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Restriction Fragment Length Polymorphisms in the Ribosomal Genes for Species Identification and Subtyping of Aerotolerant *Campylobacter* Species

JULIA A. KIEHLBAUCH,[1]* BRIAN D. PLIKAYTIS,[2] BALASUBRAMANIAN SWAMINATHAN,[3] DANIEL N. CAMERON,[1] AND I. KAYE WACHSMUTH[1]

Enteric Diseases Branch,[1] Biostatistics and Information Management Branch,[2] and Meningitis and Special Pathogens Branch,[3] Division of Bacterial and Mycotic Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

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Whole-cell chromosomal digests of 84 strains of aerotolerant *Campylobacter* (AC) were examined by using *Pvu*II restriction fragment length polymorphisms of rRNA genes followed by hybridization with *Escherichia coli* 16S and 23S rRNA (ribotyping). The AC strains belonged to *Campylobacter cryaerophila* ($n = 13$) and a newly defined species, "*C. butzleri*" ($n = 64$). Strains of *C. cryaerophila* belonged to two hybridization groups: DNA group 1A (including the type strain of *C. cryaerophila*) and DNA group 1B (J. A. Kiehlbauch, D. J. Brenner, M. A. Nicholson, C. N. Baker, C. M. Patton, A. G. Steigerwalt, and I. K. Wachsmuth, J. Clin. Microbiol. 29:376–385, 1991). Six AC strains not classified as *C. cryaerophila* or "*C. butzleri*" were also included. All 35 sporadic human and animal isolates of "*C. butzleri*" sent to the Centers for Disease Control for identification showed different ribotype patterns. However, most "*C. butzleri*" strains contained common bands at ~3.0, 6.2, 12.0, and 15.0 kb; the 3.0-kb band was present in all but four strains. An additional 23 strains of "*C. butzleri*," isolated as part of special studies, contained the 3.0-kb band. Thus, on the basis of visual identification of the 3.0-kb band, 94% of available strains were correctly identified as "*C. butzleri*." Ribotyping demonstrated that *C. cryaerophila* strains (DNA groups 1A and 1B) were different from *C. butzleri* strains. All *C. cryaerophila* strains demonstrated a common ribosomal DNA restriction fragment of 3.2 kb; DNA group 1B strains contained an additional common band at 2.6 kb. Ribotyping patterns of AC species were easily distinguished from patterns of other *Campylobacter*, *Helicobacter*, and *Wolinella* species. Thus, ribotyping patterns were useful in discriminating between AC strains and genospecies, as well as between AC species and other *Campylobacter* species that may share some phenotypic characteristics. Quantitation of ribotyping results, as described here, allowed comparisons of strains electrophoresed on different gels.

As a general rule, *Campylobacter* isolates are relatively inactive phenotypically and definitive strain identification is based on limited phenotypic characterization. Because of this narrow set of phenotypic tests, a single atypical characteristic can result in misidentification of a strain; e.g., a strain of *Campylobacter jejuni* that is hippurate negative would be identified as *C. coli* (41). This often necessitates laborious DNA-DNA hybridization for definitive species identification. In addition, subspecies typing techniques for strain discrimination in epidemiologic studies are generally available only for *C. jejuni* and *C. coli*. We found recently that typing based on restriction fragment length polymorphisms (RFLPs) of rRNA genes (ribotyping) was one of the most sensitive of 10 methods used for discrimination among an epidemiologically defined group of *Campylobacter* strains (26). Also, common rRNA gene restriction fragments were observed within a particular *Campylobacter* species. This allowed discrimination of atypical *C. jejuni* and *C. coli* isolates from typical *C. jejuni* isolates.

In this study, we applied ribotyping to a group of aerotolerant *Campylobacter* (AC) organisms recently classified by DNA-DNA hybridization. DNA-DNA hybridization indicated the presence of at least two species of AC: *C. cryaerophila* and "*C. butzleri.*" *C. cryaerophila* was genotypically and phenotypically heterogeneous, containing 14 strains which were difficult to separate by DNA hybridization; 5 strains were more closely related to the type strain of *C. cryaerophila* (DNA hybridization group 1A), and 8 strains were more closely related to one another (DNA hybridization group 1B). Two DNA group 1B strains phenotypically resembled the type strain of *C. cryaerophila*; the remaining six strains more closely resembled "*C. butzleri*" (14). Our objectives were to determine whether ribotyping would be useful in distinguishing between these closely related organisms and the possible usefulness of this technique in discriminating among strains of AC for epidemiologic purposes. In addition, we devised a scheme for quantitative analysis for evaluation of the rRNA banding data.

