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Rapid Identification of *Campylobacter* Species by Restriction Fragment Length Polymorphism Analysis of a PCR-Amplified Fragment of the Gene Coding for 16S rRNA

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Restriction fragment length polymorphism analysis of a PCR-amplified DNA fragment of the gene coding for 16S rRNA was performed on 148 previously characterized strains of *Campylobacter*, *Helicobacter*, *Arcobacter*, and *Wolinella succinogenes* and 13 *Campylobacter*-like isolates. These strains included clinical, animal, and environmental isolates. PCR amplification generated a 283-bp fragment from all species. The amplicon from each strain was digested with six restriction endonucleases (*Acc*I, *Ava*I, *Dde*I, *Hae*III, *Hpa*II, *Xho*I). *Dde*I was useful for the initial grouping of the strains. Additional discrimination within the different *Dde*I groups was obtained with *Acc*I, *Hae*III, *Hpa*II, and *Xho*I digestions. The PCR-restriction fragment length polymorphism analysis allowed for the discrimination of members of the genus *Campylobacter* from members of closely related genera and discrimination between *Campylobacter* species. The proposed method is simple and rapid and can be useful for the routine identification of *Campylobacter*-like organisms in clinical or epidemiologic studies.

The genera *Campylobacter*, *Arcobacter*, *Helicobacter*, *Wolinella*, and "*Flexispira*" constitute, within the class *Proteobacteria*, a separate eubacterial lineage identified as rRNA superfamily VI (24). Vandamme et al. (25) have described differential features for all of the genera of this superfamily.

The bacteria in the genus *Campylobacter* do not ferment or oxidize carbohydrates and are inert in most biochemical media commonly used to characterize bacterial isolates. Therefore, only a few morphologic and physiologic criteria can be applied to the classification of *Campylobacter* species. Identification to the species level is often not possible even after extensive biochemical tests and determination of cellular fatty acid (CFA) profiles (11). Recently, genetic methods have been used to identify some *Campylobacter* species (4, 5, 9, 16–18). DNA-DNA hybridization provides definitive species identification; however, the method is not suitable for routine identification purposes.

Bacterial 16S rRNA sequences are attractive targets for developing diagnostic tests for the clinical laboratory. Because 16S rRNA molecules have crucial structural constraints, certain conserved regions of the 16S rRNA sequence are found in all bacteria. PCR primers can be designed to target these conserved bacterial 16S rRNA gene sequences and can be used to amplify intervening variable regions that may have diagnostic potential (26, 27).

We describe the development of a PCR-based restriction fragment length polymorphism (RFLP) method for the differentiation of *Campylobacter*, *Helicobacter*, *Arcobacter*, and *Wolinella* species from one another by using the endonucleases *Dde*I, *Acc*I, *Ava*I, *Hae*III, *Hpa*II, and *Xho*I. PCR amplification of the target sequence does not require a large number of cells or lengthy DNA extraction procedures.

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MATERIALS AND METHODS

PCR-RFLP grouping bacterial strains. A total of 161 *Campylobacter* and *Campylobacter*-like isolates from the *Campylobacter* Reference Laboratory at the Centers for Disease Control and Prevention were studied. Of these, 148 were phenotypically characterized and represented 17 species and subspecies of *Campylobacter*, 5 *Helicobacter* species, 4 *Arcobacter* species, and *Wolinella succinogenes*. The remaining 13 isolates were phenotypically characterized but could not be identified to the species level.

DNA hybridization. DNAs from type strains of *Campylobacter curvus* CDC D2712, *Campylobacter sputorum* subsp. *sputorum* CDC D2908, and *Campylobacter concisus* CDC D2717 were labeled in vitro with ^{32}P by nick translation with a commercial kit (Bethesda Research Laboratories, Gaithersburg, Md.) and were tested for their relatedness to unlabeled DNA from each of the *C. curvus* test strains. Homologous control reactions were included in all experiments. For DNA-DNA hybridization experiments, each strain was cultured onto 25 to 50 plates (15 by 150 mm) of Mueller-Hinton agar containing 5% defibrinated sheep blood. The plates were incubated for 72 to 120 h at 36°C in a microaerobic atmosphere of approximately 5% O_2, 7.5% CO_2, 7.5% H_2, and 80% N_2. DNA extraction, purification, and hybridization were done by previously described procedures (2). Reassociation reactions were done at 50°C (optimum temperature).

PCR-RFLP. (i) Bacterial extracts. Strains were stored at −70°C in tryptic soy broth containing 20% (vol/vol) glycerol. Bacteria were grown for 48 h at 36°C on heart infusion agar or Mueller-Hinton agar containing defibrinated sheep blood under microberobic conditions. *Campylobacter rectus* and *W. succinogenes* were grown anaerobically in GasPak jars (BBL GasPak Anaerobic Systems; Becton Dickinson and Co., Cockeysville, Md.). The growth from one plate was harvested with a cotton swab and was suspended in 1 ml of sterile double-distilled water, vortexed, and boiled for 20 min. The suspension was centrifuged at 3,700 × *g* for 20 min, and the supernatant was used as the source of target DNA for PCR amplification.

