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Identification and Characterization of *Campylobacter jejuni* Outer Membrane Proteins

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Outer membrane proteins from isolates of *Campylobacter jejuni* were examined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Sarcosinate-insoluble membrane preparations were outer membrane enriched based on increased ketodeoxyoctonate concentrations, the presence of surface-exposed ^{125}I-labeled proteins that were hydrophobic, and similarity to membrane vesicle (bleb) sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles. Most isolates contained a single major band with molecular weight of 41,000 to 45,000. Profiles of *C. jejuni* and *Campylobacter coli* isolates were indistinguishable, but either could be easily differentiated from *Campylobacter fetus* and *Campylobacter faecalis*. The profiles were stable for strains under a variety of growth, incubation and passage conditions. We classified 110 isolates from patients with sporadic campylobacter enteritis into nine subtypes based on differences in outer membrane sodium dodecyl sulfate-polyacrylamide gel electrophoresis profiles. Two categories accounted for 76% of the isolates. Complete concordance was observed in subtypes of strains obtained from epidemiologically related cases. Thus, comparison of the major outer membrane proteins of *C. jejuni* is a useful technique for investigating the transmission of this organism and may provide a basis for immunological characterization of the outer membrane proteins.

Campylobacter jejuni is a major cause of acute enteritis in humans (6). Nevertheless, major questions concerning the transmission of this agent and the pathophysiology of the infection it causes remain unanswered. Recent studies have shown that isolates from humans are heterogeneous in their biochemical (13) and serological (28) characteristics.

As with other gram-negative organisms (19), *Campylobacter* species contain hydrophobic outer membrane proteins (OMPs) that can be identified, characterized, and isolated (17). Using appropriate methods, the OMP profiles of isolates from various sources can be compared. For other gram-negative pathogens such as *Neisseria meningitidis* (24), *Neisseria gonorrhoeae* (8), and *Haemophilus influenzae* (3), this approach has added a useful marker for epidemiological purposes. Considering the vast reservoir for *C. jejuni* and the diversity of serotypes expressed. OMP profiles as markers may allow for separation of isolates with different origins.

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Another reason for performing such studies would be to identify OMPs that many or all *C. jejuni* strains share in common. At present, no group antigens have been identified for *C. jejuni*. Recognition of a common protein may provide a group antigen that can be used for serological assays for *C. jejuni* infections or for development of vaccines.

Our studies showed that we could isolate outer membrane-enriched fractions of *C. jejuni* cells and that the proteins resolved were largely stable when strains underwent a variety of growth, incubation, and passage conditions. We also found that by using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (SDS-PAGE), most *C. jejuni* isolates could be differentiated into one of nine types based upon their OMP profiles and that a single major OMP band was similar in all strains tested.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *Campylobacter* strains used in this study were either from the culture collection of the Denver Veterans Administration Medical Center *Campylobacter* laboratory (DCL)

or were the prototype strains used in the Penner system of serotyping on the basis of heat-stable hemagglutinating antigens (28). All individual strains used in this study were identified by their DCL number or number in the Penner serotyping system. *C. jejuni*, *Campylobacter coli*, *Campylobacter fetus*, and *Campylobacter faecalis* strains had been identified according to standard criteria (13, 14). Most strains had been passaged multiple times on artificial media. For several experiments, strains isolated from chronically infected mice were compared with laboratory-passaged strains. All strains were maintained frozen at −70°C in brucella broth containing 15% glycerol. Working stocks of strains were obtained by culturing the freezer stock on sheep blood agar plates (PASCO, Wheat Ridge, Colo.) followed by transfer to Mueller-Hinton agar. Cultures were grown statically at 37 or 42°C and incubated in an atmosphere with 5% oxygen, 10% carbon dioxide, and 85% nitrogen (14) for periods ranging from 18 to 120 h. Cells from plates were harvested in 50 ml of sterile distilled water, centrifuged twice at 5,000 × *g* for 10 min at 25°C, and then suspended in 0.01 M Tris (pH 7.4). The optical density at 450 nm of the suspension was adjusted to 26% transmission and 30 ml of this suspension was recentrifuged and the pellet frozen at −20°C until the membrane preparations were made.