MATERIALS AND METHODS

Bacterial strains. Eighty-four isolates of AC were included in this study. Seventy-eight of these isolates were characterized by both DNA hybridization and phenotypic traits, as described in detail elsewhere (14). An additional six strains which have not been classified in any currently defined DNA hybridization group of AC were also included. These strains were provided by S. D. Neill, Belfast, Ireland, and were from the investigation that originally described *C. cryaerophila* (19). In addition, type and reference strains representing all currently recognized species and hybridization groups of *Campylobacter* were included in this study. Two groups of *Campylobacter* not currently recognized as validly pub-

* Corresponding author.

lished species were included, "*C. upsaliensis*" (34) and CLO1B. Strains belonging to *C. cinaedi* and CLO1B are designated as *C. cinaedi*/CLO1B, with the exception of the reference strain of CLO1B designated by P. Totten (40), since they cannot be differentiated by currently available phenotypic tests.

Isolates of AC were cultured on heart infusion agar containing 5% rabbit blood (BBL Microbiology Systems, Cockeysville, Md.) unless otherwise indicated. Plates were incubated at 30°C in a microaerobic atmosphere consisting of approximately 5% O_2, 7.5% CO_2, 7.5% H_2, and 80% N_2. Isolates of type and reference strains were incubated at 36°C, with the exception of *C. nitrofigilis*, which was incubated at 25°C under the same conditions.

DNA extraction and purification. Two DNA isolation methods were used in preparing the DNA used in this study.

(i) Large-scale preparation. Preparation of large quantities of purified DNA for use in DNA hybridization studies is described elsewhere (4, 14). Aliquots (2 μg) of purified DNA were utilized for restriction digestions.

(ii) Extraction of DNA by using guanidium thiocyanate. A method of DNA extraction which utilizes guanidium thiocyanate for cell lysis was included because it appeared to eliminate the high levels of endonucleases often encountered when working with *Campylobacter* species other than *C. jejuni* or *C. coli*. The procedure was performed essentially as described by Pitcher et al. (30), with three exceptions. First, most *Campylobacter* strains required larger cell pellets (the size of cooked rice grains), and *C. concisus*, *C. cinaedi*/CLO1B, and *Helicobacter pylori* required substantially larger cell pellets (approximately pea sized), than those indicated (rice grain sized) in the publication by these researchers. Second, lysis of *Campylobacter* organisms occurred almost immediately, and it was necessary to place suspensions on ice quickly to prevent loss of DNA. Third, we found that three washes with 70% ethanol were sufficient to remove contaminating guanidium thiocyanate.

Restriction endonuclease digestion of DNA. Preliminary experiments were conducted with 10 enzymes (*Bam*HI, *Bgl*II, *Cla*I, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, *Pvu*II, *Sma*I, and *Xho*I) on a small subset of *C. jejuni* and AC organisms to determine the most appropriate enzyme for restriction digestion. Subsequent evaluation of *Ava*I, *Dra*I, *Eco*RV, *Hae*III, *Hha*I, *Hin*cII, *Hpa*I, *Nco*I, *Sal*I, and *Sst*I did not reveal a more suitable enzyme.

An aliquot of each DNA sample was examined spectrophotometrically to determine concentration and purity. Approximately 2 μg of DNA was digested with 2 μl of enzyme (*Pvu*II [4 to 20 U/μl] or *Cla*I [4 to 10 U/μl]; New England BioLabs, Beverly, Mass.) in a volume of 20 μl for a total of 4 h at the temperature recommended by the manufacturer. One microliter of enzyme was added prior to incubation and again after 2 h of incubation, and this was followed by an additional 2 h of incubation. Preliminary experiments indicated that a 10× buffer consisting of 100 mM Tris (pH 7.4), 100 mM $MgCl_2$, 1 mg of bovine serum albumin per ml, 10 mM dithiothreitol, 500 mM NaCl, and 1 mM spermidine (Sigma Chemical Co., St. Louis, Mo.) was superior to other buffers for *Pvu*II digestion of DNA from some *Campylobacter* species (particularly AC, *C. cinaedi*/CLO1B, *C. concisus*, and *C. mucosalis*). Consequently, this buffer was used in all further *Pvu*II endonuclease digestion reactions of DNA from *Campylobacter* organisms.