(ii) Primers. Primers were synthesized on an Applied Biosystems Synthesizer at the Biotechnology Core Facility Branch, Centers for Disease Control and Prevention. The genes coding for 16S rRNA were amplified by PCR with the following primers in the conserved regions within the 16S rRNA gene: forward primer, PLO6 (12a), 5′-GGTTAAGTCCCGCAACGAGCGC-3′; reverse primer, CAMPC5 (1), 5′-GGCTGATCTACGATTACTAGCGAT-3′.

(iii) PCR. PCR amplification was carried out with 100-μl samples consisting of 10 μl of bacterial extract and 90 μl of the amplification cocktail, which contained the following components: 15 pmol of each primer, 200 μM (each) the four

TABLE 1. Source and PCR-RFLP patterns of 139 study strains

Species	No. (source) of strains	Group[a]	PCR-restriction patterns[b]					
			*Dde*I	*Acc*I	*Ava*I	*Hae*III	*Hpa*II	*Xho*I
Campylobacter jejuni	18 (16 human, 2 animal)	A	1	1	2	3	2	2
Campylobacter jejuni subsp. *doylei*	7 (7 human)	A	1	1	2	3	2	2
Campylobacter coli	19 (7 human, 11 animal, 1 unknown)	A	1	1	2	3	2	2
Campylobacter lari	5 (4 human, 1 animal)	A	1	1	2	2	2	2
Campylobacter curvus	3 (human)	A	1	1	1	3	3	1
Campylobacter mucosalis	3 (animal)	A	1	1	1	3	NT[c]	1
Campylobacter showae	1 (1 human)	A	1	1	1	3	NT	1
Campylobacter rectus	5 (human)	A	1	2	1	3	NT	1
Campylobacter fetus subsp. *fetus*	5 (4 human, 1 animal)	B	2a	NT	1	2	2	1
Campylobacter fetus subsp. *veneralis*	5 (4 human, 1 unknown)	B	2a	NT	1	2	2	1
Campylobacter hyointestinalis	5 (3 human, 2 animal)	B	2a	NT	2	2	2	2
Campylobacter concisus	3 (human)	B	2a	1	1	2	3	1
Campylobacter sputorum biotype sputorum	6 (5 human, 1 unknown)	B	2a	1	1	2	3	1
Campylobacter sputorum biotype bubulus	4 (3 animal, 1 unknown)	B	2a	1	1	2	3	1
Campylobacter sputorum biotype fecalis	5 (animal)	B	2a	1	1	2	3	1
Arcobacter cryaerophilus DNA group 1A	2 (animal)	B	2a	NT	1	2	1	1
Arcobacter cryaerophilus DNA group 1B	3 (animal)	B	2a	NT	1	2	1	1
Arcobacter skirrowii	5 (animal)	B	2a	NT	1	2	1	1
Arcobacter nitrofigilis	1 (environment)	B	2a	NT	1	2	1	1
Campylobacter upsaliensis	5 (4 human, 1 animal)	C	3a	NT	NT	2	NT	2
Campylobacter helveticus	2 (animal)	C	3a	NT	NT	2	NT	2
Arcobacter butzleri	5 (4 human, 1 animal)	D	3b	NT	1	2	NT	1
Wolinella succinogenes	1 (human)	E	2b	1	1	3	1	1
Helicobacter pylori	5 (human)	F	4	1	1	2	1	1
Helicobacter cinaedi	5 (human)	F	4	NT	NT	2	NT	1
Helicobacter fennelliae	5 (human)	F	4	NT	NT	2	NT	1
Helicobacter mustelae	5 (animal)	F	4	NT	NT	2	NT	1
Helicobacter muridarum	1 (animal)	F	4	NT	NT	2	NT	1

[a] Groups A through F were determined by the use of the *Dde*I digestion pattern only.
[b] 1, no restriction site; 2, 3, and 4, number of fragments observed; 2a, 2b, 3a, and 3b, restriction fragments of different lengths.
[c] NT, not tested.

deoxyribonucleotides, 10 μl of GeneAmp PCR buffer (Perkin-Elmer, Norwalk, Conn.), and 2.5 U of *Taq* DNA polymerase (Perkin-Elmer).

The PCR amplification was performed in a PC-100 Thermal Controller (MJ Research, Watertown, Mass.). Samples were incubated for 2 min at 96°C to denature the target DNA and were cycled 30 times at 94°C for 30 s, 50°C for 30 s, and 72°C for 1 min. The samples were then incubated at 72°C for 10 min for a final extension and were maintained at 4°C until they were tested.