Fractionation of cells. Frozen cell pellets were thawed and resuspended in 30 ml of 0.01 M Tris (pH 7.4). A 1.0-ml portion was saved for analysis of whole cell proteins. The remaining cells were sonicated on ice four times with a Branson Sonifier (model S-75; Branson Instruments, Danbury, Conn.) for 30 s with 30-s rests. The preparation was then centrifuged two times at 5,000 × *g* for 20 min to remove whole cells, and the supernatant was centrifuged for 1 h at 100,000 × *g* at 4°C (L8-70 ultracentrifuge; Beckman Instruments, Inc., Fullerton, Calif.). The pellet was suspended in 3 ml of sterile distilled water, and 200 μl was saved for analysis of crude membranes. The remainder was added to 20 ml of 1% sodium lauryl sarcosinate (Sarkosyl; Ciba-Geigy Corp., Greensboro, N.C.) in 7 mM EDTA and incubated for 20 min at 37°C as described by Fillip et al. (11). This suspension was centrifuged at 100,000 × *g* for 2 h, and the supernate (Sarkosyl-soluble) was saved for later studies. The pellet was suspended in the Tris buffer and centrifuged at 100,000 × *g* for 2 h, and the resulting pellet (Sarkosyl-insoluble) was suspended in 1.0 ml of sterile distilled water and stored at 4°C.

Radioiodination of intact cells. Cells from 24-h cultures grown confluently on two Mueller-Hinton plates were added to 30 ml of Dulbecco phosphate-buffered saline (30). The suspension was adjusted with Dulbecco phosphate-buffered saline to an optical density of 1.0 (10% transmission) at 450 nm, and then 1.5 ml was transferred to a 1.5-ml polypropylene centrifuge tube and spun at 12,800 × *g* in an Eppendorf model 5412 centrifuge (Brinkman Instruments, Westbury, N.Y.) to pellet the cells. Cells were radiolabeled by the method of Swanson (30). In brief, 40 μl of Dulbecco phosphate-buffered saline and 5 μl of 10^{-5} M KI were added to the pellet, and the suspension was transferred to an Iodogen-coated tube (Pierce Chemical Co., Rockford, Ill.) to which 500 μCi of ^{125}I was added. The suspension was agitated for 10 min at 25°C, added to 1.0 ml of Dulbecco phosphate-buffered saline at 4°C in a 1.5-ml centrifuge tube, spun at 12,800 × *g*, and suspended in 30 ml of 0.01 M Tris (pH 7.4), and cells were fractionated as described above.

Preparation of blebs. Frozen cells were subcultured onto blood agar. With a sterile swab, cells were transferred to the diphasic medium in a 75-cm^2 tissue culture flask (Falcon 3024; BBL Microbiology Systems, Cockeysville, Md.). The diphasic medium consisted of 24 ml of Mueller-Hinton agar overlaid with 36 ml of brucella broth. All brucella broths had been incubated at 42°C for 24 h to check sterility before use in the diphasic media. Inoculated media were incubated and the cells were harvested, and after two centrifugations at 5,000 × *g* for 20 min, the supernatant was centrifuged at 100,000 × *g* for 1 h at 4°C. The pellet was then suspended in 0.01 M Tris and centrifuged, and this pellet was suspended in 1.0 ml of sterile distilled water and stored at 4°C until used.

Analytical methods. Protein concentrations were measured by using the Markwell et al. modification (18) of the Lowry method for membrane proteins. The thiobarbituric acid method (27) was used for determining the 2-keto-3-deoxyoctonate (KDO) concentrations of fractions. Succinic dehydrogenase activity was determined spectrophotometrically by measuring the decrease in absorbance at 700 nm of dichlorophenolindophenol according to the method of Mizuno and Kageyama (21). Gross morphology of cells or cell fractions was examined by using phase-contrast microscopy or carbol-fuchsin stain.

SDS-PAGE. Whole cell and membrane preparations were analyzed by SDS-PAGE in a modified Laemmli gel system as described by Ames (1). Electrophoresis materials were obtained from Bio-Rad Laboratories, Richmond, Calif., using a Vokam power source (Shandon Southern Instruments, Ltd., Camberley, Surrey, England). Discontinuous SDS-PAGE was done in 1.5-mm-thick slab gels with a 4.5% stacking gel and a 10% separating gel; for several experiments, either 7 or 15% separating gels were used. Membrane fractions were suspended in sample buffer containing SDS (4%), bromphenol blue (0.1%), glycerol (20%), and Tris base (0.38%) at pH 6.8, by the method of Ohman et al. (26) and then boiled at 100°C for 3 min. Samples with 1 to 2 μg of protein were applied to each gel lane. Electrophoresis was carried out with a constant current of 35 mA for about 2 h, and the temperature was maintained at 8°C by circulating cold water.

