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Differentiation of *Campylobacter* Species by Protein Banding Patterns in Polyacrylamide Slab Gels

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Soluble protein extracts of 37 catalase-positive strains of *Campylobacter* species were examined by polyacrylamide slab gel electrophoresis (PAGE). Electrophoretic banding patterns showed good correlation with biochemical tests and with available DNA homology data in distinguishing species of *Campylobacter* but did not differentiate subspecies or biotypes. PAGE patterns indicated that *Campylobacter coli* is a distinct species. Furthermore, the PAGE patterns indicated that *C. jejuni* and nalidixic acid-resistant thermophilic *Campylobacter* species (*C. laridis*) are each distinct species. The protein banding patterns of *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* strains were distinctly different from those of the three thermophilic species.

Polyacrylamide gel electrophoresis (PAGE) of soluble proteins is a sensitive method and a practical aid for the classification of microorganisms (4, 5, 7, 8, 13–15, 17, 24, 28, 32). The use of appropriate PAGE reference patterns is sufficiently specific for primary identification of bacterial isolates (5, 24). In addition, the method is sufficiently sensitive to differentiate serotypes of anaerobic coryneforms (8).

Since the genus *Campylobacter* was first proposed by Véron and Chatelain (34) to include *Vibrio fetus* (31) and other similar vibrios (18) which were microaerophilic and neither oxidized nor fermented carbohydrates, investigators have used a number of classification systems to identify these organisms as to species (27). This situation probably resulted from the limited number of biochemical tests which were useful for distinguishing among these species and a lack of DNA-DNA homology data at that time. The accepted nomenclature is now that of Véron and Chatelain (34) and recent DNA-DNA homology studies have supported this scheme (1, 10, 11, 22, 26, 33). We have used PAGE to determine whether the electrophoretic patterns of soluble protein extracts of *Campylobacter* species are sufficiently distinct to be used as a basis of differentiation at the species, subspecies, or biotype level. The biotypes of *Campylobacter jejuni* and the nalidixic acid-resistant thermophilic strains (*C. laridis*) were described by Skirrow and Benjamin (30) and Benjamin et al. (2).

This investigation characterizes the protein patterns of 37 strains of *Campylobacter* species representing *C. fetus* subsp. *fetus*, *C. fetus* subsp. *venerealis*, *C. jejuni* biotypes 1 and 2, *C. coli*, and *C. laridis*.

MATERIALS AND METHODS

Bacterial strains. The 37 strains of *Campylobacter* species examined included 8 strains of *C. fetus* subsp. *fetus*, 2 strains of *C. fetus* subsp. *venerealis*, 15 strains of *C. jejuni* biotype 1 (one nalidixic acid-resistant strain), 4 strains of *C. jejuni* biotype 2, 7 strains of *C. coli*, and 1 strain of *C. laridis*.

The sources of the strains used in this study were as follows: *C. fetus* subsp. *venerealis* strains 147-80 (= ATCC 19438; American Type Culture Collection, Rockville, Md.; type strain) and 145-80 (= VPI 998; Virginia Polytechnic Institute and State University, Blacksburg); *C. fetus* subsp. *fetus* strains 149-80 (= ATCC 27374; type strain), 146-80 (= ATCC 15296), 148-80 (= ATCC 25936), 144-80 (= VPI PEDRO), 74-79 (= Butzler V44; J.-P. Butzler, St. Pierre Hospital, Brussels, Belgium; from human diarrheal feces), 90-81 (= Skirrow, 4620; M. B. Skirrow, Worcester Royal Infirmary, Worcester, England), 91-81 (= Skirrow 85979), and 92-81 (= Skirrow 3034); *C. jejuni* biotype 1 strains 173-79, 111-79, 112-79, 225-79, 94-79, 118-79, 26-80, and 5-80 isolated from human diarrheal stool and strain 90-79 isolated from the feces of a healthy chicken in our laboratory, strains 231-81 and 218-81 isolated from human diarrheal stool and strain 214-81 isolated from feces of a healthy dog (A. Folkens, Methodist Medical Center Hospital, Peoria, Ill.), 14-81 (University of Colorado Hospital 60; University of Colorado Hospital, Denver; human diarrheal stool), 10-81 (University of Colorado Hospital strain 57; human diarrheal stool), and 198-81 (= Falsen, EF 10357; E. Falsen, Institute of Medical Microbiology, Göteborg, Sweden; nalidixic acid resistant); *C. jejuni* biotype 2 strains 217-81 and 219-81 from human diarrheal stool (Folkens, Peoria, Ill.), 147-81 (= Falsen EF 10246), and 7981 (= Falsen EF 10259); *C. coli* strains 156-79, 220-79, and 221-79 from human diarrheal stool (isolated in our laboratory), 11-81 (= University of Colorado Hospital 55), 86-81 (= Falsen EF 7741), 89-81 (= Skirrow 5636), and 188-81 (= Rogol 7489; M. Rogol, Government Central Laboratories, Jerusalem, Israel; human diarrheal stool); *C. laridis* strain 94-81 (= Watson E149879; K. C. Watson, Central Microbiological Laboratories, Edinburgh, Scotland).

Identification. Strains were identified by phenotypic characteristics described by Holdeman et al. (12), Skirrow and Benjamin (30), and Lambe et al. (21). The test for hippurate hydrolysis (30) was modified by including aerobic overnight incubation at 37°C before addition of ninhydrin solution at 4°C. Development of a dark purple color in the upper ninhydrin layer after incubation for 15 min at room temperature indicated a positive test. The ninhydrin remained yellow in a negative test. This modification eliminated misidentification of weakly positive *C. jejuni* strains and false-positive *C. coli* strains and gave 100% correlation with PAGE banding patterns. The biochemical characteristics of catalase-positive *Campylobacter* species are shown in Table 1.

Organisms were grown at 37°C for 24 h in tissue culture flasks in a biphasic medium as described by Rollins et al. (D. M. Rollins, J. C. Coolbaugh, and R. I. Walker, Abstr.