(iv) Restriction of the amplified DNA. Ten microliters of each PCR-amplified product was digested with restriction endonucleases as recommended by the manufacturer. Six restriction enzymes were used: *Dde*I, *Hae*III, and *Xho*I (Boehringer-Mannheim, Indianapolis, Ind.) and *Acc*I, *Ava*I, and *Hpa*II (New England BioLabs, Inc., Beverly, Mass.).

(v) Electrophoresis. Amplified DNA was detected on a 2.5% agarose gel (1% agarose [GIBCO-BRL, Gaithersburg, Md.] plus 1.5% NuSieve agarose [3:1] [NuSieve; FMC BioProducts, Rockland, Maine]) in 1× Tris-borate-EDTA buffer at 90 V for 90 min, while the restriction fragments were separated on 5% NuSieve agarose (3:1) at 90 V for 210 min. The gels were stained with ethidium bromide and photographed.

(vi) Calculation of the molecular weight of the fragments. Photographs or negatives of the gels were analyzed visually and by a Bio Image System (Bio Image, Ann Arbor, Mich.) equipped with Whole Band Analysis software. Fragment sizes were assessed against *Msp*I digests of pBR322 DNA (U.S. Biochemicals, Cleveland, Ohio) and a 10-bp DNA ladder (GIBCO-BRL, Grand Island, N.Y.).

RESULTS

The PCR restriction patterns for the study strains obtained with six restriction endonucleases are summarized in Table 1. The strains were divided into six groups (groups A to F) on the basis of their *Dde*I restriction patterns. All *Campylobacter* strains clustered in one of three *Dde*I groups (groups A to C). *Arcobacter butzleri* gave a unique *Dde*I restriction pattern (pattern D), unlike the other *Arcobacter* species, which had *Dde*I patterns identical to those of the *Campylobacter* strains in *Dde*I group B. *W. succinogenes* gave a *Dde*I restriction pattern (pattern E) different from those of the other strains. All *Helicobacter* strains clustered in *Dde*I group F. All of the restriction patterns are shown in Fig. 1. A scheme for the identification of *Campylobacter* species and their discrimination from closely related genera based on the use of five restriction enzymes (*Dde*I, *Acc*I, *Hae*III, *Hpa*II, and *Xho*I) is shown in Fig. 2. *Ava*I was not useful for the identification.

The 139 strains that were used to develop the identification scheme shown in Fig. 2 were characterized by the PCR-RFLP method two to four times to ensure the reproducibilities of the patterns. All patterns were reproducible.

With the exception of *C. curvus*, all strains within a species gave identical restriction profiles. Of five *C. curvus* strains studied, two (strains D4319 and D4320) had PCR-RFLP profiles similar to that of the *C. curvus* type strain (strain D2712), and the profiles of two strains (strains D4321 and D4322) were similar to those of *C. sputorum* and *C. concisus*. We characterized D4319, D4321, and D4322 by DNA-DNA hybridization and showed that strain D4319 was 83% related to *C. curvus*, whereas strains D4321 (98% related) and D4322 (87% related) were related to *C. sputorum*.

To determine the reliability of the PCR-RFLP method in identifying *Campylobacter* and related organisms, nine coded strains, *Campylobacter fetus* D233, *Campylobacter hyointestinalis* D2189, *Campylobacter jejuni* D1420, *Campylobacter lari* D71, *C. rectus* D4332, *Campylobacter upsaliensis* D2173, *Helicobacter cinaedi* D1576, *Arcobacter butzleri* D2703, and *Arcobacter cryaerophilus* D2891 (seven of which had been previously identified by DNA-DNA hybridization), were tested by the PCR-RFLP method. All isolates were correctly identified to