After electrophoresis, gels were fixed and proteins were resolved by using the modified silver stain of Oakley et al. (25). The modified silver stain achieves a 100-fold increase in sensitivity compared with Coomassie blue stain; some gels were stained with Coomassie blue for comparison. The molecular weights of the membrane polypeptides were estimated from a calibration curve prepared with myosin (200,000), β-galactosidase (116,250), phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400) as standards. Carbohydrates were stained with periodate according to the method of Tsai and Frasch (31) or with Stains-All (Eastman Kodak Co., Rochester, N.Y.) (15).

For resolution of radiolabeled proteins, gels fixed overnight in 50% methanol and 10% acetic acid were rehydrated in water and then dried on a Bio-Rad gel dryer for 90 min. The dried gel was applied to X-ray

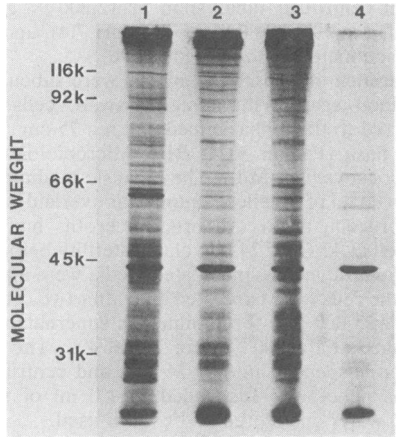

FIG. 1. SDS-PAGE of several preparations of proteins of the PEN 1 strain of *C. jejuni*. All gels are shown with the silver stain by the methods of Oakley et al. (25). The preparations were from 24-h growth of the strain on brucella agar incubated at 42°C and represent whole cell (lane 1), crude membrane (lane 2), Sarkosyl-soluble membrane (lane 3), and Sarkosyl-insoluble membrane (lane 4) fractions. Each lane contained 1 μg of protein and the gel contained 10% acrylamide. In each preparation, a major protein band is seen migrating with a molecular weight of 43,700. The concentration of that protein is increased in lane 4; the concentration in lane 3 is less than that in lane 2. In comparison to the other preparations, the Sarkosyl-insoluble preparation shows relatively few protein bands. Five minor OMPs are seen migrating between 60,000 and 73,000 and three other minor bands are seen between 29,000 and 32,000.

film (Kodak XRP-5) and exposed with an intensifying screen at −70°C.

RESULTS

Characterization of detergent-insoluble membrane preparations. The Sarkosyl-insoluble membrane preparations derived from the PEN 1 isolate of *C. jejuni* contained a single major protein band with a molecular weight of 43,700 (Fig. 1). In comparison to the SDS-PAGE profile of the whole cell, crude membrane, and Sarkosyl-soluble membrane preparations, the number of distinct protein bands was markedly reduced; gels stained with Coomassie blue showed a pattern identical to that obtained with the silver stain. Studies of the PEN 2 and PEN 3 strains show similar results. The solubility of this major protein band in PAGE was enhanced by both heating and pretreatment with SDS (Fig. 2). Adding 2-mercaptoethanol (0.1% to 1.0%) did not change the migration characteristics of the proteins resolved.

Analysis of the KDO content of cellular and subcellular preparations of four *C. jejuni* strains is shown in Table 1. The concentrations of KDO in crude and Sarkosyl-insoluble membrane preparations were higher than those of the whole cell preparations; bleb and Sarkosyl-insoluble membrane KDO concentrations were similar. Incubation of crude membrane preparation with Sarkosyl or SDS removed all succinic dehydrogenase activity; thus, relative amounts of this enzyme activity could not be used as a criterion to assess the composition of the Sarkosyl-soluble and -insoluble preparations.

Radioiodination of intact *C. jejuni* cells with subsequent fractionation showed that major surface-exposed peptides migrated at 44,000 and 30,000 (Fig. 3). These migrations were nearly identical to those observed for two major peptide bands resolved in the Sarkosyl-insoluble membrane preparation.