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TABLE 1. Biochemical characteristics of catalase-positive *Campylobacter* species[a]

Species (no. of strains)	Growth at: 25°C	Growth at: 42°C	Growth in the presence of nalidixic acid (50 μg/ml)	Growth in the presence of 2,3,5-triphenyltetrazolium chloride (400 μg/ml)[b]	Hippurate hydrolysis[c]	H_2S produced in metabisulfite-sulfate-containing medium[c]	Growth in 1% glucine
C. fetus subsp. *venerealis* (2)	+ (100)[d]	− (0)	+ (100)	− (0)	− (0)	− (0)	− (0)
C. fetus subsp. *fetus* (8)	+ (100)	− (0)	+ (100)	− (0)	− (0)	− (0)	+ (100)
C. jejuni biotype 1 (15)	− (0)	+ (100)	− (6.7)	+ (100)	+ (100)	− (0)	+ (100)
C. jejuni biotype 2 (4)	− (0)	+ (100)	− (0)	+ (100)	+ (100)	+ (100)	+ (100)
C. coli (7)	− (0)	+ (100)	− (0)	+ (100)	− (0)	− (0)	+ (100)
C. laridis (1)	− (0)	+ (100)	+ (100)	+ (100)	− (0)	+ (100)	+ (100)

[a] All strains in this study conform to the biochemical characteristics of the genus *Campylobacter* (12).
[b] Lambe et al. (21).
[c] Skirrow and Benjamin (30).
[d] Values in parentheses are percent positive for each character.

Annu. Meet. Am. Soc. Microbiol. 1982, D10, p. 49). The effect of growth conditions upon protein banding patterns was examined by comparing selected strains grown at 37 and 42°C and by comparing strains grown in biphasic medium with those grown in thioglycolate agar containing 5% sheep blood.

Preparation of soluble fractions. Cells were harvested by centrifugation at 8,000 × *g* for 30 min and suspended in 2 ml of 0.15 M Tris-hydrochloride buffer, pH 7.0. Cells were disrupted by sonication for a total of 2 min with 8 × 15-s bursts in an MSE sonic oscillator fitted with a 19-mm titanium probe (O. H. Johns Scientific, Toronto, Ont.). The probe was switched off for 30 s between bursts for cooling. The bacterial suspension was maintained at 4°C in an ice-water–ethanol bath; the amplitude of the sonicator was set at 1.4 nm. After centrifugation at 8,000 × *g* for 30 min, the protein content of the soluble fraction was estimated by the method of Lowry et al. (23) and adjusted to 3 mg/ml by dilution. Sucrose was added to a final concentration of 30% (wt/vol). Fractions were stored at −15°C until used.

PAGE. Vertical anionic electrophoresis was carried out in polyacrylamide slab gels with 0.025 M Tris–0.2 M glycine electrode buffer, pH 8.3. An 8.4% acrylamide resolving gel and a 4.5% spacer gel were used. Samples of 50 μl, containing 150 μg of protein, were loaded into sample wells. Electrophoresis was carried out at 25°C, using a vertical tank electrophoresis apparatus (model 100; Aquebogue Machine and Repair Shop, Aquebogue, N.Y.). Voltage was maintained at 150 V, using a constant-voltage power supply (model ECPS 2000/300; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), until bromphenol blue tracking dye placed in the upper tank reached the bottom of the gel, approximately 2.5 h. The PAGE methods of Laemmli (20) were modified by omitting sodium dodecyl sulfate and EDTA. Gels were stained with 2% Coomassie brilliant blue in water-methanol-acetic acid (4.5:4.5:1) for 4 h at 37°C, destained by agitation in several changes of the same solvent without dye, and stored in 7% acetic acid. All chemicals were from Sigma Chemical Co. (St. Louis, Mo.) except acrylamide, *N,N'*-methylenebisacrylamide, and *N,N,N',N'*-tetramethylethylenediamine, which were obtained from Bio-Rad Laboratories (Richmond, Calif.).

RESULTS

Strain identification. The phenotypic characteristics of the catalase-positive *Campylobacter* species are given in Table 1. *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* strains, which are separable by few phenotypic tests, did display characteristic cellular morphology (16). Strain 198-81 was nalidixic acid resistant but failed to produce ferrous sulfide in the medium described by Skirrow and Benjamin (30) and possessed hippurate hydrolase activity; it was therefore identified as *C. jejuni* biotype 1 rather than *C. laridis*.

PAGE. Figures 1 and 2 show electrophoretic protein banding patterns for representative strains of *C. jejuni* biotype 1, *C. jejuni* biotype 2, *C. coli*, *C. laridis*, *C. fetus* subsp. *venerealis*, and *C. fetus* subsp. *fetus*. The arrowheads (Fig. 1) indicate the protein bands present in all strains of a species which are distinct for strains of that species. The remaining bands are shared among two or more species or are variable among strains within a species and are, therefore, not useful as distinguishing features.

The various species can be distinguished from one another by considering a single region of each lane or run, although distinctive bands occur at various points throughout the length of a lane or run (Fig. 1, lanes 1 to 6, arrowheads). In the region between the top of the gel and 1 cm, strains of *C. jejuni* biotype 1 (Fig. 1, lane 1) and *C. jejuni* biotype 2 (Fig. 1, lane 2) share a single sharply focused band at approximately 6 mm. All strains of *C. jejuni* biotypes 1 and 2 examined shared this band (Fig. 3 and 4). The *C. coli* strain (Fig. 1, lane 3) lacks the band at 6 mm but has a thin dense band at 1 cm. All seven strains of *C. coli* examined (Fig. 5, lanes 4 to 10), obtained from varied locations throughout the world, lacked the band at 6 mm. Three strains (Fig. 5, lanes 4 and 6) isolated from diarrhea patients in Johnson City, Tenn., showed the band at 1 cm, whereas the other four strains isolated from patients outside of Tennessee lacked any sharply focused bands in the 0- to 1-cm region. The *C. laridis* strain (Fig. 1, lane 4) also lacks the band at 6 mm which is distinctive for the *C. jejuni* biotype 1 and 2 strains. The *C. laridis* strain (94-81) did, however, show a distinctive banding pattern in the 0- to 1-cm region of the gel. Thin double