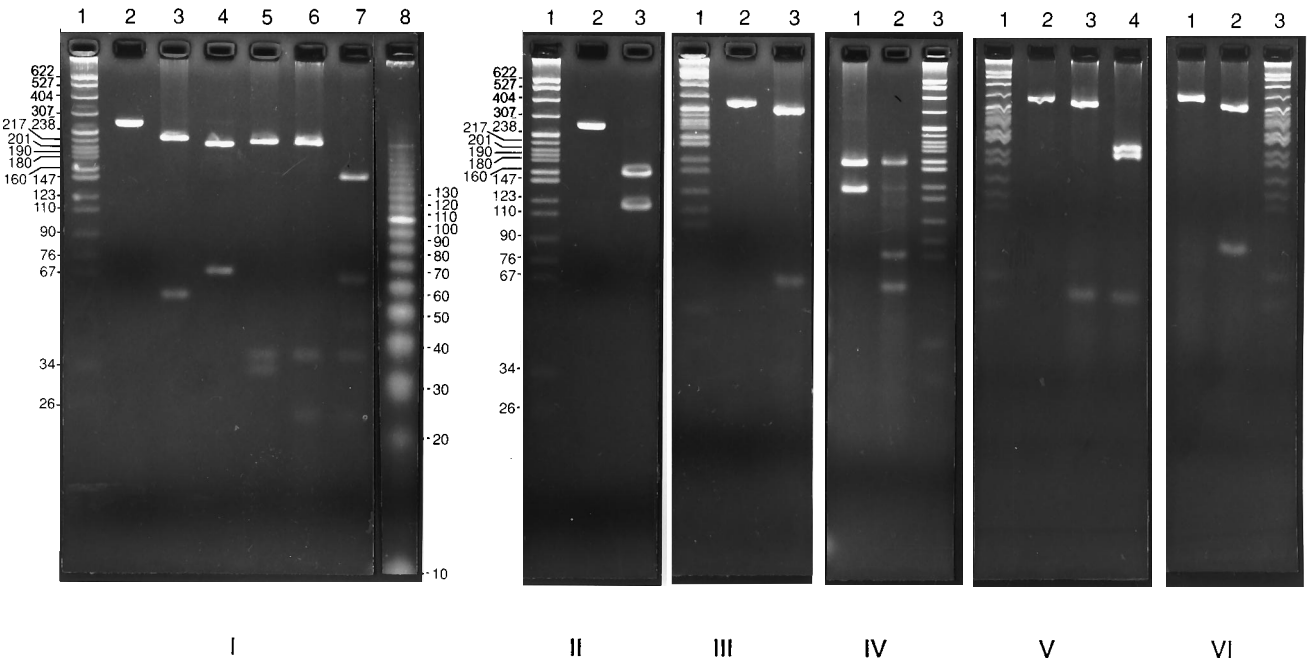

FIG. 1. PCR-RFLP patterns of *Campylobacter* spp., *Arcobacter* spp., *W. succinogenes*, and *Helicobacter* spp. The numbers to the right and left of the gel are in base pairs. (I) Lane 1, *Msp*I digest of pBR322 DNA; lane 8, 10-bp ladder; lanes 2 to 7, *Dde*I digest; lane 2, group A (uncut, 283 bp); lane 3, group B (229 and 58 bp); lane 4, group E (213 and 69 bp); lane 5, group C (219, 39, and 33 bp); lane 6, group D (217, 37, and 24 bp); lane 7, group F (152, 65, 39, and 24 bp). (II) Lane 1, *Msp*I digest of pBR322 DNA; lanes 2 and 3, *Acc*I digest; lane 2, pattern 1 (uncut, 283 bp); lane 3, pattern 2 (160 and 118 bp). (III) Lane 1, *Msp*I digest of pBR322 DNA; lane 4, 10-bp ladder; lanes 2 and 3, *Ava*I digests, lane 2, pattern 1 (uncut, 283 bp); lane 3, pattern 2 (242 and 43 bp). (IV) Lane 3, *Msp*I digest of pBR322 DNA; lanes 1 and 2, *Hae*III digests; lane 1, pattern 2 (156 and 120 bp); lane 2, pattern 3 (157, 68, and 52 bp). (V) Lane 1, *Msp*I digest of pBR322 DNA; lane 2 to 4, *Hpa*II digests, lane 2, pattern 1 (uncut, 283 bp); lane 3, pattern 2 (256 and 29 bp); lane 4, pattern 3 (134, 121, and 29 bp). (VI) Lane 3, *Msp*I digest of pBR322 DNA; lanes 1 and 2, *Xho*I digests; lane 1, pattern 1 (uncut, 283 bp); lane 2, pattern 2 (236 and 47 bp). See Table 1 for information on *Dde*I groups and pattern types obtained with different restriction enzymes.

the species or species group (composed of more than one species) level by the PCR-RFLP method.

When the PCR-RFLP method was applied for the characterization of 13 atypical *Campylobacter*-like strains (Table 2), 11 were identified to the species or species group level. Two isolates (isolates D3880 and D3911) could not be identified and must be further characterized by DNA-DNA hybridization studies.

DISCUSSION

The members of the family *Campylobacteraceae* are important from a public health standpoint; many fall in the category of emerging human and animal pathogens (20, 23, 24).

Phenotypic identification of *Campylobacter* species has always been problematic because they are generally biochemically inactive in comparison with many other bacteria. They do not utilize sugars or produce indole, but they all produce oxidase and many of them produce catalase (19). Often, it is difficult to identify campylobacters to the species level because of atypical or inconclusive biochemical reactions even in the few tests that are useful for their identification (6, 7, 11–14, 21).

Furthermore, the outcomes of tests based on biochemical reactions for *Campylobacter* species identification may be influenced by inoculum size (10^6 versus 10^7 to 10^8 CFU/ml) and the basal medium used, leading to erroneous conclusions (15). The extreme variations noted in the tolerance of *C. fetus* subsp. *fetus* to 1.5% sodium chloride (0 versus 95%) and the arylsulfatase activity of *C. upsaliensis* (5 versus 100%) may have been due to one or both of these factors (3, 15).

