins of *C. vinosum* (18, 19) and *R. sphaeroides* (4-6).

The cell wall of *Rhodopseudomonas capsulata* deserves special notice. Strain-specific bacteriophages (39) and a so-called "gene transfer agent" (38), a defective phage, are known for this species. An unusually high susceptibility of *R. capsulata* cells to β-lactam antibiotics was reported (40). Our knowledge of this wall, however, is restricted to the chemical composition of lipopolysaccharides from three different strains of *R. capsulata* (29, 41). The presence of external cell envelope layers in *R. capsulata* was indicated light microscopically by the use of India ink (40). Recently, the capsule and slime polysaccharides were visualized electron microscopically by the use of specific antisera against two *R. capsulata* strains (30).

In this paper, the isolation and analytical-chemical characterization of the cell wall of *R. capsulata* St. Louis (= ATCC 23782), including the polypeptide pattern of the outer membrane, are reported. A heteropolysaccharide, very likely the capsule of the strain, is present in large amounts in cell wall fractions.

MATERIALS AND METHODS

Bacteria. *R. capsulata* St. Louis was kindly provided by H. Gest, Indiana University, Bloomington. For cell enve-

lope preparation, bacteria were grown chemotrophically in R8AH medium (8) in a New Brunswick Microferm fermentor. Cells were harvested at late exponential growth phase (optical density at 660 nm, about 1.6) and washed once with distilled water.

Extraction of bacteria in saline. Cells were grown phototrophically in the light and harvested at an optical density at 660 nm of about 1.5. With modifications, extraction of cells in saline was done as described previously (42). Freshly harvested cells were suspended in saline (0.9% NaCl, 5 mM NaN₃) and incubated at 37°C for 2 h with slight stirring. Cells were removed by centrifugation at 10,000 × *g* for 30 min. Large cell debris was sedimented by centrifugation at 30,000 × *g* for 30 min, and the supernatant was concentrated in an evaporator. Centrifugation at 105,000 × *g* for 4 h (three times) resulted in the sedimentation of gel-like material which, for chemical determinations (not for polyacrylamide gel electrophoresis [PAGE]), was lyophilized.

Isolation of the various cell envelope fractions. The methods used for isolation of the cell envelope and the cell wall fractions were, with modifications, as reported by Oelze et al. (28). Freshly harvested cells were suspended in buffer (20 mM Tris-hydrochloride [pH 8.0], 0.3 M sucrose, 2 mM EDTA) at about 1 g of wet cells per 3 ml and frozen at -20°C for 2 to 3 days. Thawed cells were broken at 0°C by double passage through a French pressure cell at 16,000 lb/in². DNase and RNase were added before the second passage. All of the following steps were carried out at 4°C in 20 mM Tris-hydrochloride, pH 8.0. The homogenate was diluted with 3 volumes of cold buffer and centrifuged twice at 7,500 × *g* for 20 min; the latter supernatant was centrifuged at 64,000 × *g* for 1 h. The final sediment representing the cell envelope fraction (about 20 mg of protein in 3 ml of buffer) was loaded onto a discontinuous sucrose density gradient, 55:43:35:19% (wt/wt) sucrose in 5:4:8:5 ml of buffer, respectively, and centrifuged at 113,000 × *g* for 90 min. The lower bands (pooled) and the upper band of the three distinct bands obtained were isolated separately, dialyzed against buffer at

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4°C for 24 h, and recovered by centrifugation at $100,000 \times g$ for 1 h. The density gradient centrifugation was repeated once with the isolated fractions. The respective bands were recovered and dialyzed (48 h), washed with distilled water (twice), and lyophilized.

Triton-insoluble cell walls, free of cytoplasmic membrane (35), were prepared as follows: cell walls (lower band 2 of the above-indicated density gradient centrifugation) were incubated in 2% (wt/vol) Triton X-100–10 mM $MgCl_2$ –10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethylsulfonic acid (HEPES) buffer, pH 7.4, at 23°C for 20 min (protein concentrations, 2 mg/ml). The purified cell walls were sedimented at $100,000 \times g$ for 1 h and washed twice with 10 mM $MgCl_2$ and five times with distilled water. Outer membranes were obtained by digestion of Triton-insoluble cell walls for 6 h with lysozyme in 10 mM ammonium acetate, pH 7.4 (ratio of cell walls/lysozyme, 50:1, on a dry-weight basis). Membranes were recovered by centrifugation at $150,000 \times g$ for 1 h and washed three times with distilled water.

Determination of buoyant density of membrane fractions. Cell wall and cytoplasmic membrane fractions, isolated by repeated discontinuous sucrose density gradients, were loaded separately on a continuous sucrose gradient (25 to 60%, wt/wt) and were centrifuged to equilibrium (SW41 rotor; $150,000 \times g$, 14 h). Gradient bands were punctured with a syringe, and the buoyant densities were determined by measuring the respective refraction index.