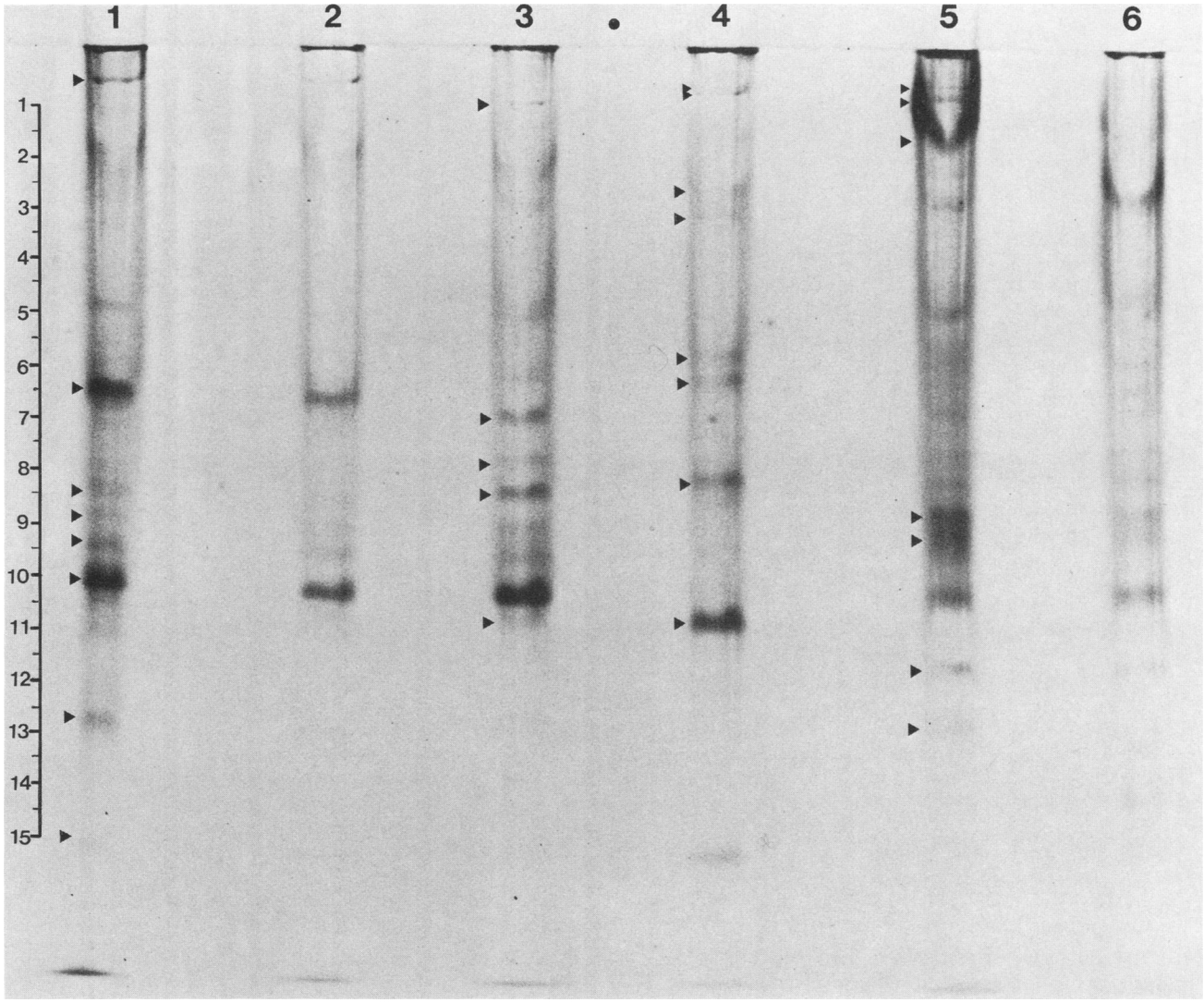

FIG. 1. Protein patterns from representative strains of *C. jejuni*, *C. coli*, *C. laridis*, and *C. fetus*. All strains were grown in biphasic medium at 37°C for 24 h. Lanes: (1) *C. jejuni* biotype 1, strain 111-79; (2) *C. jejuni* biotype 2, strain 217-81; (3) *C. coli*, strain 156-79; (4) *C. laridis*, strain 94-81; (5) *C. fetus* subsp. *venerealis*, strain 147-80 (type strain); (6) *C. fetus* subsp. *fetus* strain 149-80 (type strain). Arrowheads indicate characteristic bands for each species.

lines at 7.5 and 8.0 mm are unique to this strain (Fig. 1, lane 4; Fig. 2, lanes 8 and 9; Fig. 5, lane 1). Strain 198-81 (*C. jejuni* biotype 1) was resistant to nalidixic acid; otherwise, it was identical to other strains of *C. jejuni* biotype 1 in biochemical reactions and banding patterns in PAGE. *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* likewise showed a unique pattern in the 0- to 1-cm region. All strains of both subspecies of *C. fetus* showed widely spaced thin dense bands at 7 and 9 mm (Fig. 1, lanes 5 and 6; Fig. 6).

The effects of media and temperature on protein banding patterns of several strains were investigated. *C. jejuni* biotype 1, strains 173-79 (Fig. 4, lanes 1 and 2) and 10-81 (Fig. 4, lanes 3 and 4), and *C. jejuni* biotype 2, strains 219-81 (Fig. 3, lanes 12 and 13; Fig. 4, lanes 7 and 8) and 147-81 (Fig. 4, lanes 11 and 12), were grown in biphasic medium at 37 and 42°C. Identical bands were seen in both preparations, although the size and relative intensity of the band at 98 mm were diminished in the organisms grown at 42°C. A third strain of *C. jejuni* biotype 2 (strain 79-81) did not show a decrease in the intensity of this band at 42°C (Fig. 4, lanes 9 and 10). No shared bands were gained or lost by varying the temperature of growth for the strains tested.

C. fetus subsp. *venerealis* strain 145-80 (Fig. 6, lanes 1 and 2), *C. fetus* subsp. *fetus* strain 144-80 (Fig. 6, lanes 11 and 12), and *C. jejuni* biotype 2 strain 217-81 (Fig. 4, lanes 5 and 6) were grown at 37°C in the biphasic medium and on thioglycolate agar supplemented with 5% sheep blood to determine whether growth on a solid-phase substrate would alter the banding patterns of the strains. There were no qualitative or quantitative differences in the banding patterns of either preparation.