Electrophoresis and Southern blotting of restricted DNA fragments. Following restriction, samples were heated at 65°C for 10 min and cooled on ice for 5 min, and 5 μl of loading buffer (type II [17]) was added to each sample. Standards representing molecular size fragments were included in at least one lane on each gel (0.2 μg of lambda and 0.1 μg of a 1-kb ladder; Bethesda Research Laboratories, Inc.). DNA fragments were separated by horizontal electrophoresis in 1.0% agarose gels in Tris acetate buffer (0.04 M Tris acetate, 0.002 M EDTA, pH 8.1). Following electrophoresis, gels were stained with ethidium bromide (1 μg/ml) and photographed under UV light. DNA fragments were denatured in the gel and then transferred to membranes by the method of Southern (37), modified by the use of nylon membranes (MSI magnagraph; MSI, Westboro, Mass.). Nylon membranes were rinsed briefly in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), air dried, and baked for 2 h at 80°C prior to hybridization.

Preparation of radioactive probes. (i) Preparation of *E. coli* rRNA probe. Commercially prepared ribosomal *E. coli* 16S and 23S RNA (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) was purified by high-performance liquid chromatography (HPLC) to remove contaminating 5S RNA. All aqueous solutions were treated with diethylpyrocarbonate (Sigma) to inactivate RNases. Following HPLC, purified RNA was dried and redissolved in 50% ethanol.

Before labeling, HPLC-purified RNA (1 μg) was dried and resuspended in diethylpyrocarbonate-treated water (20 μl of a 0.1% solution). RNA was hydrolyzed for 5 min at 90°C in the presence of 0.2 mmol Tris, pH 9.5, and chilled on ice for 5 min. RNA was end labelled with [γ-^{32}P]ATP (Dupont/New England Nuclear Research Products, Boston, Mass.), using polynucleotide kinase (3′-phosphatase-free; Boehringer Mannheim Biochemicals) and RNA kinase buffer as described by Maniatis et al. (17). Labelled RNA was purified by passage through a commercial Sephadex G-50 column (nick column; Pharmacia LKB Biotechnology, Piscataway, N.J.), following directions provided by the manufacturer and using 0.1× SSC containing 0.1% diethylpyrocarbonate as the mobile phase.

(ii) Preparation of labelled molecular size standards. Fragments of a 1-kb ladder (1 μg) and *Hin*dIII-digested fragments of lambda phage DNA (2 μg; Bethesda Research Laboratories, Inc.) were labelled with [α-^{32}P]dCTP, using a nick translation kit (Bethesda Research Laboratories) according to the manufacturer's directions. Labelled fragments were separated from unincorporated label by passage through a commercial Sephadex G-50 column as described above, using 0.1× SSC as the mobile phase.

Hybridization and autoradiography. Nylon membranes with immobilized DNA restriction fragments were pretreated with 50 ml of hybridization solution (6× SSC, 5× Denhardt solution [1× Denhardt is 0.02% bovine serum albumin, 0.02% polyvinylpyrrolidone, and 0.02% Ficoll], 32% formamide [deionized], 0.02 M Tris [pH 7.4], 0.1% sodium dodecyl sulfate [SDS]) and incubated for 1 to 2 h before addition of the RNA probe. Labelled RNA probe (5×10^6 to 1×10^7 cpm) was added. Following overnight hybridization at 37°C with shaking, 10^6 cpm of marker probe was added and allowed to hybridize for 2 to 3 h. The membranes were rinsed and then washed twice for 15 min each at 37°C in 2× SSC containing 0.1% SDS, followed by two additional washes in 0.1× SSC–0.1% SDS, also for 15 min each at 37°C. Membranes were blotted briefly, wrapped in Saran Wrap, and exposed to XAR Omat film (Kodak, Rochester, N.Y.) at −70°C in the presence of intensifying screens.