CFA profiles facilitate the identification of *Campylobacter* species (10). The determination of major CFAs and the respiratory quinone content is strongly recommended for the characterization of new *Campylobacter* species (22). Nevertheless, the combination of biochemical tests and CFA profiles is often insufficient for definitive identification at the species level. Also, many laboratories may not have access to the instruments required for CFA analyses.

Several molecular identification methods (DNA probes, DNA-DNA hybridization, and partial or complete 16S rRNA sequencing) provide definitive identification of *Campylobacter* species and allow for the characterization of new species. However, these methods are too complex and time-consuming for routine diagnostic identification in clinical laboratories (5, 8, 9, 16).

The PCR-RFLP method described here differentiates between the related genera *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella*. This method also identifies *Campylobacter* isolates to the species or species group (composed of more than one species) level. PCR amplification generated an amplicon of 283 bp from all isolates tested (Fig. 1). *Dde*I restriction analysis of the amplicon placed an isolate into one of six groups (Table 1). On the basis of its *Dde*I restriction pattern, an isolate could then be identified by using one or two additional restriction enzymes. The PCR-RFLP method described here does not distinguish *C. jejuni* from *Campylobacter coli*, *C. concisus* from *C. sputorum*, or *Campylobacter showae* from *Campylobacter mucosalis* and *C. curvus* (Fig. 2). A proposed scheme for the identification of *Campylobacter* species is shown in Fig. 2.