DISCUSSION

The catalase-positive *Campylobacter* species can be divided into two groups; thermophilic which grow at 42°C but not at 25°C, and nonthermophilic which grow at 25°C but not at 42°C. The thermophilic strains, which include the frank pathogens for humans (6, 27, 29), have been the most

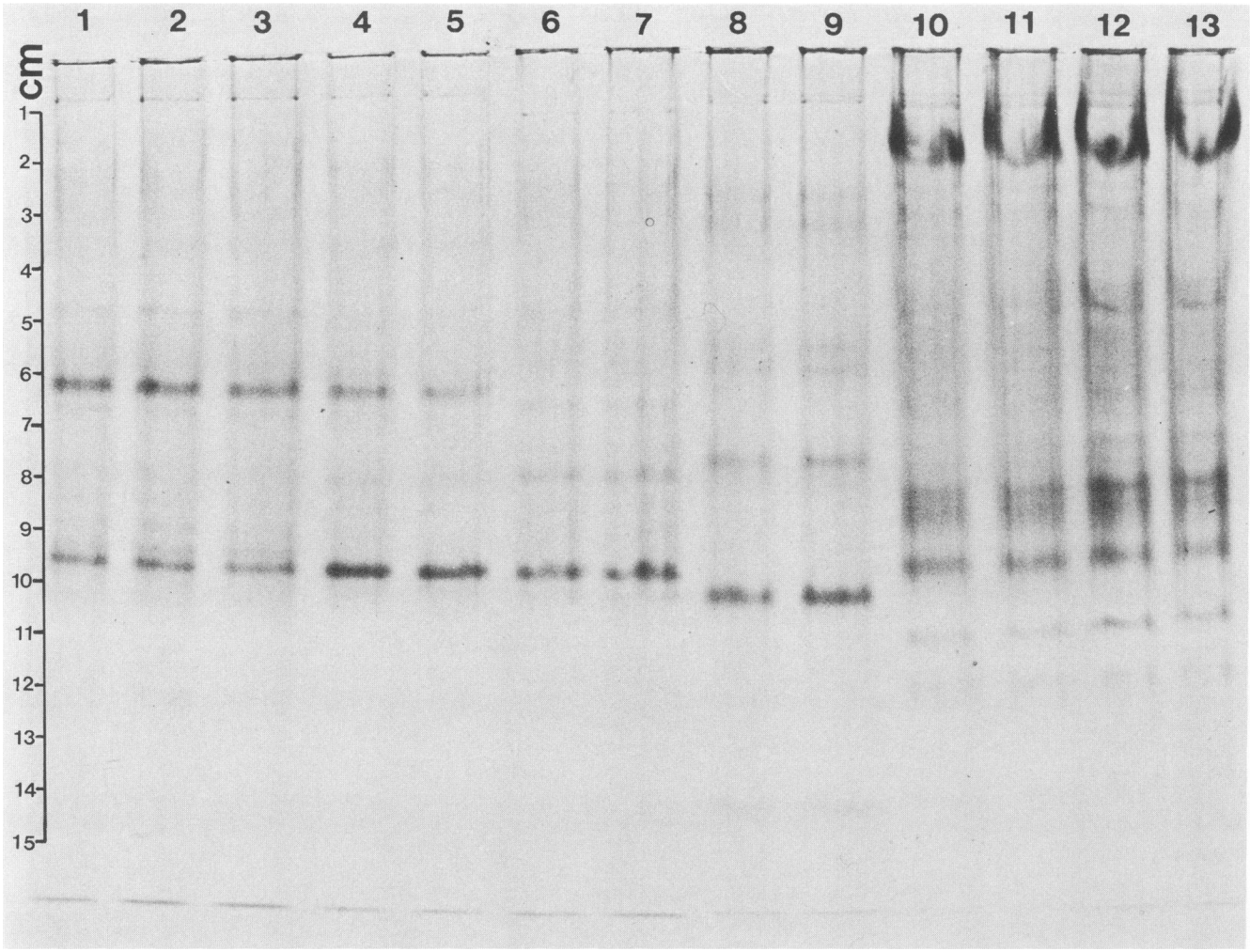

FIG. 2. Protein patterns of representative strains of *C. jejuni*, *C. coli*, *C. laridis*, and *C. fetus* run in duplicate. Growth conditions were as in the legend to Fig. 1. Lanes: (1 to 3) *C. jejuni* biotype 1, strain 111-79; (4, 5) *C. jejuni* biotype 2, strain 217-81; (6, 7) *C. coli*, strain 156-79; (8, 9) *C. laridis*, strain 94-81; (10, 11) *C. fetus* subsp. *venerealis*, strain 147-80 (type strain); (12, 13) *C. fetus* subsp. *fetus*, strain 91-81.

difficult to identify as species. Table 1 shows phenotypic characteristics which are useful for separating strains into species, subspecies, and biotypes. Our data suggest that, on the basis of soluble protein profiles in polyacrylamide slab gels, the nomenclature of Véron and Chatelain (34) properly describes the relationships of these organisms. These findings agree well with results from recent DNA-DNA homology studies (1, 10, 11, 22, 26, 33).

Each of these studies concludes that *C. coli* and *C. jejuni* are separate species. Although separation of these species in the clinical laboratory is usually based on a single test (hippurate hydrolysis), DNA-DNA homology studies correlated well with this characteristic (11, 33). DNA-DNA homology analysis of our *C. coli* strains 156-79 and 220-79 show a high degree of homology (84 and 71%, respectively) with reference *C. coli* DNA and lower homology (47 and 39%, respectively) with reference *C. jejuni* DNA (10).

The thermophilic species differ markedly from the nonthermophilic *C. fetus* strains in temperature optima, phenotypic properties (Table 1), morphology (16), pathogenic potential (27), and serological cross-reactivity (3, 19). The DNA-DNA homology studies confirm these differences (1, 10, 11, 22, 26, 33). Our PAGE data provided an additional basis for differentiation (Fig. 1 and 2). The marked similarity of the protein banding patterns of *C. fetus* subsp. *venerealis* and *C. fetus* subsp. *fetus* supports the suggestion of Harvey and Greenwood (10) that they are not sufficiently different in DNA-DNA homology to warrant separation into subspecies. The thermophilic species (*C. coli* and *C. jejuni*), however, are both distinctly different in their protein banding patterns and can readily be differentiated from each other and from *C. fetus* strains.