Quantitative analysis of ribosomal DNA (rDNA) patterns. Fragment sizes, based on migration distances of fragments and kilobase standards included in each gel, were calculated

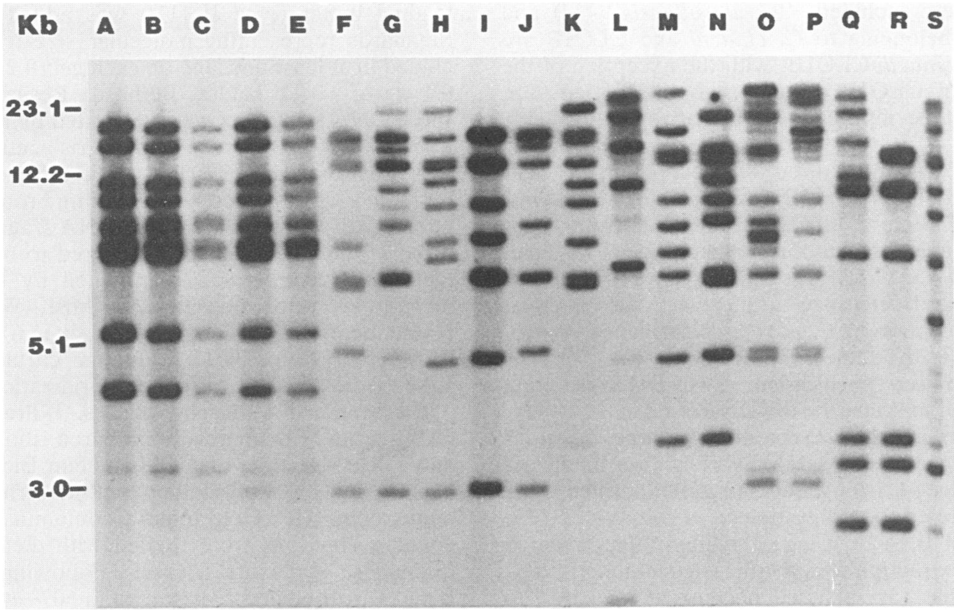

FIG. 1. rDNA patterns following *Pvu*II digestion of type and reference strains of *Campylobacter* and related species. Lanes: A, *H. pylori*; B, *Wolinella succinogenes*; C, *W. recta*; D, *W. curva*; E, *C. nitrofigilis*; F, *C. cryaerophila*; G, *C. fetus* subsp. *fetus*; H, *C. sputorum* subsp. "*fecalis*"; I, *C. sputorum* subsp. *bubulus*; J, *C. cinaedi*; K, CLO1B; L, *C. fennelliae*; M, *C. jejuni*; N, *C. lari*; O, "*C. upsaliensis*"; P, *C. hyointestinalis*; Q, *C. coli*; R, *C. mucosalis*; S, *C. concisus*.

by using the DNASTAR program (DNASTAR, Inc., Madison, Wis.) and a digitizer (Science Accessories Corp., Madison, Wis.). Fragment sizes were used to construct dendrograms based on clustering, using the Dice similarity coefficient (8) as described by Plikaytis et al. (32). A tolerance window equal to 5% of kilobase pair number was used in determining relationships between strains electrophoresed on different gels.

RESULTS

Quantitative analysis of RFLPs of rDNA was used to determine relatedness of human and animal strains of AC. *Pvu*II was the only enzyme tested that was capable of restricting DNA from strains of all recognized species of *Campylobacter*, *Wolinella*, and *Helicobacter* (Fig. 1). Digestion did not occur when *Eco*RI or *Sma*I was used; few bands were demonstrated following restriction with *Xho*I, *Kpn*I, and *Bam*HI. Good digestion was generally seen when *Hin*dIII, *Bgl*II, and *Pst*I were used; however, the resulting patterns following hybridization with the rRNA probe did not produce good discrimination or distribution of fragments. Suitable digestion and discrimination were seen when *Cla*I and *Pvu*II were used. However, *Cla*I failed to digest DNA isolated from reference strains of *C. cinaedi*, CLO1B, and *H. pylori*, and ideal discrimination was not always seen when *Pvu*II was used. Thus, it appears that *Pvu*II is the most appropriate enzyme for ribotyping of *Campylobacter* and related species; strains that demonstrate identical *Pvu*II ribotype patterns may be further subdivided by using a second enzyme, such as *Cla*I.