Analysis of polypeptide pattern. Sodium dodecyl sulfate (SDS)-PAGE was carried out according to reference 23. Linear separating gels had 13% acrylamide; gradient gels (11 to 20% acrylamide) were prepared by the use of a peristaltic pump. All slab gels (11 by 22 cm) were 1 mm thick with a stacking gel (2 cm) of 3% acrylamide. Samples (about 2 mg of protein per ml) were solubilized in sample buffer (62 mM Tris-hydrochloride [pH 6.8], 2% [wt/vol] SDS, 10% [wt/wt] glycerol, 5% [vol/vol] 2-mercaptoethanol) at 100°C for 5 min or under different conditions as stated in the figure legends. A 10- to 50- μg portion of protein for each sample was usually applied to the gel. A small amount of bromophenol blue was added to the electrolyte buffer (0.19 M glycine, 0.1% [wt/vol] SDS, 0.025 M Tris-hydrochloride [pH 8.3]) as tracking dye. PAGE was run at 10 mA (constant) and stopped when the tracking dye had reached the end of the gel. Gels were stained in 0.1% Coomassie R250–25% 2-propanol–7.5% acetic acid for 2 h at room temperature. Gels were destained in 20% ethanol–7.5% acetic acid for 3 to 4 h at room temperature.

Activity of SDH. The activity of succinate dehydrogenase (SDH; EC 1.3.99.1) was estimated by the phenazine methosulfate-mediated reduction of 2,6-dichlorophenolindophenol at 578 nm (1). The enzyme assay contained 50 mM potassium phosphate (pH 7.6), 2 mM potassium cyanide, 0.06 mM 2,6-dichlorophenolindophenol, 0.4 mM phenazine methosulfate, and 6 mM sodium succinate. After preincubation of the reaction mixture, including the sample, at 30°C for 2 min, the assay was started by addition of substrate (succinate).

Analytical-chemical determinations. Fatty acids were analyzed by transesterification (concentrated HCl/methanol, 1:5 [vol/vol], at 100°C for 16 h) as their methyl ester derivatives on a CPsil-5 or OV-101 glass capillary column (length, 25 m each; inner diameter, 0.25 mm each; column temperatures, 175 and 150°C, respectively, isotherm; flow rate, 1 ml of N_2 or H_2 per s, respectively) and on an EGSSX column (column temperature, 165°C, isotherm; flow rate, 0.5 ml of N_2 per s).

Neutral sugars (hexoses) were estimated by a modification of the anthrone-sulfuric acid method (37). Determination of

individual sugars as their alditol acetates was performed by gas-liquid chromatography as described elsewhere (30).

Amino sugars and amino acids were liberated by hydrolysis in 4 N HCl at 110°C for 18 h, neutralized over NaOH in vacuo, and estimated on an automatic amino acid analyzer, Durrum model D-500. Hexosamine content was also determined according to reference 34.

For estimation of total lipids, membranes or cell walls were extracted with chloroform-methanol-water (1:2:0.8, vol/vol/vol) (16). Extracted lipids were quantified by weight. Protein content was determined according to Lowry et al. (21); phosphorus was determined as reported in reference 20.

Electron microscopy. Negative staining was performed with 1% phosphotungstic acid, pH 7.0. Preparation of ultrathin sections was according to reference 11.

RESULTS

Isolation of the cell envelope. Ultracentrifugation of cell homogenates, free of whole cells, resulted in sedimentation of a slightly reddish material with a jelly-like appearance. This consistency accounted for the low dry weight/wet weight ratio of the sediment, the former representing only 2 to 3% of the latter.

Chemical analyses showed that the sediment, designated as "cell envelope," contained typical cell wall components such as glucosamine and muramic and diamino-pimelic acids (Table 1). Part of glucosamine, the fatty acids 3-OH- $C_{10:0}$, $C_{12:1}$, and 3-oxo- $C_{14:0}$, and the neutral sugar acofriose (3-O-methylrhamnose) (44) indicated the presence of lipopolysaccharide (29, 41, 44). However, the very high neutral sugar content, about 300 μg per mg (dry weight) of cell envelope, could not be contributed exclusively by lipopolysaccharide. Furthermore, one of the major sugars identified, galactose, is not a constituent of the lipopolysaccharide of *R. capsulata* St. Louis (29). Thus, the presence of an additional polysaccharide was indicated.

Octadecenoic acid, the dominating fatty acid in phospholipids of membranes from *R. capsulata* (7, 36, 45), was the main fatty acid in the cell envelope fraction (Table 1). Therefore, it was concluded that the fraction contained cytoplasmic (and possibly intracytoplasmic) membranes in addition to the cell wall.

Separation of cell wall from cytoplasmic membrane. Three different bands, denoted as upper band, lower band 1, and lower band 2 (Fig. 1), were obtained on discontinuous sucrose gradient centrifugation of the cell envelope fraction. Separation of the cytoplasmic membrane from the crude cell wall was monitored by measuring the SDH activity and the distribution of hexosamines. The sedimentation profile in Fig. 1B reveals that the specific activity of SDH was concentrated in the upper band which, therefore, represented the cytoplasmic membrane (intracytoplasmic membranes, which might have been formed to some extent, were included within the upper band).