C. laridis (strain 94-81) differed substantially in its protein banding pattern from other species tested. Our PAGE patterns and DNA-DNA homology findings of others (2, 10) show that these strains represent a separate species. Belland and Trust (1), however, reported significant DNA relatedness between two reference strains of *C. jejuni* and one nalidixic acid-resistant thermophilic strain. It should be noted that nalidixic acid resistance is not a consistent characteristic for distinguishing *C. laridis*. One isolate of *C. jejuni* (strain 198-81) obtained from Sweden was nalidixic acid resistant; otherwise, it was identical to other strains of *C. jejuni* in other biochemical reactions and had a protein banding pattern which was typical of *C. jejuni* but not of *C. laridis*. Other workers have reported rare nalidixic acid-

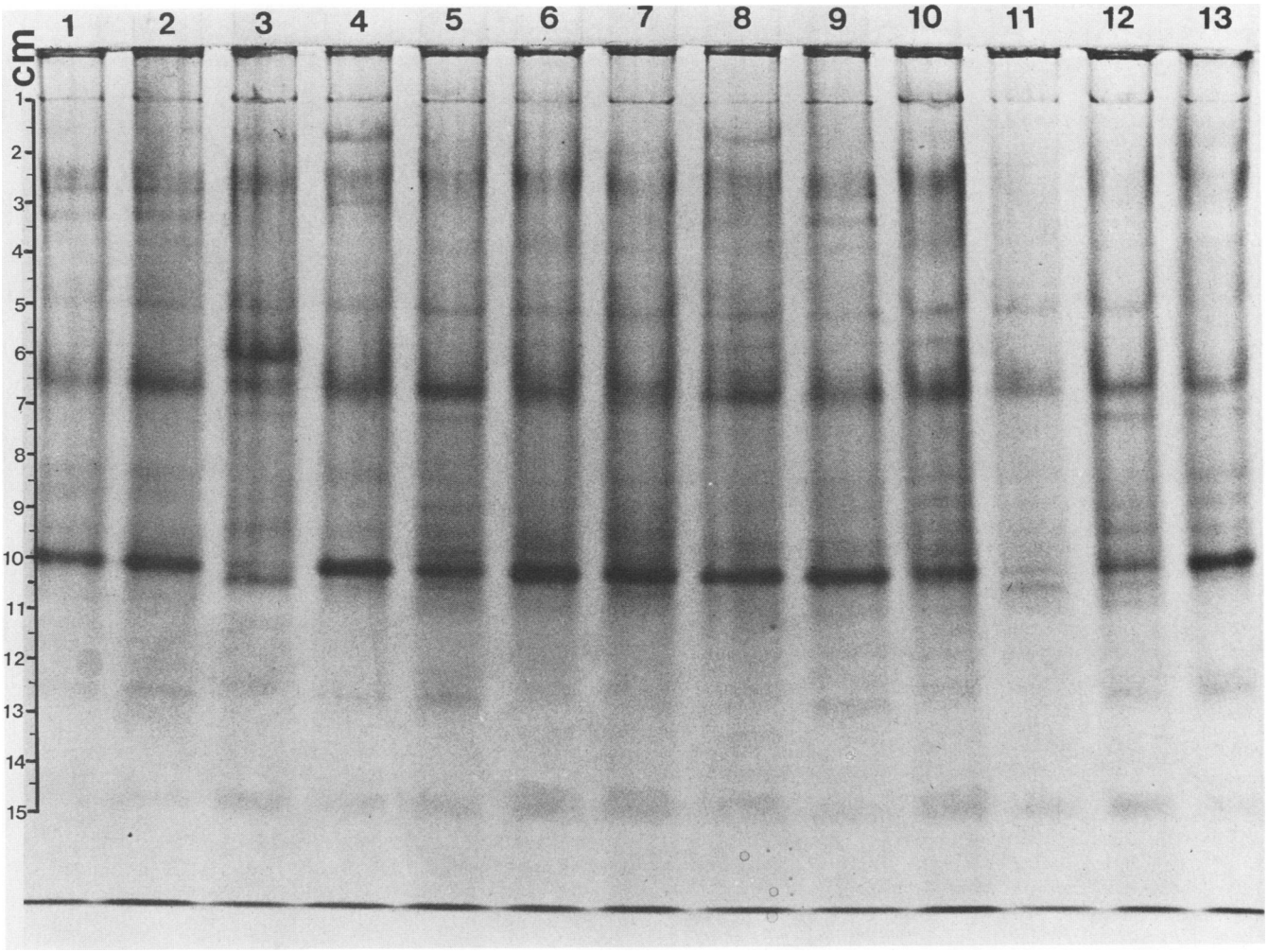

FIG. 3. Protein patterns of strains of *C. jejuni* biotype 1. Growth conditions were as in the legend to Fig. 1, except as noted below. Lanes 1 to 11—*C. jejuni* biotype 1: (1) strain 214-81; (2) 218-81; (3) 26-80; (4) 94-79; (5) 111-79; (6) 112-79; (7) 14-81; (8) 225-79; (9)5-80; (10) 90-79; (11) 173-79; lanes 12 and 13—*C. jejuni* biotype 2: (12) strain 219-81 (42°C); (13) 219-81.

resistant *C. jejuni* strains by using DNA-DNA homology (33). Because of reported nalidixic acid resistance in strains of *C. jejuni*, hippurate hydrolysis and H_2S production in iron metabisulfite medium (30) are more reliable phenotypic markers than nalidixic acid resistance for distinguishing *C. laridis* from strains of *C. jejuni* and *C. coli*.