Stability of SDS-PAGE patterns. When assessed by gel electrophoresis, the major Sarkosyl-insoluble proteins were found to be stable under a variety of natural and experimental conditions. Membrane preparations derived from the PEN 1 and PEN 2 isolates harvested

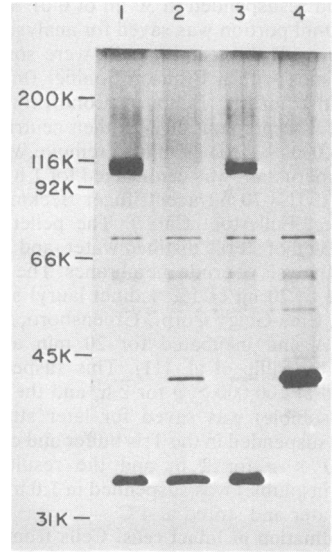

FIG. 2. Influence of heating and SDS on migration characteristics of Sarkosyl-insoluble membrane proteins of the PEN 1 strain of *C. jejuni* in PAGE (10% acrylamide). The membrane preparation was divided and portions were solubilized at 25 or 100°C in the presence or absence of 1% SDS. The solubilization treatments were: 25°C, no SDS (lane 1); 25°C, SDS (lane 2); 100°C for 3 min, no SDS (lane 3); 100°C for 3 min, SDS (lane 4). Without pretreatment, the major protein band seen at 42,000 in lane 4 and several of the minor bands were insoluble in the gel. Treatment with SDS alone increased the solubility of the major band protein more than did treatment with heat alone. For lanes 1 to 3, in which both modalities were not used, a major band was resolved at 32,000.

TABLE 1. KDO concentrations of subcellular fractions for four *C. jejuni* strains

Strain designation	Fraction (μg of KDO/mg of protein)				
	Whole cell	Crude membrane	Sarkosyl-soluble membrane	Sarkosyl-insoluble membrane	Bleb
PEN 1	2.5	5.6	3.9	8.8	ND[a]
92858	5	14	7	9	10
79-151	4	9	0	11	9
79-406	4	9	5	13	8
Mean (±SE)	3.9 ± 0.5	9.4 ± 1.7	4.0 ± 1.5	10.5 ± 1.0	9.0 ± 0.6

[a] ND, Not done.

from cultures incubated for 24 to 120 h on Mueller-Hinton agar showed identical SDS-PAGE profiles. For the PEN 3 strain, production of a major protein reached a maximum when the cells were incubated for 48 h and then declined (Fig. 4). Nevertheless, for all three strains the SDS-PAGE profiles changed minimally, despite a complete change in the morphology of the cells from the vibrio form to the coccoid form observed after 48 h of incubation (Fig. 5). Although the SDS-PAGE profiles remained relatively constant, in quantitative studies the amount of Sarkosyl-insoluble membrane protein peaked when cells had been incubated for 48 h.

Although SDS-PAGE profiles of the Sarkosyl-insoluble membrane preparations were the same for *C. jejuni* strains grown in brucella and Mueller-Hinton broths and on sheep blood, brucella, and Mueller-Hinton agar plates, the temperature of incubation affected the protein concentration. For six strains incubated for 24 h, the mean protein concentration at 37°C (1,258 ± 288 μg per ml of suspension) was significantly less than that at 42°C (2,114 ± 437 μg per ml of suspension; $P < 0.005$, paired analysis of variance); however, the SDS-PAGE profiles were concordant for the six strains grown at the two incubation temperatures.

The SDS-PAGE profile of a strain (PEN 1)

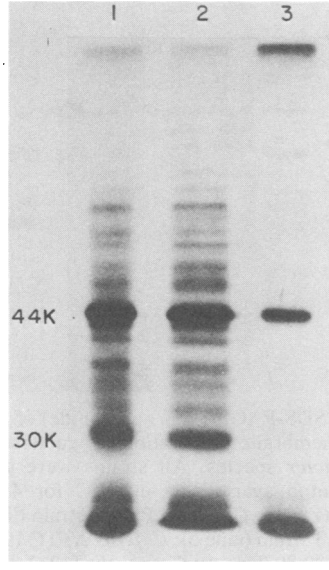

FIG. 3. Autoradiograph of ^{125}I-labeled PEN 1 strains of *C. jejuni*. Intact whole cells were labeled with ^{125}I and then fractionated as described in the text; fractions were run in 10% acrylamide SDS-PAGE. The preparations were: whole cells (lane 1); Sarkosyl-soluble membrane (lane 2); Sarkosyl-insoluble membrane (lane 3). A major band migrating at 44,000 is surface exposed as shown by this extrinsic radiolabeling technique. A surface-exposed major band at 30,000 is present in the whole cell and Sarkosyl-soluble membrane preparations. Radiolabel at the bottom of the gel represents degraded peptides or peptides not resolved in the gel.