The two lower bands of the gradient had low SDH activities (Fig. 1B). Together with a high content in hexoses and hexosamines, this indicated enrichment in cell wall (Fig. 1A and B). Lower fractions from gradient 1 were pooled and, like the cytoplasmic membrane (upper band), submitted separately to a second gradient centrifugation. Since repeated density gradient centrifugation revealed a higher specific activity of SDH with lower band 1 than with lower band 2 (0.3 and 0.13 $\mu mol \text{ min}^{-1} \text{ mg of protein}^{-1}$, respectively), the latter seemed to be less contaminated with cytoplasmic membrane. For this reason the cell wall fraction will be

TABLE 1. Chemical composition and activity of SDH of various cell envelope fractions of *R. capsulata* St. Louis

Component	μg/mg. dry wt					
	Cell envelope	Cytoplasmic membrane	Lower band 1 ^a	Lower band 2 ^a	Triton-insoluble cell wall	Outer membrane
Protein (total)	322	440	257	302	284	446
Lipids (total) ^b	205	239	151	112	ND ^c	ND
Fatty acids (total)	127	184	60	56	26	26
3-OH-C _{10:0}	10	3	8	8	5	5
C _{12:1}	6	2	5	3	2	3
3-Oxo-C _{14:0}	5	1	10	11	4	2
C _{16:0}	9	12	4	4	2	2
C _{16:1}	4	7	3	1	1	1
C _{18:0}	8	11	4	4	2	2
C _{18:1}	84	146	25	23	8	8
Others	1	2	1	2	2	2
Neutral sugars (total)	300	53	292	384	370	366
Acofriose	11	5	7	13	8	6
Rhamnose	184	25	193	246	246	238
Galactose	75	8	67	98	108	103
Glucose	30	17	25	27	8	19
Glucosamine	8	0	15	13	12	9
Muramic acid	6	0	12	11	13	
Diaminopimelic acid	8	0	14	13	16	2
Phosphorus	8	17	3	3	2	2
SDH activity ^d	0.96	1.74	0.13	0.03	ND	ND
Total analyzed	781	694	653	782	723	851

^a See Fig. 1.

^b Including pigments.

^c ND, Not determined.

^d Micromoles of 2,6-dichlorophenolindophenol reduced per minute per milligram of protein.

referred to as lower band 2 herein, although lower band 1 was almost identical with respect to chemical composition (Table 1).

Chemical analysis of cell envelope, cytoplasmic membrane, and cell wall. The cell wall and cytoplasmic membrane fractions were different, especially in their peptidoglycan contents (Table 1). Peptidoglycan was enriched in the cell wall fraction, whereas it was lacking in the cytoplasmic membrane. Surprisingly, the higher galactose and rhamnose contents remained associated with the cell wall; both sugars were accumulated 10-fold over the respective amounts in the cytoplasmic membrane. The fatty acids of the cell wall comprised only 50 μg per mg (dry weight) of material. This marked decrease relative to the cell envelope accounted mainly for reduction in octadecenoic acid. Nearly 50% of the remaining fatty acids were contributed by those typical for lipopolysaccharide (3-OH-C_{10:0}, C_{12:1}, and 3-oxo-C_{14:0}) (29). The relatively high fatty acid content of the cytoplasmic membrane was mainly due to octadecenoic acid.

The cell wall was extracted with Triton X-100 in the presence of Mg²⁺ (35) to remove cytoplasmic membrane contaminants and is referred to as the Triton-insoluble cell wall fraction. Triton-insoluble cell walls had a chemical composition similar to that of the cell wall (Table 1) except for a 50% reduction of the fatty acid content. Mainly octadecenoic acid was removed, accompanied by a loss of lipopolysaccharide-specific fatty acids. Furthermore, the phosphorus content decreased to about half of the amount found in the cell wall.

Isolation and chemical analysis of the outer membrane. Lysozyme digestion of the Triton-insoluble cell wall led to the isolation of a peptidoglycan-free outer membrane fraction (Table 1). The glucosamine found is very likely to be ascribed to lipid A. Again, it should be noted that the high

neutral sugar content (mainly rhamnose and galactose) remained associated with the outer membrane.

Isolation of a lipopolysaccharide-protein(-lipid?) complex from whole cells. Parts of the outer membrane of *R. capsulata* St. Louis could be obtained from whole cells without cell breakage. Extraction of cells in saline resulted in solubilization of a lipopolysaccharide-protein(-lipid?) complex (Table 2). Components such as acofriose, 3-OH-C_{10:0}, and glucosamine indicated the presence of lipopolysaccharide. The relative amounts of fatty acids present showed considerable variation. The polypeptide pattern of the isolate was simple: essentially the major cell wall protein only (*M_r* 33,000; Fig. 2A and B) was present.

Polypeptide pattern of cell envelope fractions. The cell envelope and cytoplasmic membrane fractions showed a complex polypeptide pattern on SDS-PAGE (Fig. 2A). The polypeptide composition of the cell wall fractions, however, was much simpler (Fig. 3). After solubilization at 100°C, its pattern revealed only one major protein (*M_r* 33,000) among 20 to 25 different polypeptides (many of them very faint). Polypeptides of *M_r* 24,000 and 23,000 were also usually observed in the cell wall and outer membrane fractions. Triton extraction did not affect the protein composition (Fig. 3). Outer membranes very likely retained some of the lysozyme used for peptidoglycan digestion, resulting in an additional band (*M_r* about 15,000) in the gel (Fig. 3). Lysozyme is known for strong attachment to cell envelopes (31). The other alterations in the polypeptide pattern provoked by lysozyme action on Triton-insoluble cell walls were a slight shift upwards in molecular weight of the *M_r* 24,000 protein and some diffuse banding.