Ribotyping patterns generally demonstrated the presence of a common species band(s) for each AC DNA hybridization group. Typical patterns for each of the AC hybridization groups (14) are shown in Fig. 2. Most "*C. butzleri*" strains demonstrated an ~3.0-kb band in *Pvu*II digests (Fig. 2, lanes K, M, N, O, and P). This band was not seen (or was very faint) in 4 of 64 "*C. butzleri*" isolates tested (data not shown). These four strains demonstrated other bands common to "*C. butzleri*" (such as those at ~6.2, 12.0, and 15.0 kb), but not the 3.0-kb band. *C. cryaerophila* isolates all demonstrated the presence of a common band at ~3.2 kb; isolates of DNA group 1B which phenotypically resembled "*C. butzleri*" demonstrated an additional common band at ~2.6 kb. These common bands may be useful in species identification. Figure 2 demonstrates the applicability of this technique to 12 strains originally identified as *C. cryaerophila*. Four strains demonstrated the 3.2-kb band common to *C. cryaerophila* (Fig. 2, lanes A to D); the 3.2-kb band was only faintly demonstrated by one strain of *C. cryaerophila* (Fig. 2, lane A). Two strains demonstrated the 2.6- and 3.2-kb bands common to *C. cryaerophila* hybridization group 1B strains (Fig. 2, lanes E and F); four strains demonstrated the 3.0-kb band common to "*C. butzleri*" (Fig. 2, lanes K, M, N, and O). The two strains shown in lanes C and D were more closely related to DNA group 1B by DNA hybridization, but were determined phenotypically to resemble more closely the type strain of *C. cryaerophila* (DNA group 1A [14]). Ribotyping patterns (Fig. 2, lanes C and D) also indicated that these strains belonged to DNA group 1A, rather than DNA group 1B. Two strains (Fig. 2, lanes G and H) demonstrated a 2.6-kb band, but not the 3.2-kb band; these strains have been shown by DNA hybridization to be unrelated at the species level to either hybridization group of *C. cryaerophila* (unpublished data). An additional strain (lane L) is shown which demonstrates a band slightly higher than 3.0 kb; this strain was not related at the species level to "*C. butzleri*" (unpublished data).

A dendrogram based on quantitative analysis of these patterns showed that AC strains were grouped in six clusters (Fig. 3, clusters A to F). Cluster A contained one strain of *C. cryaerophila* hybridization group 1B and one strain which

