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Species-Specific Identification of Campylobacters by PCR-Restriction Fragment Length Polymorphism and PCR Targeting of the Gyrase B Gene▽†

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PCR-restriction fragment length polymorphism (RFLP) analysis of a 960-bp fragment of the *Campylobacter gyrB* gene with either DdeI or XspI restriction enzymes generated unique digestion patterns for 12 different *Campylobacter* species. In addition, PCR assays using species-specific primer sets targeting *gyrB* were specific for the respective *Campylobacter* species. Therefore, PCR-RFLP analysis and species-specific PCR assays based on the *gyrB* gene provide valuable tools for rapid and unambiguous identification of the majority of *Campylobacter* species.

Campylobacter spp. are an important cause of bacterial gastrointestinal infections worldwide. Travel to developing countries, contact with household pets, and consumption of contaminated vegetables, shellfish, water, and especially poultry are risk factors for infection (2, 6, 11). The genus *Campylobacter* consists of 16 species and 6 subspecies (12), which may cause disease in humans and animals (10). An additional species has recently been described (3). *C. jejuni* accounts for the majority of human morbidity, although the other thermotolerant species, *C. coli*, *C. lari*, and *C. upsaliensis*, have also been isolated from clinical samples. Other species have been linked with diarrheal illness and periodontal disease (*C. concisus*, *C. gracilis*, *C. rectus*, and *C. showae*) as well as meningitis and septicemia (*C. fetus*) (10). *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus* have been associated with food- and waterborne outbreaks of gastroenteritis (1, 4, 8). Due to the technical limitations of cultural and phenotypic methods employed for detection, isolation, and typing, the incidence of *Campylobacter* spp., especially non-*jejuni* species, is likely underreported.

The *gyrB* gene encodes the subunit B protein of DNA gyrase, a type II topoisomerase that catalyzes the negative supercoiling of bacterial DNA. Because the frequency of base substitutions in *gyrB* exceeded that of 16S rRNA within the species *Pseudomonas putida*, analysis based on *gyrB* was more discriminating than that based on 16S rRNA (19). Species identification and detection methods based on *gyrB* have been developed for *Bacillus* spp. and *Vibrio* spp. (15, 18). In this study, we identified the sequence polymorphisms in the *Campylobacter gyrB* gene and developed species-specific PCR assays and PCR-restriction fragment length polymorphism (RFLP) using the restriction enzymes DdeI and XspI to differentiate 12 *Campylobacter* species.

PCR amplification and sequencing and analysis of the *Campylobacter gyrB* gene. Bacterial strains used to sequence the *gyrB* gene and to determine the specificity of the resultant *gyrB*-based PCR assays are listed in Table S1 in the supplemental material. The DNA was isolated as described previously (16) or was purified using PrepMan Ultra reagent (Applied Biosystems, Foster City, CA). PCR amplification of the *gyrB* gene and direct sequencing of the PCR products were performed using a GeneAmp 9700 thermal cycler (Applied Biosystems). The universal primer set for the PCR amplification of ca. 1,250 bp (1,253 or 1,256 bp) of the *gyrB* gene region from all strains was 5′-TAATACGACTCACTATAGGGGTCGACCAYGCNGGNGGNAARTTYGA-3′ (T7-FWD; the T7 promoter sequence attached to the 5′ end is underlined) and 5′-GATTTAGGTGACACTATAGCTCGAGCCRTCNACRTCNGCRTCNGTCAT-3′ (SP6-REV; the SP6 promoter sequence attached to the 5′ end is underlined). The DNA template (1 μl) was PCR amplified in a 100-μl reaction volume containing 1× PCR buffer, 4 mM $MgCl_2$, 0.625 U *rTaq* DNA polymerase (Takara Bio Inc., Shiga, Japan), a 0.2 mM concentration (each) of the four deoxynucleoside triphosphates (dNTPs), and a 0.4 μM concentration of each primer. The amplification conditions were as follows: initial denaturation (95°C for 5 min), followed by 30 cycles each of denaturation (95°C for 1 min), annealing (60°C for 1 min), and extension (72°C for 1 min). The primers used for the DNA sequencing were 5′-TAATACGACTCACTATAGGGGTCGAC-3′ (T7kai) and 5′-GATTTAGGTGACACTATAGCTCGAG-3′ (SP6kai). DNA sequences were determined from both strands by extension from the attached promoter sequences (T7kai and SP6kai primers) and by primer walking using the ABI Prism dye terminator cycle sequencing kit (Applied Biosys-

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TABLE 1. Sequences used to prepare the universal primer mix for amplification of the 960-bp *gyrB* gene sequence for each *Campylobacter* species