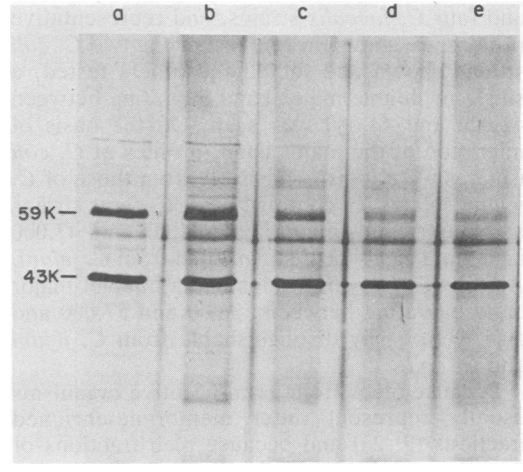

FIG. 4. SDS-PAGE (10% acrylamide) of outer membrane preparations of the PEN 3 strain of *C. jejuni*. Cells were incubated for 24 h (lane a), 48 h (lane b), 72 h (lane c), 96 h (lane d), and 120 h (lane e) and then outer membrane fractions were prepared as previously described. The peptide band migrating at 59,000 reached its highest concentration in cells incubated for 48 h and then diminished progressively.

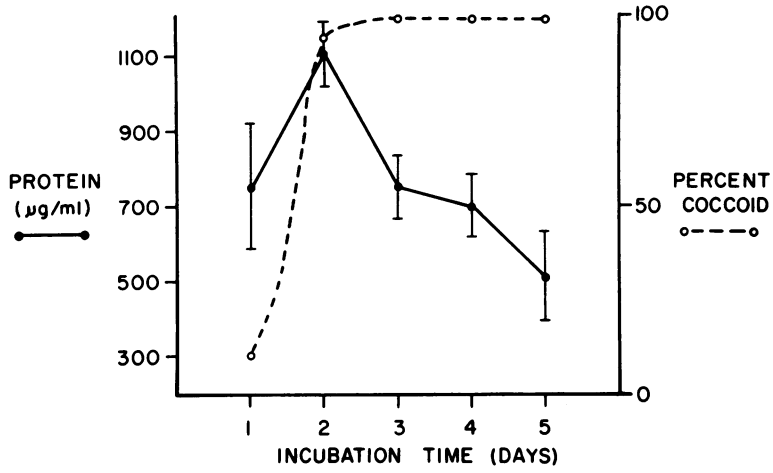

FIG. 5. OMP production and morphology of three strains of *C. jejuni* (PEN 1, PEN 2, and PEN 3) by length of incubation. Strains were incubated at 42°C for 24 to 120 h, Sarkosyl-insoluble membranes were prepared in a standard fashion (see text), and protein concentrations were measured. For the protein concentration, each point represents the mean of one determination for each of the three strains tested.

isolated from a mouse that was chronically colonized with *C. jejuni* was no different from that of the same strain passed 10 times on a plate medium (data not shown).

Diversity of SDS-PAGE patterns of various *Campylobacter* strains. We next examined the protein profiles seen in the Sarkosyl-insoluble membrane preparations of several *Campylobacter* strains representing other catalase-positive *Campylobacter* species. In total, we examined eight *C. coli*, seven *C. fetus* subsp. *fetus*, and four *C. faecalis* strains, and representative profiles are shown in Fig. 6. For the two *C. coli* strains shown and for all the others tested, a single or double major band migrating between 42,000 and 44,000 was seen. On the basis of migration of the major band, profiles of *C. coli* isolates were indistinguishable from those of *C. jejuni* isolates. In contrast, most *C. fetus* strains had major bands migrating at 44,000 and 47,000 and could easily be distinguished from *C. jejuni*. All four *C. faecalis* strains had a doublet major band migrating between 52,000 and 57,000 and also were easily distinguishable from *C. jejuni* and *C. coli*.

Because blebs from gram-negative organisms usually represent outer membrane-enriched fractions (9, 23) and because bleb fractions of the *Campylobacter* cells we examined were KDO enriched (Table 1), we compared SDS-PAGE profiles observed in bleb and Sarkosyl-insoluble membrane fractions. A comparison of bleb and Sarkosyl-insoluble membrane fractions for four *C. coli* strains showed identity of the major bands and close approximation of most of the minor bands (Fig. 7). Studies with *C. jejuni*