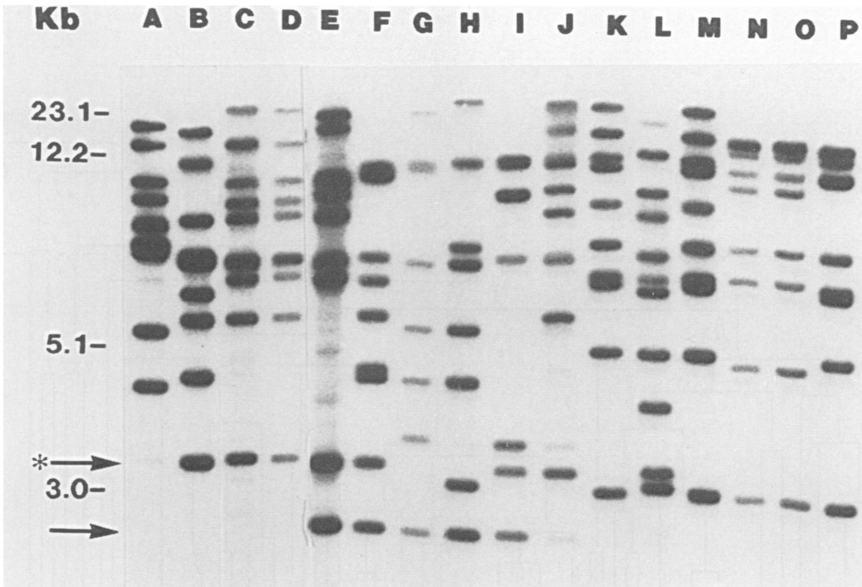

FIG. 2. Autoradiograph of hybridization resulting from *Pvu*II digests of animal strains originally designated *C. cryaerophila* and subsequently placed into multiple DNA hybridization groups. Lane B contains the type strain of *C. cryaerophila* (ATCC 43158); lanes A, C, and D represent hybridization group 1A (*C. cryaerophila*); lanes E and F represent hybridization group 1B (*C. cryaerophila*); lanes I and J contain hybridization group 1B strains isolated from humans; lanes K and M to O represent "*C. butzleri*"; lane P contains the reference strain for "*C. butzleri*." Lanes G, H, and L contain strains that were not classified into *C. cryaerophila* or "*C. butzleri*" by DNA hybridization. Lanes G and H demonstrate the presence of the 2.6-kb band but not the 3.2-kb band seen in all hybridization group 1B strains. Lane L demonstrates the presence of a band ~0.1 kb higher than that seen for "*C. butzleri*" isolates. Arrow (at left) with asterisk indicates location of 3.2-kb fragments; arrow without asterisk indicates location of 2.6-kb fragments.

was not classified by DNA hybridization, in addition to all but two of the "*C. butzleri*" strains. The two strains of "*C. butzleri*" that did not demonstrate the 3.0-kb band were not included in cluster A. Quantitative analysis of rDNA patterns also indicated two large subclusters of "*C. butzleri*" strains, A1 and A2 (Fig. 3). Human clinical isolates from the United States, Australia, Canada, and Thailand and the primate isolates were randomly distributed in both subclusters.

Clusters B to F were related to cluster A at a genetic distance of >0.5. These clusters contained strains of both phenotypic types of *C. cryaerophila*, in addition to other strains not classified by DNA hybridization.

All of 35 sporadic "*C. butzleri*" isolates from the United States, Canada, and Australia had different ribotypes. When two additional groups of strains isolated at two separate facilities were examined, several duplicate patterns were found; five pairs and one trio of strains appeared to be identical within a group of 15 strains isolated in Thailand (39), and 4 of 12 isolates from a primate facility were found to have the same pattern, which was clearly distinguishable from the other 8 isolates (33). These two studies indicate that ribotyping is useful in distinguishing between AC strains isolated from the same location and may be useful in an epidemic setting. Quantitative analysis of patterns indicated that patterns visually judged to be identical were clustered at a genetic distance of ≤0.1 by dendrogram analysis.

DISCUSSION

Analysis of RFLPs with or without subsequent probing for specific fragments has been used with many bacterial species, including *Campylobacter*; however, application of these techniques has generally been limited to the more common species of *Campylobacter*, such as *C. jejuni*, *C. coli*, *C. fetus* subsp. *fetus*, *C. lari* (formerly *C. laridis*), and *H. pylori* (formerly *C. pylori*) (3, 5–7, 12, 13, 15, 16, 18, 21–25, 27, 30, 36). Restriction digests by themselves are difficult to interpret visually because >100 fragments may be present in each digest. RFLPs of specific genes, on the other hand, usually result in <15 bands and are much easier to interpret.

The use of RFLPs in the genes encoding rRNA has been described in detail by other investigators (10, 38). To realize the full potential of this technique, the restriction enzyme should provide maximum discrimination between strains from different geographic locations as well as between closely related strains. We believe that *Pvu*II is the best enzyme for use with *Campylobacter* and related genera; however, strains that demonstrate identical patterns following restriction with *Pvu*II should be restricted with a second enzyme such as *Cla*I. Our investigation of enzymes (*Hin*dIII, *Hae*III, *Xho*I, *Bst*EII, and *Bgl*II) used by other researchers for *Campylobacter* revealed poor discrimination among strains of the same serotype or incomplete digestion of DNA isolated from all *Campylobacter* and related species (unpublished data).