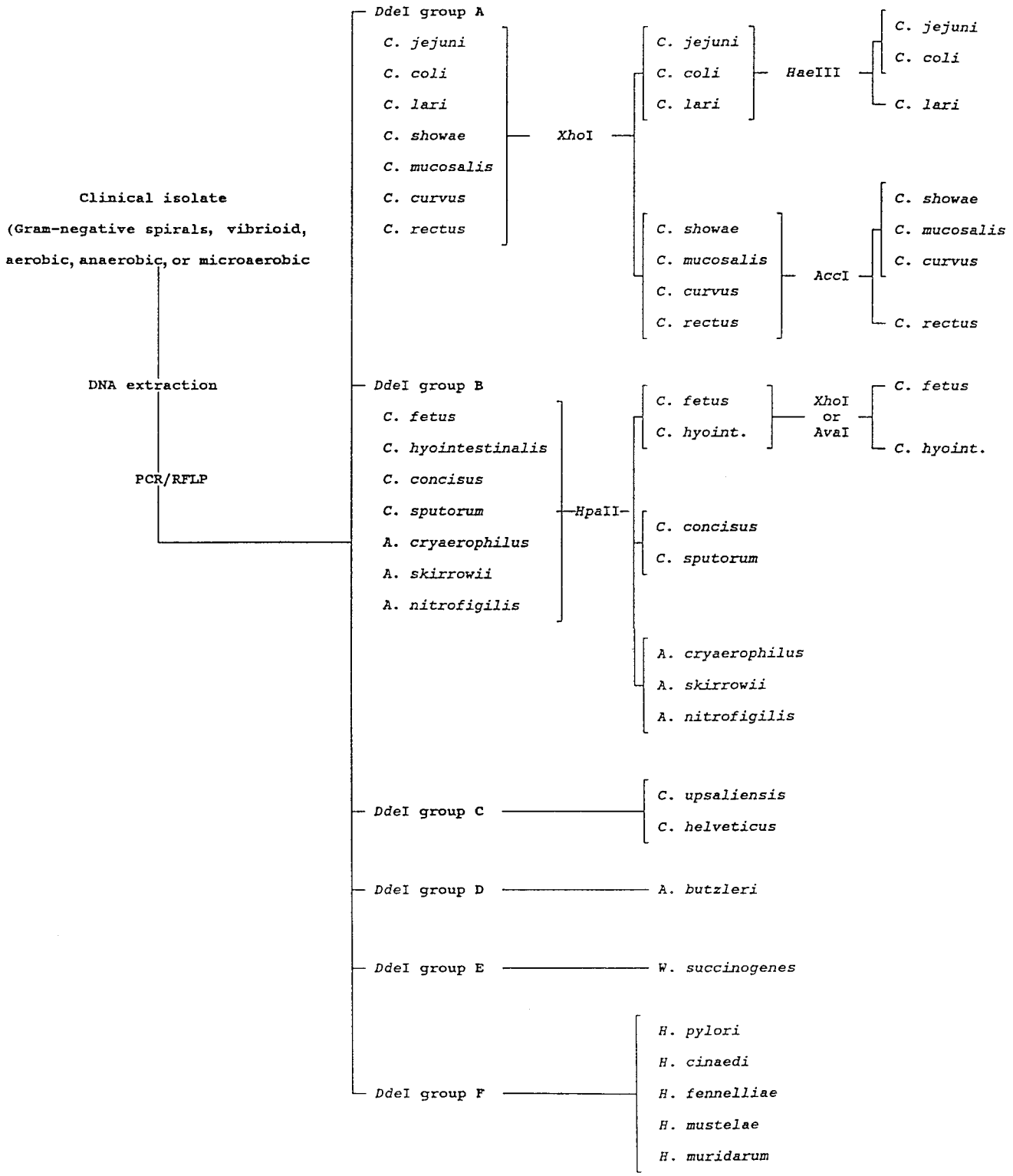

FIG. 2. Scheme for rapid identification of the genera *Campylobacter*, *Arcobacter*, *Wolinella*, and *Helicobacter* by PCR-based RFLP.

C. mucosalis and *C. concisus*, which are difficult, often impossible, to distinguish by phenotypic tests, were clearly separated into two *Dde*I groups by the PCR-RFLP analysis. We analyzed 22 *Campylobacter*-like organisms with the phenotypic characteristics of *C. concisus* and *C. mucosalis* by the PCR-RFLP method and were able to separate these two organisms into two distinct *Dde*I groups (3a).

No variation in the restriction profiles of strains of the same

TABLE 2. Application of PCR-RFLP to the identification of *Campylobacter*-like organisms

CDC no.[a]	Group by PCR-RFLP (*Dde*I)	Pattern with other restriction enzymes (no. of restriction fragments)[b]					PCR-based RFLP identification	Phenotypic characterization[c]	
		*Hae*III	*Xho*I	*Hpa*II	*Acc*I	*Ava*I		CFAs	Biochemical tests and DNA analysis
D1843	A	3	2				*C. jejuni*/*C. coli*	*C. coli*/*C. upsaliensis*, *C. lari*/*C. hyointestinalis*	Resembles *H. cinaedi*; not *C. upsaliensis* by DNA hybridization studies; NA and CF susceptible
D2974	A	3	2				*C. jejuni*/*C. coli*	*C. coli*/*C. upsaliensis*, *C. lari*/*C. hyointestinalis*	Resembles *C. coli*/*C. upsaliensis*; NA and CF susceptible; strongly catalase positive
D2976	A	3	2				*C. jejuni*/*C. coli*	*C. coli*/*C. upsaliensis*, *C. lari*/*C. hyointestinalis*	*C. lari* variant; urease positive
D4248	A		1		1		*C. curvus*/*C. mucosalis*/*C. showae*	*C. concisus*/*C. mucosalis*	Unable to identify; NA and CF resistant; catalase negative; H_2S in TSI agar
D2949	B			3			*C. concisus*/*C. sputorum*	*C. sputorum*	Resembles catalase-negative anaerobic campylobacters
D3884	B			3			*C. concisus*/*C. sputorum*	*C. sputorum*	*C. sputorum* biotype bubulus
D2831	B		2	2			*C. hyointestinalis*	*Campylobacter*-like	Resembles *C. hyointestinalis*; beta-hemolytic; H_2S in TSI agar
D2604	B		2	2			*C. hyointestinalis*	*C. fetus*	Resembles *C. fetus*/*H. cinaedi*; H_2S in TSI agar
D2739	B		2	2			*C. hyointestinalis*	*C. fetus*	Resembles *C. fetus*/*H. cinaedi*; H_2S in TSI agar
D2953	F						*Helicobacter* sp.	*Campylobacter*-like, similar to *C. fetus*	Resembles *C. fetus*; Urease negative
D3828	F						*Helicobacter* sp.	*H. cinaedi*	*H. cinaedi* by DNA-DNA hibridization; NA and CF resistant
D3880	B	2	2	5	1	2	Unknown *Hpa*II RFLP pattern	Varies slightly from *C. fetus*	Resembles *C. fetus* subsp. *fetus*; growth at 25°C
D3911	Unknown	2	1	3	1	1	*Unknown Dde*I RFLP pattern	*C. sputorum*	Resembles *C. sputorum* biotype bubulus; H_2S in TSI agar

[a] COC, Centers for Disease Control and Prevention.
[b] Only the RFLP patterns useful for the differentiation are shown.
[c] NA, nalidixic acid; CF, cephalothin; TSI, triple sugar iron.

species was observed by the PCR-RFLP method. In the one instance in which variation was observed between strains that were thought to be *C. curvus*, DNA-DNA hybridization experiments showed the variant strains to be *C. sputorum* subsp. *sputorum*, validating the results of PCR-RFLP.