Some distinct changes occurred when the conditions used for solubilization were altered. At temperatures below 60°C (Fig. 4), major amounts of the total protein applied were

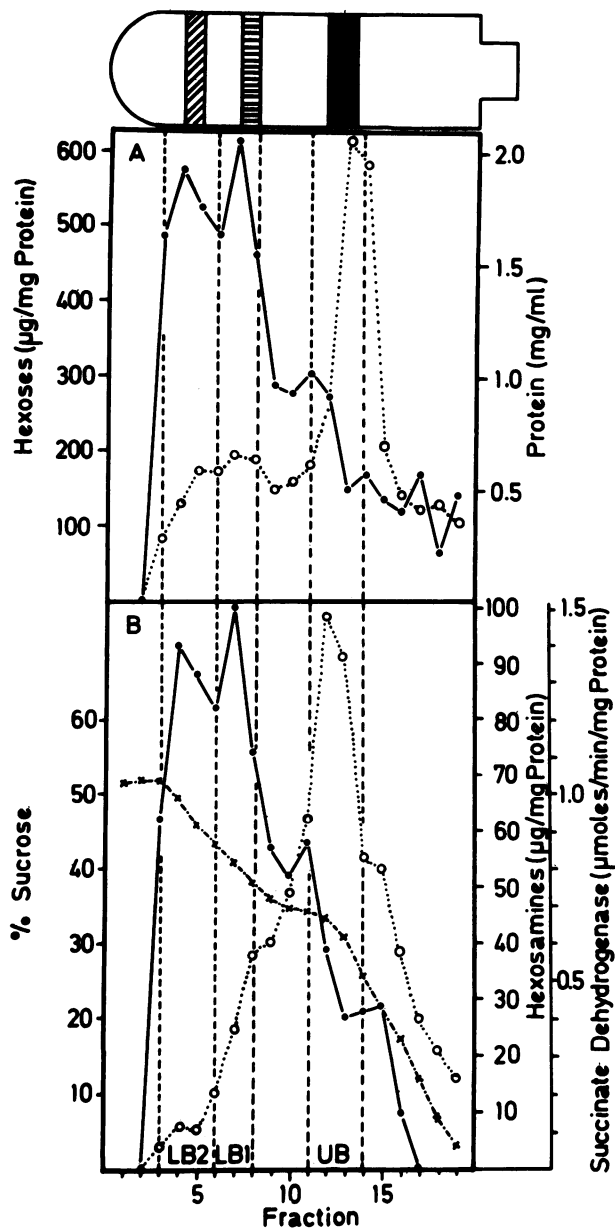


FIG. 1. Separation of the cell envelope of *R. capsulata* St. Louis into cytoplasmic membrane and cell wall fractions. Samples were loaded on a discontinuous sucrose gradient and were centrifuged at $113,000 \times g$ for 1.5 h. Gradients were fractionated (1.25 ml per fraction) from bottom to top with a peristaltic pump. Symbols: (A) Hexoses, solid line; protein, dotted line. (B) Hexosamines, solid line; specific activity of SDH, dotted line; sucrose concentration (weight/weight), dashed line. UB, Upper band (cytoplasmic membrane); LB1 and LB2, lower bands 1 and 2 (cell wall fractions). The vertical, dashed lines indicate respective fractions which were pooled and submitted to a second gradient centrifugation.

retained in the upper part of the gel and the major protein (M_r 33,000) was almost completely absent. By raising the temperature ($>62^\circ\text{C}$; Fig. 4), the spreading of protein was reduced and the major protein became increasingly prominent. The temperature-dependent transition was between 65 and 70°C . The M_r 33,000 protein accounted for about 67% of total protein of the cell wall according to densitometric measurements of gels.

Solubilization of cell wall proteins may be influenced by metal ions (24). When 0.5 M NaCl was present on solubilization below 60°C , one protein (M_r about 69,000) dominated the pattern (Fig. 5). Above 60°C , two polypeptides (M_r about 34,000 and 33,000) became visible accompanied by a simultaneous decrease of the M_r 69,000 polypeptide. The addition of EDTA (final concentration, 10 mM) on sample solubilization shifted the transition temperature of the major protein to 30 to 50°C (Fig. 6). The M_r 24,000 protein was resolved into at least two polypeptides (Fig. 6). Samples solubilized at 100°C revealed a polypeptide of M_r 23,000, which otherwise (e.g., at 70°C or lower) was not detected (Fig. 4). Omitting 2-mercaptoethanol from the sample buffer did not result in any alteration of the polypeptide pattern.