Two earlier studies utilized PAGE to differentiate *Campylobacter* sp. strains. Morris and Park (25) analyzed the water-insoluble proteins extracted from the cell pellet after sonication by the acid phenol method. These workers found that the PAGE patterns of the acid phenol-soluble proteins produced three groups which correlated with habitat. Group I, isolated from bovine genital tracts and associated with infertility, was probably *C. fetus* subsp. *venerealis*; group II isolated from cattle feces, associated with sporadic abortion in cattle and sheep, was probably *C. fetus* subsp. *fetus*; and group III, isolated from healthy pigs and pigs with swine dysentery, was probably *C. jejuni* (27). These workers analyzed the acid phenol-soluble proteins of the cell pellet because they were unable to differentiate strains by electrophoresis of water-soluble proteins. Possible problems with their analysis of these water-soluble proteins were that (i) at least two of their groups (I and II) were closely related by DNA-DNA homology (10) and (ii) gels were electrophoresed at constant current rather than at constant voltage; thus, the migration of the protein bands was not necessarily a linear function with time. The fluctuating voltage might, therefore, result in varying patterns from run to run. Our data showed that the total water-soluble proteins produced distinctive banding patterns for each of the four species tested when anionic electrophoresis was used with constant voltage.

In a more recent study, Hanna et al. (9) used the acid phenol-soluble protein method of Morris and Park (25) to study aerotolerant and reference strains of *Campylobacter* species. These investigators distinguished *C. sputorum*, *C. fetus*, *C. jejuni*, *C. coli*, and the aerotolerant strains by this method. Unlike Morris and Park (25), they could not distinguish the subspecies of *C. fetus* by this method. Similarly, they were unable to distinguish *C. sputorum* subsp. *sputorum* from *C. sputorum* subsp. *bubulus*. *C. laridis* strains were not tested. Our water-soluble protein data agreed with the acid phenol-soluble protein data of Hanna et al. (9) and with DNA-DNA homology data (1, 2, 10, 11, 22, 26, 33) in that the thermophilic *Campylobacter* species consist of at least three species: *C. jejuni*, *C. coli*, and *C. laridis*. All three species possess unique and reproducible protein banding

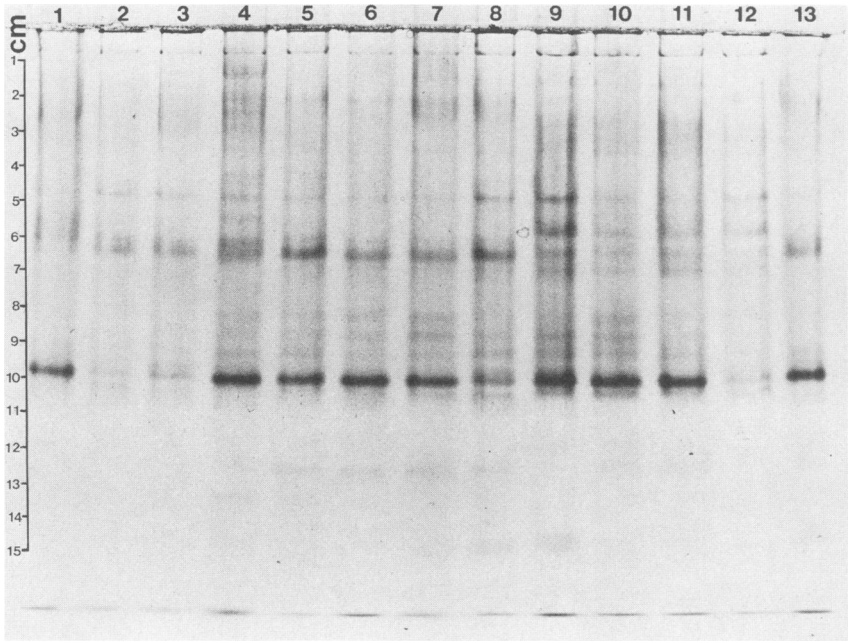

FIG. 4. Protein patterns of strains of *C. jejuni* biotype 2. Growth conditions were as in the legend to Fig. 1, except as noted below. Lanes 1 to 4; 13—*C. jejuni* biotype 1: (1) strain 173-79; (2) 173-79 (42°C); (3) 10-81 (42°C); (4) 10-81; (13) 112-79; lanes 5 to 12—*C. jejuni* biotype 2: (5) strain 217-81; (6) 217-81 (thioglycolate blood agar); (7) 219-81; (8) 219-81 (42°C); (9) 79-81 (42°C); (10) 79-81; (11) 147-81; (12) 147-81 (42°C).

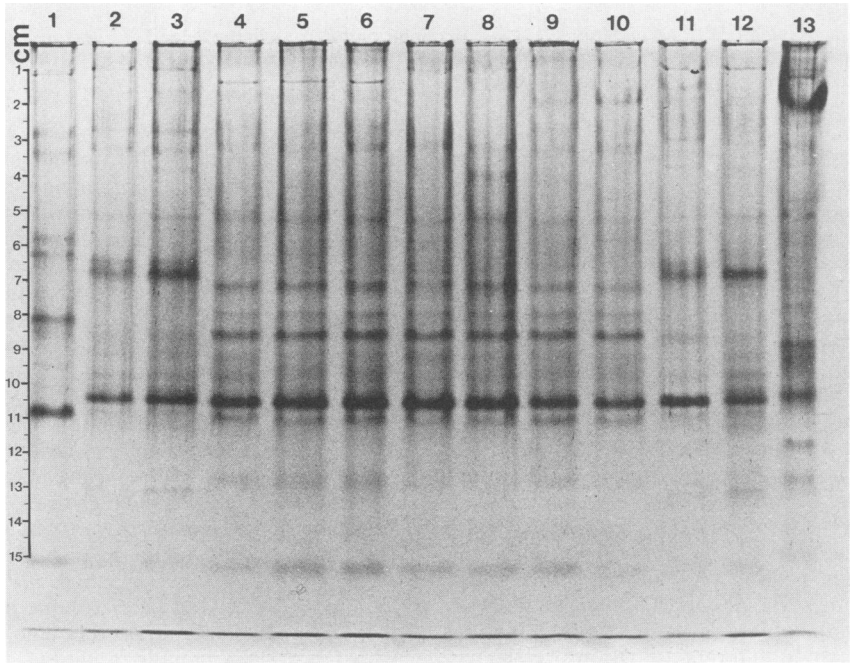

FIG. 5. Protein patterns of strains of *C. coli* and C. laridis. Growth conditions were as described in the legend to Fig. 1. Lanes 4 to 10, *C. coli*: (4) strain 156-79; (5) 220-79; (6) 221-79; (7) 89-81; (8) 188-81; (9) 11-81; (10) 86-81; lane 1, *C. laridis* 94-81; lanes 2, 3, 11, 12, *C. jejuni* biotype 1: (2) strain 214-81; (3) 218-81; (11) 94-79; (12) 111-79; lane 13, *C. fetus* subsp. *venerealis* 147-80 (type strain).