Ribotyping may also be useful in species identification, particularly for strains that are difficult to analyze phenotypically or to cultivate in quantities large enough to provide sufficient DNA for DNA-DNA hybridization. *Campylobacter* strains have a limited number of known phenotypic traits; further, atypical reactions exhibited by strains of certain species make species identification difficult. Examples include nalidixic acid-resistant *C. jejuni* strains identified as *C. lari* (1) and hippuricase-negative strains of *C. jejuni* identified as *C. coli* (41). Several reports show that common rDNA

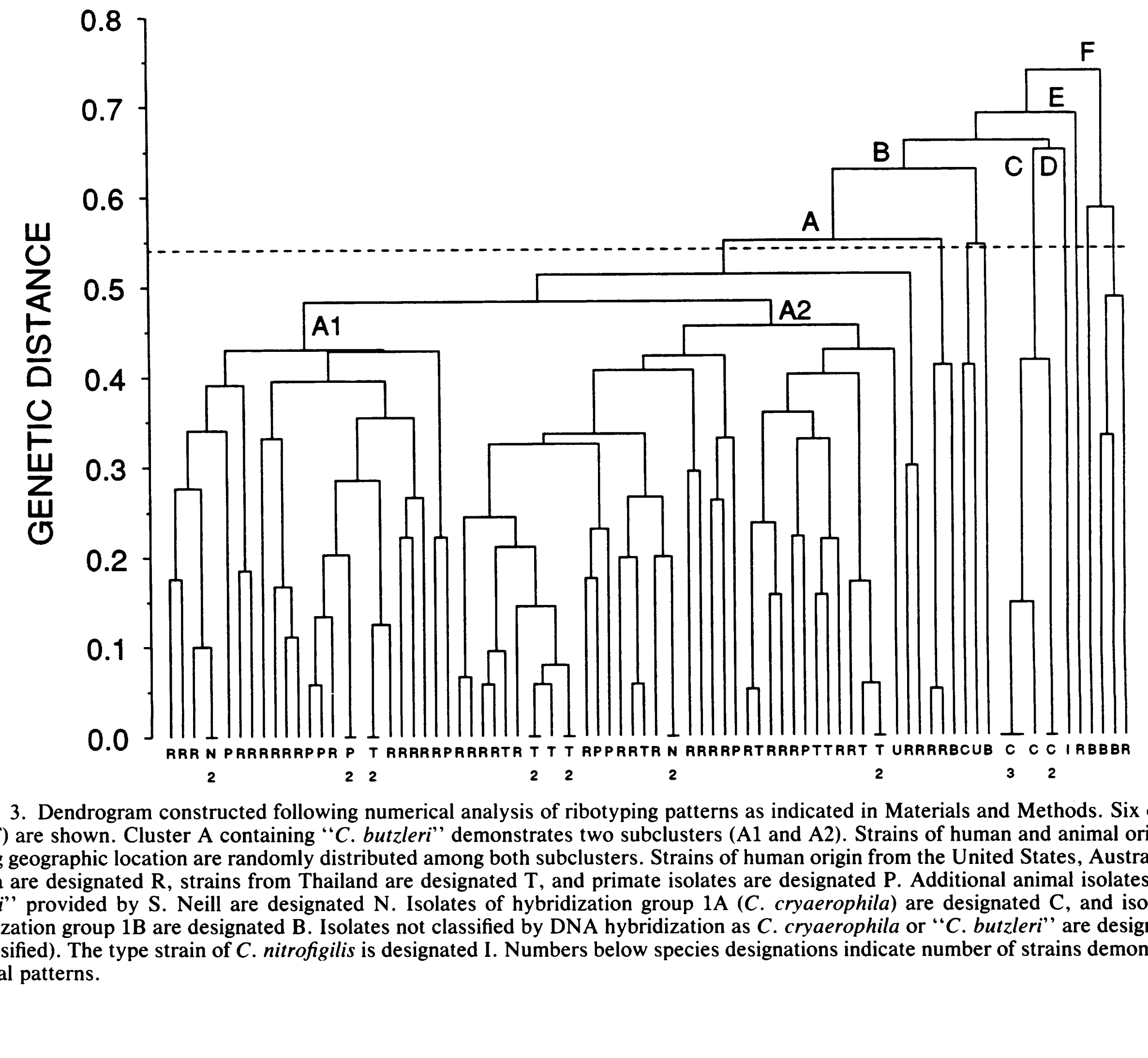

FIG. 3. Dendrogram constructed following numerical analysis of ribotyping patterns as indicated in Materials and Methods. Six clusters (A to F) are shown. Cluster A containing "*C. butzleri*" demonstrates two subclusters (A1 and A2). Strains of human and animal origin and varying geographic location are randomly distributed among both subclusters. Strains of human origin from the United States, Australia, and Canada are designated R, strains from Thailand are designated T, and primate isolates are designated P. Additional animal isolates of "*C. butzleri*" provided by S. Neill are designated N. Isolates of hybridization group 1A (*C. cryaerophila*) are designated C, and isolates of hybridization group 1B are designated B. Isolates not classified by DNA hybridization as *C. cryaerophila* or "*C. butzleri*" are designated U (unclassified). The type strain of *C. nitrofigilis* is designated I. Numbers below species designations indicate number of strains demonstrating identical patterns.