Electron microscopy of isolated cell envelope fractions. In ultrathin sections, cell envelope, cell wall, or outer membrane fractions of *R. capsulata* St. Louis showed the expected fine structures as known for other gram-negative bacteria (7, 26, 28). It should be noted, however, that staining of cell wall and outer membrane fractions was rather difficult, likely due to the polysaccharide nature of the capsule associated with these fractions. Better staining was obtained with a phage-resistant mutant of *R. capsulata* St. Louis (*R. capsulata* St. Louis RC1⁻) lacking a firmly attached capsule (Flammann and Weckesser, manuscript in preparation).

DISCUSSION

The identification of a cell wall fraction from *R. capsulata* St. Louis is based on the following results: (i) separate banding in sucrose density gradients with a buoyant density of 1.215 g/cm^3 (cytoplasmic membrane, 1.139 g/cm^3 coupled with low SDH activity and enrichment of hexosamines (buoyant densities are in accordance with respective values for membranes of other phototrophic bacteria [26]; SDH activity accounted for about 2% only of that measured for the cytoplasmic membrane, and hexosamines could be detected only with the cell wall fractions), (ii) detection of peptidoglycan- and lipopolysaccharide-specific constituents (muramic and diaminopimelic acids as well as acofriose and 3-OH- $\text{C}_{10:0}$, $\text{C}_{12:1}$, and 3-oxo- $\text{C}_{14:0}$, respectively [29]); (iii) a characteristic polypeptide pattern different from that of the cytoplasmic membrane (as observed with outer membranes of other gram-negative bacteria [32], the protein composition is rather simple and dominated by only a few polypeptides [22]); and (iv) a characteristic appearance in ultrathin sections.

The high neutral sugar content of the cell wall and outer membrane fractions of *R. capsulata* St. Louis, mainly due to

TABLE 2. Chemical composition of a lipopolysaccharide-protein-(lipid?) complex isolated by saline extraction of whole cells

Component	$\mu\text{g/mg}$ (dry wt) of material
Neutral sugars (total)	272
Acofriose	51
Rhamnose	104
Glucose	117
Glucosamine	36
Fatty acids ^a	
3-OH- $\text{C}_{10:0}$	21-118
3-Oxo- $\text{C}_{14:0}$	24-30
Others	10-20
Protein ^a	80-140
Total analyzed ^a	443-616

^a Amounts variable in the indicated range.

rhamnose and galactose, cannot be ascribed predominantly to lipopolysaccharide (29). Recently, an acidic polysaccharide fraction (sedimented at 60 mM NaCl upon Cetavlon fractionation) was obtained from *R. capsulata* St. Louis cells and ascribed to external cell envelope layers, probably slime polysaccharide(s) (30). The acidity of the polymer(s) was contributed by a high galacturonic acid content. Galacturonic acid, however, could not be detected in the cell wall fractions of *R. capsulata* St. Louis, whereas its galactose content was about 50 times higher than that of the slime polysaccharide. Thus, the high galactose and rhamnose content of the cell wall and outer membrane resulted neither from lipopolysaccharide nor the indicated acidic polymer. Very likely, this polysaccharide represents the capsule polymer of *R. capsulata* St. Louis (Flammann et al., in preparation).

The total lipid and fatty acid contents of the cell wall of *R. capsulata* St. Louis were only 50% or lower than the respective values of the cytoplasmic membrane. Extraction of the cell wall with Triton X-100 in the presence of Mg^{2+} (35) even reduced the fatty acid content to one-half, removing mainly phospholipids represented by octadecenoic acid (17, 36). A low total lipid, phospholipid, and fatty acid

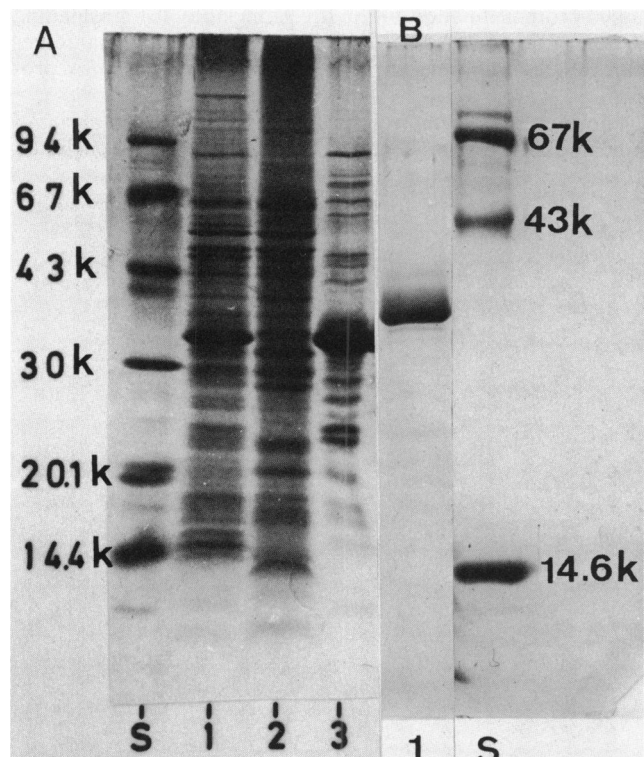


FIG. 2. Polypeptide pattern of cell envelope fractions of *R. capsulata* St. Louis. (A) SDS-PAGE: 11 to 20% acrylamide (gradient gel). Lane 1, Cell envelope, 50 μ g of protein; lane 2, cytoplasmic membrane, 50 μ g of protein; lane 3, Triton-insoluble cell wall, 30 μ g of protein; lane S, molecular weight standard proteins (top to bottom: phosphorylase b, 94,000; bovine serum albumin, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,100; α -lactalbumin, 14,400). (B) PAGE: 13% acrylamide. Lane 1, Saline extract, 20 μ g of protein; lane S, molecular weight standard proteins. Samples were solubilized in sample buffer at 100°C for 5 min.