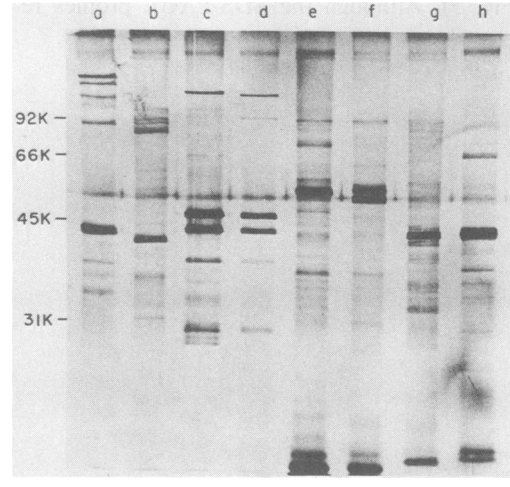

FIG. 6. SDS-PAGE (10% acrylamide) of Sarkosyl-insoluble membrane preparations of catalase-positive *Campylobacter* species. All strains were grown on Mueller-Hinton agar plates at 37°C for 48 h. The preparations were: *C. jejuni*, PEN 1 strain (lane a); *C. jejuni* PEN 3 strain (lane b); *C. fetus* ATTC 19438 (lane c); *C. fetus* 80-80 (lane d); *C. faecalis* 11212 (lane e); *C. faecalis* 11364 (lane f); *C. coli* 80-222 (lane g); *C. coli* 80-241 (lane h). The major bands for the *C. jejuni* and *C. coli* strains migrate at the same molecular weight range (42,000 to 44,000). For *C. fetus*, major bands can be seen at 47,000 and 44,000 and more minor bands at 12,000 and 39,000. For *C. faecalis*, doublet major bands can be seen between 52,000 and 57,000. For another three *C. coli*, two *C. fetus*, and two *C. faecalis* strains, the same characteristics as seen in the strains shown were present. The thermophilic *Campylobacter* species (*C. jejuni* and *C. coli*) can be readily distinguished from *C. fetus* and *C. faecalis* on the basis of the protein profiles seen in these preparations.

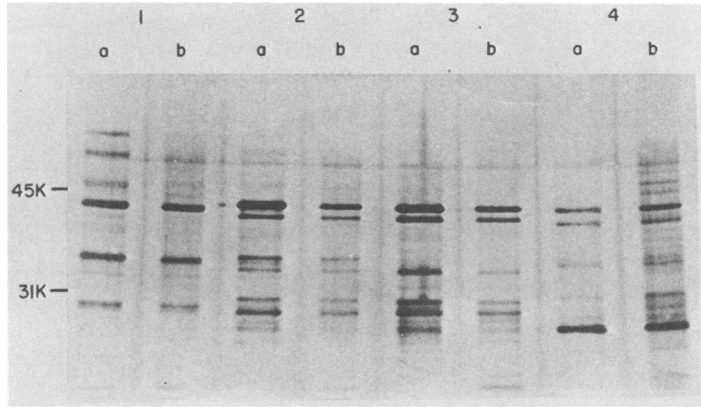

FIG. 7. SDS-PAGE (10% acrylamide) of bleb and Sarkosyl-insoluble membranes from four *C. coli* strains. Column a represents the Sarkosyl-insoluble membranes and column b represents bleb membranes. The strains tested were 80-222 (lane 1), 80-212 (lane 2), 80-218 (lane 3), and 80-236 (lane 4). For each strain there is homology of the major bands seen in the two preparations and similarities in the minor bands as well.

and *C. fetus* showed similar concordance of bleb and Sarkosyl patterns. Because bleb fractions were so much simpler to produce, we used these for the remainder of the studies.

To assess the diversity of SDS-PAGE profiles seen in a sampling of strains, we prepared bleb (29) fractions from *Campylobacter* isolates from patients with enteritis in Denver (7). Fourteen of the 124 isolates from that study could not be recovered, and thus we prepared bleb fractions from 110 strains. For all 110 strains examined, either a singlet or doublet major band migrating between 41,000 and 45,000 was seen. Based on the heterogeneity of the migration of the major band(s), a typing system was established with nine prototype strains (Fig. 8). The distribution of SDS-PAGE profiles among the 110 strains is shown in Table 2. Type 3 (52.7%) and type 2 (23.6%) accounted for the majority of the isolates. The diversity of the minor bands was enormous and was not used for developing this typing system, but minor band patterns could be used to differentiate among strains of the same major band type.