hybridization bands correlate with previously described DNA hybridization groups (5, 11, 18, 25, 28). Similarly, results of the present study indicate excellent correlation of common rDNA hybridization bands and DNA hybridization data.

In addition, a dendrogram constructed from ribotyping results placed all but two of the "*C. butzleri*" strains into a large cluster (Fig. 3, cluster A). Strains that were not related at the species level by DNA hybridization were excluded from this large cluster with one exception. The separation between "*C. butzleri*" strains and other AC strains is at a genetic distance of ~0.54. This is approximately the same genetic distance at which other investigators have noted a separation of other species when dendrograms based on multilocus enzyme electrophoresis results were used (35).

In the present study, migration distances, determined by digitizing gels, were converted to fragment sizes based on a 1-kb ladder standard before computerized analysis for genetic relatedness. Although there have been several attempts to use densitometry, or soft-laser scanning, to convert restriction digest patterns or protein profiles to quantitative data on the basis of inclusion of internal or external standards (2, 5, 18, 21, 25, 32), these methods were not practical for use in our experiments since our application of ribotyping to many species of bacteria resulted in wide ranges of fragment distribution. Previously described standards would potentially conflict with patterns obtained. The use of a digitizer introduces possibilities for operator error, and there is also a greater chance for error when measuring small rather than large migration distances (31). However, despite potential difficulties, quantitative rRNA data generated from a large number of gels correlated well with visual impressions, size calculations, and DNA hybridization data. Results appeared to be quite reproducible; examination of numerical profiles for the same strain electrophoresed on 12 gels revealed <3% variability in band sizes.

One problem we encountered when examining strains run on different gels was the presence of less intense or unstable bands that were not always present when the same DNA preparation was analyzed. These bands, which may result in falsely sensitive discrimination, could be caused by incomplete digestion and/or resolution of digested fragments, different concentrations of DNA in each sample, or less intense binding of the probe to those DNA fragments. Preliminary data indicate that a more consistent pattern may be obtained by using a digoxigenin-labelled probe than the ^{32}P-labelled probe described here (unpublished data).

Although we initially investigated four different methods of DNA extraction (4, 24, 30), variation in methodology did not appear to have much effect on the ribotyping patterns (unpublished data), with one notable exception: DNA from *H. pylori* strains extracted by using a large-scale procedure (4, 14) was not digested with *Hin*dIII, while DNA extracted by guanidium thiocyanate was digested by *Hin*dIII (data not shown). We also encountered difficulty obtaining DNA consistently from *H. pylori*, *C. cinaedi*, *C. concisus*, or *C. mucosalis* strains by using the method of Owen and Borman (24). In addition to the studies described above, we probed several species of *Salmonella* and *Campylobacter* with rRNA from Boehringer Mannheim that was not further purified by HPLC. These data correlated with data obtained by using more purified rRNA (unpublished data); thus, it appears that the HPLC purification step might not be necessary.

Although this study did not utilize a nonradioactive labelled rRNA probe, several ligands for nonradioactive labelling of the rRNA have been described by other investigators: biotin (2, 23, 29), acetylaminofluorene (9), and digoxigenin (20). Reports by these investigators indicate equivalent results for nonradioactive and radioactive probes.

In conclusion, the use of RFLPs of rDNA was successful in detecting minor differences in closely related strains and in providing information on species identification on the basis of common bands. The technique uses commercially available reagents and can be applied to a variety of gram-positive and -negative bacterial species. Further, quantitative data established potential genetic relationships between strains which correlated well with available DNA hybridization data. Work is in progress to determine the predictive value of rRNA analysis of an unknown group of *Campylobacter*-like organisms for species identification before DNA hybridization.

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