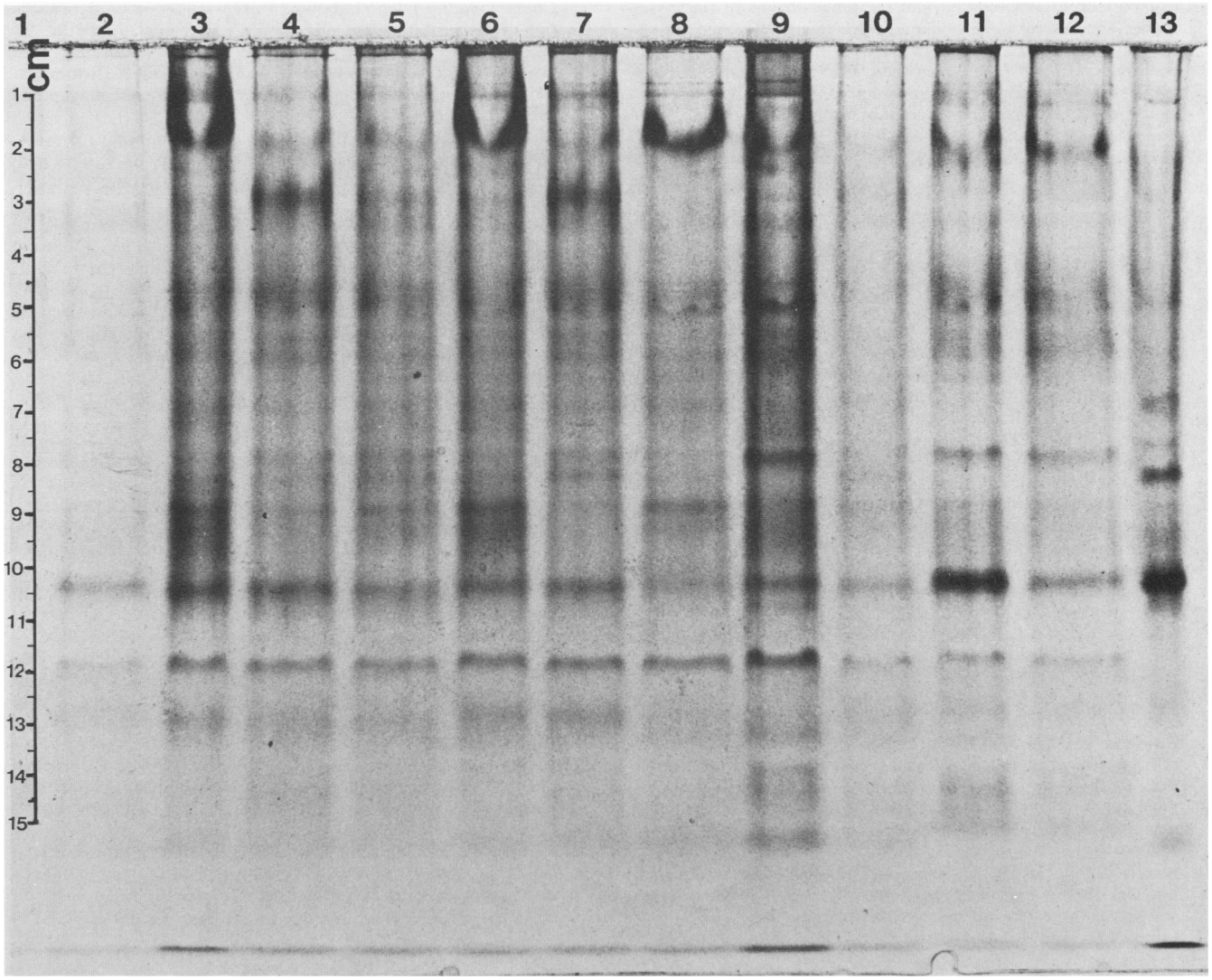

FIG. 6. Protein patterns of strains of *C. fetus*. Growth conditions were as in the legend to Fig. 1, except as noted below. Lanes 1 to 3—*C. fetus* subsp. *venerealis*: (1) strain 145-80; (2) 145-80 (thioglycolate blood agar); (3) 147-80 (type strain); lanes 4 to 12—*C. fetus* subsp. *fetus*: (4) strain 149-80 (type strain); (5) 90-81; (6) 91-81; (7) 92-81; (8) 148-80; (9) 146-80; (10) 74-79; (11) 144-80; (12) 144-80 (thioglycolate blood agar); lane 13, *C. coli*, strain 221-79.

patterns. Although identification of *Campylobacter* as to species was readily achieved by electrophoresis of water-soluble proteins, it was not possible to differentiate the biotypes of *C. jejuni* nor the subspecies of *C. fetus* by this method.