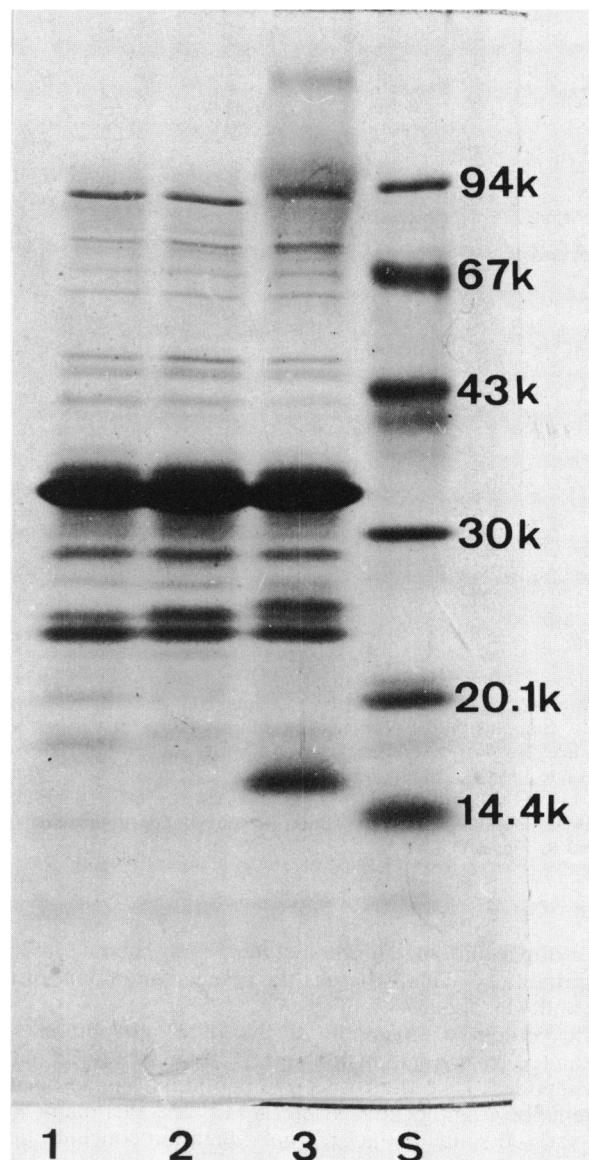


FIG. 3. Polypeptide pattern of cell wall fractions of *R. capsulata* St. Louis. SDS-PAGE: 11 to 20% acrylamide (gradient gel). Lane 1, Cell wall isolated by repeated sucrose density gradient centrifugation; lane 2, Triton-insoluble cell wall; lane 3, outer membrane; lane S, molecular weight standard proteins (see legend to Fig. 2A). Samples were solubilized in sample buffer at 100°C for 5 min; 30 μ g of each protein was applied to the gel.

content was also observed with cell walls of other phototrophic bacteria (3, 10, 28) and various other gram-negative bacteria (33). Octadecenoic acid is the predominant fatty acid in membranes of *R. capsulata* (17, 36). For one strain of *R. capsulata*, this major fatty acid was identified as $^{11}\Delta$ - C_{18} -monoenoic acid (45). In strain St. Louis, octadecenoic acid represented 81% of the fatty acid content of the cytoplasmic membrane. In the cell wall fraction, the percentage of octadecenoic acid was halved; about 50% of total fatty acids were contributed by lipopolysaccharide (3-OH- $C_{10:0}$, $C_{12:1}$, and 3-oxo- $C_{14:0}$; Table 1). Cytoplasmic membranes of phototrophic bacteria are known for their high unsaturated fatty acid content (27). Cell walls, however, contain more saturated fatty acids. The same is true for *R. capsulata* St. Louis:

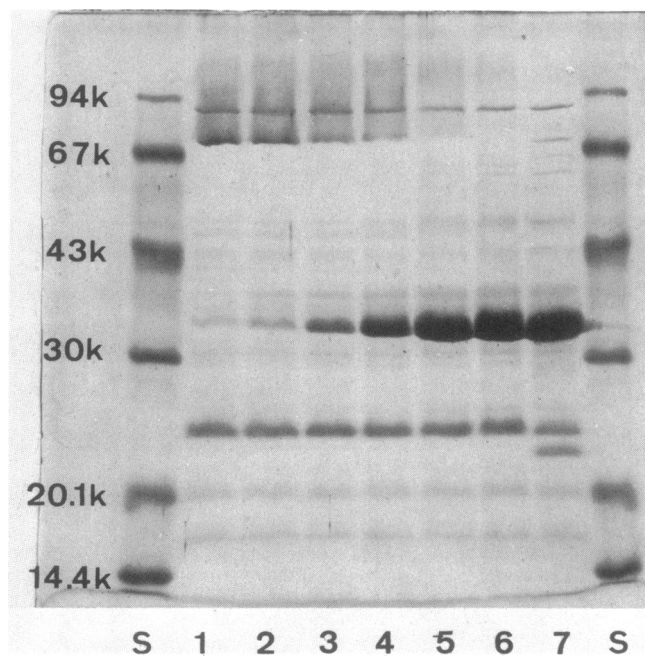


FIG. 4. Influence of solubilization temperature on cell wall polypeptide pattern of *R. capsulata* St. Louis. SDS-PAGE: 13% acrylamide. Samples were solubilized in sample buffer at various temperatures for 5 or 20 min; 30 μ g of each protein was applied to the gel. Lane 1, 55°C, 20 min; lane 2, 60°C, 20 min; lane 3, 62°C, 20 min; lane 4, 65°C, 20 min; lane 5, 67°C, 20 min; lane 6, 70°C, 20 min; lane 7, 100°C, 5 min; lane S, molecular weight standard proteins (see legend to Fig. 2A).

the cytoplasmic membrane contains only about 15% of saturated fatty acids, whereas the respective amount of the cell wall was about 48% (Table 1).

The protein composition of the outer membrane was dominated by a single major protein among 20 to 25 minor polypeptides. Like the respective peptidoglycan-associated proteins of gram-negative bacteria (13, 22), this major protein showed some distinct features: high ion concentrations affected its solubilization, and the electrophoretic mobility on SDS-PAGE was changed by heating (from M_r 69,000 at below 70°C to M_r 33,000 at higher temperatures). At low temperatures, e.g., 60°C, it was not solubilized in SDS solution and was hardly detectable as a distinct band in SDS-PAGE (Fig. 4) except that the ionic strength of the sample buffer was high (Fig. 5). This suggests ionic interactions between the protein and other cell wall components, for example, lipopolysaccharide and peptidoglycan (14). Variable electrophoretic mobility in SDS-PAGE (Fig. 4), on the other hand, very likely is due to conformational changes in the protein together with increased binding of SDS (25). The temperature transition range of the major protein of *R. capsulata* St. Louis was between 65 and 70°C, similar to OmpF protein (e.g., O-9 protein) of *Escherichia coli* K-12 (25). The shift in electrophoretic mobility of the major protein to lower temperatures by the addition of EDTA might reflect the role of (divalent) cations for stabilization of outer membrane proteins (24). Whether the major cell wall protein of *R. capsulata* St. Louis as revealed by SDS-PAGE is homogeneous cannot yet be decided on the basis of SDS-PAGE. Visible splitting into two bands (Fig. 5) might indicate different polypeptides as well as effects of solubilization conditions (0.5 M NaCl included in the sample buffer).

Possible explanations of different banding of the same protein in SDS-PAGE is discussed in references 18 and 19.

Cell walls of *R. capsulata* Ala⁻ contain one major protein of M_r 60,000 (9). Solubilization, however, was performed only at room temperature. Polypeptide patterns of outer membranes of the other phototrophic bacteria investigated so far are also all dominated by essentially one major protein. It is peptidoglycan associated in the cases of *R. capsulata* (this study) and *R. sphaeroides* (5, 6) but not in *C. vinosum* (18). All of these proteins are heat modifiable, as are porins of *Enterobacteriaceae* (25). Rupture of intermolecular associations by heat to allow more interaction with SDS has been discussed for *C. vinosum* (18). An M_r 37,400 protein of this outer membrane migrated at M_r 52,500 if solubilized at 100°C. Similarly, an M_r 58,500 polypeptide in *R. sphaeroides* was altered to M_r 83,000 upon heating to 75°C or above. The alteration was discussed to reflect a change in conformation or in SDS binding capacity or both (2). The outer membrane of *R. sphaeroides* showed major aggregates of about M_r 68,000 when solubilized at room temperature (2). Heating at 100°C before SDS-PAGE resulted in a different pattern and was interpreted as the dissociation of aggregates of a shared major M_r 47,000 subunit with additional, different polypeptides attached (2). Recently, this subunit was established to be a proteolipid with one fatty acid amidically linked to L-Ala at the NH₂ terminus (6). No such evidence is available as yet for the *R. capsulata* M_r 33,000 protein. In addition, in the same study (6), misleading M_r determination on SDS-PAGE was revealed by guanidine-thiocyanate-urea solubilization of the *R. sphaeroides* protein.