Since blebs of gram-negative organisms are known to be enriched in lipopolysaccharide

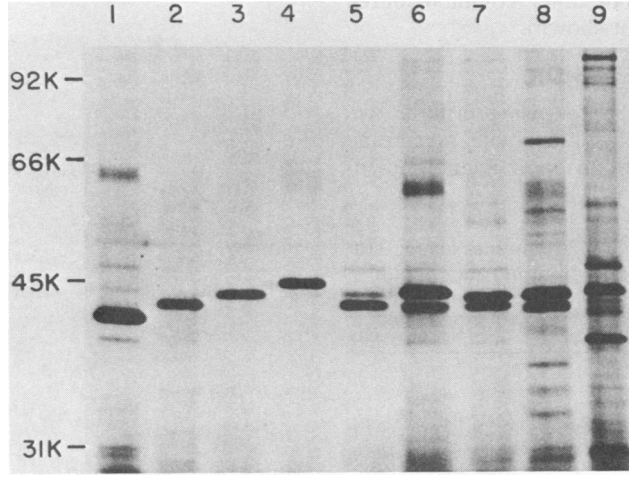

FIG. 8. SDS-PAGE (7.5% acrylamide) of OMPs of prototype *C. jejuni* strains. The identification of each strain (with the molecular weight[s] of the major band used for discrimination shown in parentheses) is as follows: type 1, 902-26 (40,300); type 2, 92858 (41,800); type 3, 79-151 (42,300); type 4, 3113 (43,800); type 5, 79-406 (41,400 and 42,100); type 6, 79-12 (41,000 and 41,800); type 7, 79-296 (41,400 and 41,800); type 8, 78-22 (45,800, 42,300, and 66,600); type 9, 80-01 (39,700 and 43,200).

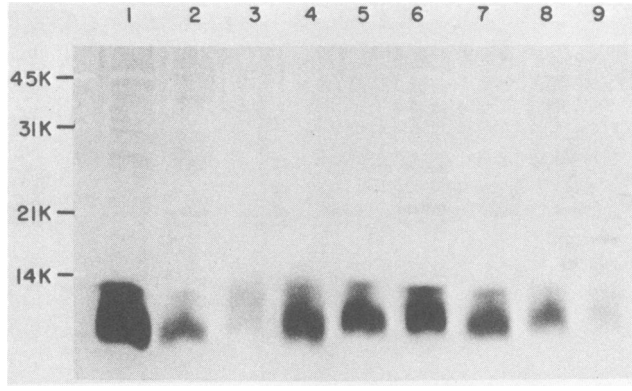

FIG. 9. SDS-PAGE (15% acrylamide) of prototype *C. jejuni* strains showing LPS stained with silver according to the methods of Tsai and Frasch (31). Lanes 1 through 9 show the same bleb fractions as shown in Fig. 8 lanes 1 through 9.

(LPS) (23), we stained preparations from the nine prototype strains to assess the characteristics of the *C. jejuni* LPS (Fig. 9). Although each lane contained an equivalent amount of protein, the concentration of LPS visualized varied considerably. For all strains tested, the LPS appeared as a single diffuse band migrating below 14,000. For prototype strain 9, three discrete bands migrating between 14,000 and 21,000 could be seen faintly on this preparation; in other preparations these bands were more noticeable. Gels stained with Stains-All (15) showed a similar appearance for the LPS.

Five strains of *C. jejuni* isolated from ill persons during a milkborne outbreak of enteritis (5) showed concordant profiles (Fig. 10) as did three isolates from a foodborne outbreak (4) and two isolates each from other common-source outbreaks (5) (data not shown).

DISCUSSION

On the basis of the following criteria, we believe that the Sarkosyl-insoluble membrane preparation represents an outer membrane-enriched cell fraction: (i) few protein bands are present; (ii) the major protein is hydrophobic and may be surface exposed as shown by ^{125}I impermeant cell labeling; (iii) KDO concentrations are increased over whole cell concentrations; and (iv) this preparation closely resembles the membrane vesicle (bleb) preparations known to be endotoxin-enriched portions of membrane (9, 23). These outer membane fractions thus have characteristics similar to those obtained from other gram-negative organisms, and results of recent studies reported by Logan and Trust (17) and Austen and Trust (2) were nearly identical. The major band accounted for more than 90% of the total OMP and thus probably represents the major structural protein of the outer

TABLE 2. Distribution of OMP profiles among 110 isolates of *C. jejuni* obtained from patients with sporadic infections in Colorado, 1978 to 1980

Profile type	No. of strains
1	2
2	26
3	58
4	1
5	5
6	3
7	4
8	4
9	5
Miscellaneous	2