ACKNOWLEDGMENT

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LITERATURE CITED

1. **Belland, R. J., and T. J. Trust.** 1982. Deoxyribonucleic acid sequence relatedness between thermophilic members of the genus *Campylobacter*. J. Gen. Microbiol. **128**:2515–2522.
2. **Benjamin, J., S. Leaper, R. J. Owen, and M. B. Skirrow.** 1983. Description of *Campylobacter laridis*, a new species comprising the nalidixic-acid resistant thermophilic *Campylobacter* (NARTC) group. Curr. Microbiol. **8**:231–238.
3. **Berg, R. L., J. W. Jutila, and B. D. Firehammer.** 1971. Revised classification of *Vibrio fetus*. Am. J. Vet. Res. **32**:11–22.
4. **Calhoon, D. A., W. R. Mayberry, and J. Slots.** 1981. Cellular fatty acid and soluble protein composition of *Actinobacillus actinomycetemcomitans* and related organisms. J. Clin. Microbiol. **14**:376–382.
5. **Cato, E. P., D. E. Hash, L. V. Holdeman, and W. E. C. Moore.** 1982. Electrophoretic study of *Clostridium* species. J. Clin. Microbiol. **15**:688–702.
6. **Dekeyser, P., M. Gossuin-Detrain, J. P. Butzler, and J. Sternon.** 1972. Acute enteritis due to related *Vibrio*: first positive stool culture. J. Infect. Dis. **125**:390–392.
7. **Fox, R. H., and D. E. McClain.** 1974. Evaluation of the taxonomic relationship of *Micrococcus cryophilus*, *Branhamella catarrhalis*, and *Neisseriae* by comparative polyacrylamide gel electrophoresis of soluble proteins. Int. J. Syst. Bacteriol. **24**:172–176.
8. **Gross, C. S., D. A. Ferguson, Jr., and C. S. Cummins.** 1978. Electrophoretic protein patterns and enzyme mobilities in anaerobic coryneforms. Appl. Environ. Microbiol. **35**:1102–1108.
9. **Hanna, J., S. D. Neill, J. J. O'Brien, and W. A. Ellis.** 1983.

Comparison of aerotolerant and reference strains of *Campylobacter* species by polyacrylamide gel electrophoresis. Int. J. Syst. Bacteriol. **33**:143–146.

10. **Harvey, S. M., and J. R. Greenwood.** 1983. Relationships among catalase-positive campylobacters determined by deoxyribonucleic acid-deoxyribonucleic acid hybridization. Int. J. Syst. Bacteriol. **33**:275–284.
11. **Hébert, G. A., D. G. Hollis, R. E. Weaver, A. G. Steigerwalt, R. M. McKinney, and D. J. Brenner.** 1983. Serogroups of *Campylobacter jejuni*, *Campylobacter coli*, and *Campylobacter fetus* defined by direct immunofluorescence. J. Clin. Microbiol. **17**:529–538.
12. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
13. **Holdeman, L. V., W. E. C. Moore, P. J. Churn, and J. L. Johnson.** 1982. Descriptions of *Bacteroides oris* sp. nov. and *Bacteroides buccae* sp. nov. isolated from human periodontitis and other human infections. Int. J. Syst. Bacteriol. **32**:125–131.
14. **Howard, C. J., D. H. Pocock, and R. N. Gourlay.** 1981. Polyacrylamide gel electrophoretic comparison of the polypeptides of ureaplasmas isolated from cattle and humans. Int. J. Syst. Bacteriol. **31**:128–130.
15. **Jarvis, A. W., and J. M. Wolff.** 1979. Grouping of lactic streptococci by gel electrophoresis of soluble cell extracts. Appl. Environ. Microbiol. **37**:391–398.
16. **Karmali, M. A., A. K. Allen, and P. C. Fleming.** 1981. Differentiation of catalase-positive campylobacter with special reference to morphology. Int. J. Syst. Bacteriol. **31**:64–71.
17. **Kersters, K., and J. DeLey.** 1975. Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. J. Gen. Microbiol. **87**:333–342.
18. **King, E. O.** 1957. Human infection with *Vibrio fetus* and a closely related *Vibrio*. J. Infect. Dis. **101**:119–128.
19. **Kosunen, T. U., D. Danielsson, and J. Kjellander.** 1980. Serology of *Campylobacter fetus* ss. *jejuni* ("related" *Campylobacters*). Acta Pathol. Microbiol. Scand. Sect. B **88**:207–218.
20. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
21. **Lambe, D. W., Jr., D. A. Ferguson, Jr., S. L. Wiener, and J. P. Butzler.** 1981. *Campylobacter fetus* ssp. *jejuni* isolation from patients with gastroenteritis. South. Med. J. **74**:157–161.
22. **Leaper, S., and R. J. Owens.** 1982. Differentiation between *Campylobacter jejuni* and allied *Campylobacters* by hybridization of deoxyribonucleic acids. FEMS Microbiol. Lett. **15**:203–208.
23. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
24. **Moore, W. E. C., D. E. Hash, L. V. Holdeman, and E. P. Cato.** 1980. Polyacrylamide slab gel electrophoresis of soluble proteins for studies of bacterial floras. Appl. Environ. Microbiol. **39**:900–907.
25. **Morris, J. A., and R. W. Park.** 1973. A comparison using gel electrophoresis of cell proteins of *Campylobacters* (Vibrios) associated with infertility, abortion and swine dysentery. J. Gen. Microbiol. **78**:165–178.
26. **Owen, R. J., and S. Leaper.** 1981. Base composition, size and nucleotide-sequence similarities of genome deoxyribonucleic acids from species of the genus *Campylobacter*. FEMS Microbiol. Lett. **12**:395–400.
27. **Rettig, P. J.** 1979. *Campylobacter* infections in human beings. J. Pediatr. **94**:855–864.
28. **Seiter, J. A., and J. M. Jay.** 1980. Application of polyacrylamide gel electrophoresis to the characterization and identification of *Arthrobacter* species. Int. J. Syst. Bacteriol. **30**:460–465.
29. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
30. **Skirrow, M. B., and J. Benjamin.** 1980. Differentiation of enteropathogenic *Campylobacter*. J. Clin. Pathol. **33**:1122.
31. **Smith, T., and M. S. Taylor.** 1919. Some morphological and biological characters of Spirilla (*Vibrio fetus* n. sp.) associated with disease of fetal membranes in cattle. J. Exp. Med. **30**:299–311.
32. **Strom, A., J. K. Dyer, C. Marsh, and J. K. Tribble.** 1976. Identification and characterization of species of the family *Bacteroidaceae* by polyacrylamide gel electrophoresis. J. Dent. Res. **55**:252–256.
33. **Ursing, J., M. Walder, and K. Sandstedt.** 1983. Base composition and sequence homology of deoxyribonucleic-acid of thermotolerant *Campylobacter* from human and animal sources. Curr. Microbiol. **8**:307–310.
34. **Véron, M., and R. Chatelain.** 1973. Taxonomic study of the genus *Campylobacter* Sebald and Véron and designation of the neotype strain for the type species, *Campylobacter fetus* (Smith and Taylor) Sebald and Véron. Int. J. Syst. Bacteriol. **23**:122–134.