The high-molecular-weight aggregates of *R. sphaeroides* indicated above cross-reacted serologically among each oth-

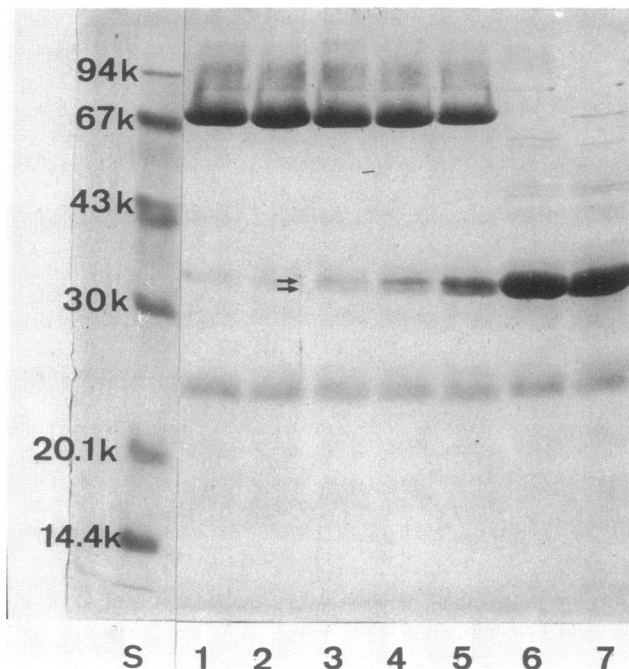


FIG. 5. Influence of high ionic strength (0.5 M NaCl) and temperature on cell wall polypeptide pattern of *R. capsulata* St. Louis. Samples were solubilized in sample buffer containing 0.5 M NaCl (final concentration) at various temperatures for 5 or 20 min; 30 μ g of each protein was applied to the gel. Lane 1, 37°C, 20 min; lane 2, 50°C, 20 min; lane 3, 60°C, 20 min; lane 4, 62°C, 20 min; lane 5, 65°C, 20 min; lane 6, 70°C, 20 min; lane 7, 100°C, 5 min; lane S, molecular weight standard proteins (see legend to Fig. 2A).

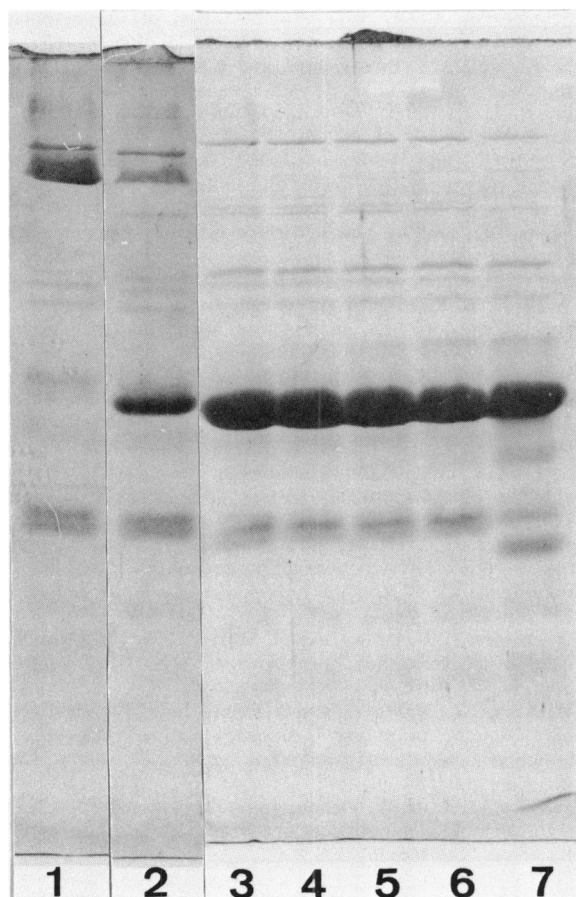


FIG. 6. Influence of EDTA and temperature on cell wall poly-peptide pattern of *R. capsulata* St. Louis. The operating gel contained 70 mM NaCl (12). Samples were solubilized in sample buffer containing 10 mM EDTA (final concentration) at various temperatures for 5 or 20 min; 30 μ g of each protein was applied to the gel. Lane 1, 30°C, 20 min; lane 2, 50°C, 20 min; lane 3, 60°C, 20 min; lane 4, 62°C, 20 min; lane 5, 65°C, 20 min; lane 6, 70°C, 20 min; lane 7, 100°C, 5 min.

er (5), but not with *R. capsulata* outer membrane proteins (4). Porin activity for the major outer membrane proteins of *C. vinosum* (18) and *R. sphaeroides* (2, 6) has been suggested. In fact, the major outer membrane proteins of both *R. sphaeroides* and *R. capsulata* show high porin activity in the phospholipid liposome swelling assay, and the pore size of the *R. capsulata* porin is significantly larger than that of the *R. sphaeroides* porin (J. Weckesser, H. Nikaido, H. T. Flammann, and E. Rosenberg, manuscripts in preparation). Thus, in spite of the close phylogenetic relationship between *R. sphaeroides* and *R. capsulata*, there seem to be differences in their major outer membrane proteins.

ACKNOWLEDGMENTS

We thank J. R. Golecki for electron microscopy, E. Metz for gas-liquid chromatography of neutral sugars, and R. Warth for amino acid and amino sugar analyses.

This work was supported by the Deutsche Forschungsgemeinschaft.

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