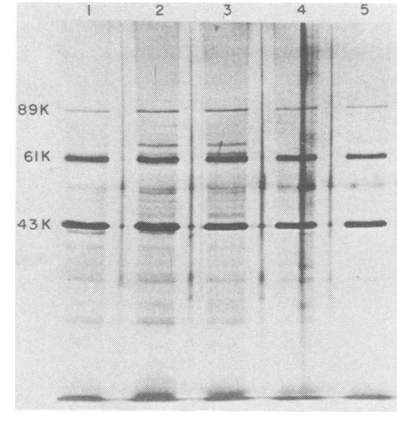

FIG. 10. SDS-PAGE (10% acrylamide) of OMPs of five *C. jejuni* strains isolated from humans during a common-source milkborne outbreak of campylobacter enteritis in Minnesota (7). All five strains show a major peptide migrating at 43,000 and minor peptides migrating at 61,000 and 89,000.

membrane. Logan and Trust have presented data suggesting that this peptide is the *C. jejuni* porin (17). It is possible that, as has been shown with *Escherichia coli* (10) and *Vibrio cholerae* (15), the major band may be resolved into separate sub-bands by using intensive methods, such as two-dimensional electrophoresis.

Our results showed that the SDS-PAGE profiles of *C. jejuni* outer membranes are stable. OMP profiles did not change when frozen samples of the same strain were grown and extracted on several occasions, after growth on different culture media, or after multiple passages on media. Various temperature and time conditions of incubation produced only minor effects on the OMP profiles. Studies of *N. meningitidis* (22) and *H. influenzae* (3, 12) also have shown that OMP profiles are stable under a variety of passage histories and experimental manipulations. A plasmid encoding for tetracycline resistance did not alter the outer membrane protein profile of a recipient *C. jejuni* strain (M. J. Blaser and D. A. Taylor, unpublished data).

Maximal recoverable membrane protein per cellular weight occurred after 48 h of incubation. Since the methods used maximized membrane yield, these results most likely reflect the time dependence of protein production rather than inefficiencies in harvesting membrane proteins at various steps of the cell metabolic cycle. As such we harvested all subsequent preparations after 48 h of incubation.

The major protein, with a molecular weight ranging from 41,000 to 45,000, was present in all *C. jejuni* strains with little apparent heterogeneity. Although we calculated the molecular weight of this band to be slightly different than that recently described by Logan and Trust, by all other parameters examined its characteristics were identical to those described for other *C. jejuni* strains (17). There was great variability in the presence or absence of the other more minor protein bands. Because some outer membrane constituents were solubilized by Sarkosyl, the SDS-PAGE profiles may not show the complete diversity present in native membranes. The small size of the LPS observed was consistent from strain to strain.

As was reported by Logan and Trust (17), we found that most *C. jejuni* and *C. coli* isolates studied had a single major OMP band. Discrepancies from the previous work in sizing the proteins probably arose from methodological differences. In a small number of *C. jejuni* or *C. coli* isolates from patients we found a doublet major band. This was not reported in the previous study, probably as a result of the smaller number of organisms studied. Our study confirms that *C. jejuni* and *C. coli* isolates cannot be differentiated from one another on the basis of OMP profiles but that these are distinct from those of *C. fetus* (20) and *C. faecalis*, two closely related catalase-positive *Campylobacter* species.

Based on migration of the major bands we recognized nine distinct profiles among the 110 strains examined. Two subtypes accounted for 76% of the isolates. Further studies are necessary to define the relative frequencies of the subtypes in different population groups and animal reservoirs.

The outer membrane profiles of other gram-negative organisms may be affected by changes in incubation conditions (32). We have only studied a small number of variations, but under the growth conditions used, the profiles of the isolates were stable and thus acceptable for development of a classification system. If minor protein bands are included, virtually every strain has a distinct profile. That strains from common source outbreaks (4, 5) had concordant profiles suggests that a system of typing *C. jejuni* based on OMP profiles could be useful for epidemiological investigations, as is true for other gram-negative pathogens (3, 8, 16). As discussed by Mocca and Frasch with reference to meningococci (22), serotyping reagents are available in only a few laboratories, whereas PAGE typing can be performed in any laboratory experienced in PAGE analysis of proteins. Development of a reference set of OMP preparations would facilitate this use.

The observation that *C. jejuni* isolates each contain a major OMP with structural characteristics that are largely conserved suggests that this marker may be useful for an immunological characterization of the organisms. Defining an OMP group antigen would facilitate development of a standard serological assay for *C. jejuni* infections and may be useful for vaccine development as well. Such studies are currently underway in our laboratory.

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