Recently, Vandamme et al. (23) transferred *Bacteroides gracilis* to the genus *Campylobacter* as *Campylobacter gracilis* comb. nov. Also, they indicated that *Bacteroides ureolyticus* can be considered a *Campylobacter* species on the basis of genotypic evidence but proposed that this taxon be considered a species incertae sedis pending the isolation and characterization of additional *B. ureolyticus*-like bacteria. We tested the type strains of *B. gracilis* and *B. ureolyticus* by the PCR-RFLP method. *B. gracilis* clustered in *Dde*I group A with *C. curvus*, *C. mucosalis*, and *C. showae. B. ureolyticus* clustered in *Dde*I group B with *C. fetus* (data not shown).

The PCR-RFLP identification method also offers significant advantages over other molecular identification methods (multilocus enzyme electrophoresis, genomic DNA micro- and macrorestriction analyses, and DNA probe-based genomic DNA microrestriction analyses), which require large quantities of cells and involve complex and time-consuming DNA extraction, purification, electrophoresis, blotting, and hybridization steps. A crude cell extract prepared by boiling the growth scraped from a plate was sufficient to serve as the target DNA for the PCR by the PCR-RFLP method.

The PCR-RFLP method was particularly useful for identifying atypical *Campylobacter*-like isolates. On the basis of the results obtained with the coded set of isolates (Table 2), some catalase-negative or weak *Campylobacter* isolates which may be misidentified as *C. upsaliensis* are probably cephalothin-susceptible *C. coli* (D1843). Additional catalase-negative or weak isolates must be examined to confirm this observation. Clinical isolates of *Campylobacter*-like organisms that had biochemical profiles similar to those of *C. fetus* or *H. cinaedi* and were *C. fetus* by fatty acid profiles had the PCR-RFLP patterns of *C. hyointestinalis* (isolates D2604 and D2739). In both instances, the PCR-RFLP results need to be validated by DNA-DNA hybridization experiments. Even when definitive identification of an isolate could not be made by the PCR-RFLP method (for isolate D3880), the information obtained by this method may facilitate the choice of reference strains (in this instance, *C. hyointestinalis*) for DNA-DNA hybridization experiments. Strain D3911 is an obvious candidate for characterization by DNA-DNA hybridization.

In conclusion, we developed a PCR-RFLP method for the rapid identification of *Campylobacter*, *Arcobacter*, *Helicobacter*, and *Wolinella* species at the genus level and for the identification of species or species group within the genus *Campylobacter*. The method is rapid and simple and can be performed with very small quantities of bacterial cultures. With the approximately 150 strains tested in the present investigation, the method proved to be sensitive and highly reliable. Additional studies with large numbers of strains are needed to validate the PCR-RFLP method. After validation, the PCR-RFLP method could be used singly or in combination with conventional identification procedures for the accurate and reliable identification of *Campylobacter* species and members of related genera.

We are attempting to increase the discriminating power of the PCR-RFLP technique by including additional amplifica-

tion targets in a multiplex PCR to enable reliable identification of all *Campylobacter* species. The same approach could be extended to the identification of the members of the rapidly expanding *Helicobacter* genus.

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REFERENCES

1. **Blom, K., C. M. Patton, M. A. Nicholson, and B. Swaminathan.** 1995. Identification of *Campylobacter fetus* by PCR-DNA probe method. J. Clin. Microbiol. **33:**1360–1362.
2. **Brenner, D. J., A. C. McWhorter, J. K. Leetekmetson, and A. G. Steigerwalt.** 1982. *Escherichia vulneris*: a new species of *Enterobacteriaceae* associated with human wounds. J. Clin. Microbiol. **15:**1133–1140.
3. **Burnens, A. P., and J. Nicolet.** 1993. Three supplementary diagnostic tests for *Campylobacter* species and related organisms. J. Clin. Microbiol. **31:** 708–710.

3a. **Cardarelli-Leite, P.** Unpublished data.

4. **Eyers, M., S. Chapelle, G. Van Camp, H. Goossens, and R. De Wachter.** 1993. Discrimination among thermophilic *Campylobacter* species by polymerase chain reaction amplification of 23S rRNA gene fragments. J. Clin. Microbiol. **31:**3340–3343.
5. **Ezaki, T., N. Takeuchi, S. Liu, A. Kai, H. Yamamoto, and E. Yabuuchi.** 1988. Small-scale DNA preparation for rapid genetic identification of *Campylobacter* species without radioisotope. Microbiol. Immunol. **32:**141–150.
6. **Figura, N.** 1993. Clinical isolates of *Campylobacter mucosalis*: author's reply. J. Clin. Microbiol. **31:**2836. (Letter to the Editor.)
7. **Figura, N.** 1994. Additional data on clinical isolates of *Campylobacter mucosalis*: author's reply. J. Clin. Microbiol. **32:**2339. (Letter to the editor.)
8. **Hall, G. S.** 1992. Probe technology for the clinical microbiology laboratory. Arch. Pathol. Lab. Med. **117:**578–583.
9. **Kiehlbauch, J. A., B. D. Plikaytis, B. Swaminathan, D. N. Cameron, and I. Wachsmuth.** 1991. Restriction fragment length polymorphisms in the ribosomal genes for species identification and subtyping of aerotolerant *Campylobacter* species. J. Clin. Microbiol. **29:**1670–1676.
10. **Lambert, M. A., C. M. Patton, T. J. Barrett, and C. W. Moss.** 1987. Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. J. Clin. Microbiol. **25:**706–713.
11. **Lastovica, A., E. Le Roux, R. Warren, and H. Klump.** 1993. Clinical isolates of *Campylobacter mucosalis*. J. Clin. Microbiol. **31:**2835–2836. (Letter to the Editor.)
12. **Lastovica, A. J., E. Le Roux, R. Warren, and H. Klump.** 1994. Additional data on clinical isolates of *Campylobacter mucosalis*. J. Clin. Microbiol. **32:** 2338–2339. (Letter to the Editor.)

12a. **Leong, D.** Personal communication.

13. **Nicholson, M. A., and C. M. Patton.** 1995. Evaluation of disk method for hippurate hydrolysis by *Campylobacter* species. J. Clin. Microbiol. **33:**1341–1343.
14. **On, S. L. W.** 1994. Confirmation of human *Campylobacter concisus* isolates misidentified as *Campylobacter mucosalis* and suggestions for improved differentiation between the two species. J. Clin. Microbiol. **32:**2305–2306.
15. **On, S. L. W., and B. Holmes.** 1991. Effect of inoculum size on the phenotypic characterization of *Campylobacter* species. J. Clin. Microbiol. **29:**923–926.
16. **Patton, C. M., I. K. Wachsmuth, G. M. Evins, J. A. Kiehlbauch, B. D. Plikaytis, N. Troup, L. Tompkins, and H. Lior.** 1991. Evaluation of 10 methods to distinguish epidemic-associated *Campylobacter* strains. J. Clin. Microbiol. **29:**680–688.
17. **Romaniuk, P. J., and T. J. Trust.** 1989. Rapid identification of *Campylobacter* species using oligonucleotide probes to 16S ribosomal RNA. Mol. Cell. Probes **3:**133–142.
18. **Salama, S. M., M. M. Garcia, and D. E. Taylor.** 1992. Differentiation of the subspecies of *Campylobacter fetus* by genomic sizing. Int. J. Syst. Bacteriol. **42:**446–450.
19. **Skirrow, M. B.** 1990. *Campylobacter*, *Helicobacter* and other motile curved gram-negative rods, p. 531–549. *In* M. T. Parker and L. H. Collier (ed.), Topley and Wilson's principles of bacteriology, virology, and immunity, 8th ed., vol. 2. Arnold, London.
20. **Skirrow, M. B.** 1994. Diseases due to *Campylobacter*, *Helicobacter* and related bacteria. J. Comp. Pathol. **111:**113–149.
21. **Totten, P. A., C. M. Patton, F. C. Tenover, T. J. Barrett, W. E. Stamm, A. G. Steigerwalt, J. Y. Lin, K. K. Holmes, and D. J. Brenner.** 1987. Prevalence and characterization of hippurate-negative *Campylobacter jejuni* in King County, Washington. J. Clin. Microbiol. **25:**1747–1752.
22. **Ursing, J. B., H. Lior, and R. J. Owen.** 1994. Proposal of minimal standards for describing new species of the family *Campylobacteraceae*. Int. J. Syst. Bacteriol. **44:**842–845.
23. **Vandamme, P., M. I. Daneshwar, F. E. Dewhirst, B. J. Paster, K. Kersters, H. Goossens, and C. W. Moss.** 1995. Chemotaxonomic analyses of *Bacteroides gracilis* and *Bacteroides ureolyticus* and reclassification of *B. gracilis* as *Campylobacter gracilis* comb. nov. Int. J. Syst. Bacteriol. **45:**145–152.
24. **Vandamme, P., and J. De Ley.** 1991. Proposal for a new family, *Campylobacteraceae*. Int. J. Syst. Bacteriol. **41:**451–455.
25. **Vandamme, P., E. Falsen, R. Rossau, B. Hoste, P. Segers, R. Tytgat, and J. De Ley.** 1991. Revision of *Campylobacter*, *Helicobacter* and *Wolinella* taxonomy: emendation of generic descriptions and proposal of *Arcobacter* gen. nov. Int. J. Syst. Bacteriol. **41:**88–103.
26. **Weisburg, W. G., S. M. Barns, D. A. Pelletier, and D. J. Lane.** 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bacteriol. **173:**697–703.
27. **Wilson, K. H., R. B. Blitchington, and R. C. Greene.** 1990. Amplification of bacterial 16S ribosomal RNA with polymerase chain reaction. J. Clin. Microbiol. **28:**1942–1946.