Strain	Universal mix forward primer	Universal mix reverse primer
C. jejuni NADC 5096	CGTCAAGAATTTTCAGAAGGTAAAGTTATC	TTTTAAAATTTTATCTAGTCTTGCTTTTTC
C. coli NADC 5095	CGCCAAGAATTTTCAGAAGGTAAAGTCATC	TTTTAAAATTTTATCTAATCTTGCTTTTTC
C. concisus ATCC 33237	AGACAAGAATTTGCAAAAGGTATCCCTCAA	CTTTAAAATTTTATCCAGTCTTGCCTTTTC
C. curvus ATCC 35224	AGGCAAGAATTTCAAAAAGGTATCCCGGTA	TTTTAAAATTTTATCGAGGCGCGATTTTTC
C. showae ATCC 51146	AGACAAGAATTTTCAAAAGGTATCCCTCAA	TTTTAAAATTTTATCTAGTCTTGCTTTTTC
C. mucosalis ATCC 49352	AGGCAAGAATTTGCAAAAGGAATTCCAGTA	TTTTAAAATTTTATCTAATCTTGATTTTTC
C. fetus subsp. *fetus* ATCC 15296	CGTCAAGAGTTTTCAAAAGGAATACCCCAA	TTTTAAAATTTTATCAAGTCTACTTTTTTC
C. hyointestinalis ATCC 35217	CGCCAAGAATTCGCCGAAGGCATACCTCAA	TTTAAGAATTTTATCAAGCCTACTTTTTTC
C. sputorum subsp. *sputorum* ATCC 33562	AGACAAGAGTTTTCAAAAGGTGTTCCTACA	TTTTAAAATTTTTTCAAGACCTGCTTTTTC
C. helveticus ATCC 51210	AGACAAGAATTTTCTAAAGGTCTAATTGCA	TTTTAAAATTTTATCCAGCCTTGCTTTTTC
C. upsaliensis ATCC 49816	CGCCAAGAATTTGCTAAAGGGCAAATAGCT	TTTTAAAATTTTATCCAGTCTTGCTTTTTC
C. lari ATCC 35221	AGACAAGAATTTTCAGAAGGAAAAGTAACA	TTTTAAAATTTTATCAAGTCTTGCTTTTTC

tems). Products were resolved on an ABI Prism 310 automated sequencer (Applied Biosystems). For phylogenetic analysis, the *gyrB* sequences of 12 species of *Campylobacter* were aligned using the DNASIS Pro program (version 2.0) (Hitachi, Tokyo, Japan). Distance matrices using the Kimura two-parameter correction and phylogenetic analysis using the neighbor-joining method (13) were performed with the CLUSTAL W program (14) on the DDBJ website (www.ddbj.nig.ac.jp/Welcome-e.html).

The major topology of the phylogenetic neighbor-joining tree constructed from the partial *gyrB* gene sequences derived in this study was similar to that previously reported for the 16S rRNA gene sequences (5, 7). However, *gyrB* provides higher resolution for *Campylobacter* species, with lower interspecies sequence similarities (ranging from 58.3 to 89.2% [see Table S2A in the supplemental material]) than those reported for the 16S rRNA gene (ranging from 89 to 99% [see Table S2B in the supplemental material]) (5). To illustrate, Gorkiewicz et al. (5) reported that the limitation of 16S rRNA analysis is its inability to differentiate *C. jejuni*, *C. coli*, and atypical *C. lari* strains, which shared identical 16S rRNA gene sequences and therefore were assigned to a common cluster. Earlier reports indicated that these thermotolerant strains exhibited a 98.1% homology based on partial 16S rRNA sequencing (9, 17). In the current study, *gyrB* gene sequence analyses discriminated these thermotolerant taxa. The *C. jejuni* isolates shared identical sequences and were clearly distinct from *C. coli*, though the two species had the highest similarity (89.2%) of the 12 *Campylobacter* species examined.

The *gyrB* sequences of *C. fetus* subsp. *fetus* ATCC 15296 from the American Type Culture Collection (ATCC), *C. fetus* subsp. *fetus* NADC 5513 from the National Animal Disease Center (NADC), *C. fetus* subsp. *venerealis* NADC 5519, and *C. hyointestinalis* ATCC 35217 had a unique 3-base insertion at positions 823 to 825 that is unlike the same region in the other *Campylobacter* species, which resulted in an additional amino acid in the protein sequence. *C. fetus* subsp. *fetus* and *C. hyointestinalis* are phylogenetically close, as inferred earlier when a 98% homology was calculated based on 16S rRNA sequence analysis (9). That *gyrB* offers higher resolution between *C. fetus* subsp. *fetus* and *C. hyointestinalis* (84% similarity [see Table S2A in the supplemental material]) than 16S rRNA should expedite the identification of these two species. *C. fetus* subsp. *fetus* and *C. fetus* subsp. *venerealis* strains shared identical *gyrB* gene sequences, however, suggesting that *gyrB* may not be a suitable marker for the identification of these subspecies. A similar conclusion was made following a comparison of *rpoO* and 16S rRNA sequences in the two *C. fetus* subspecies (9).

The primary objective of this study was to determine if the *gyrB* gene sequences were sufficiently unique to serve as suitable targets for *Campylobacter* species identification. Multiple alignments of the 12 *Campylobacter gyrB* sequences were performed, a matrix representing the sequence variations among the strains was calculated, and a dendrogram was constructed from these data (see Fig. S1 in the supplemental material). Analysis of the dendrogram showed that all 12 *Campylobacter* species were clearly differentiated in the constructed phylogenetic tree. The major topology of the tree based on the partial *gyrB* gene sequences was similar to that of one previously reported that was based on 16S rRNA gene sequence analyses (5).

PCR-RFLP for the differentiation of *Campylobacter* species. A universal primer mix prepared using 12 primer sets complementary to the *gyrB* sequence of each species (Table 1) was used to amplify a 960-bp *gyrB* fragment from each *Campylobacter* strain. The DNA template (1 μl) was amplified in a 100-μl reaction volume containing 1× PCR buffer, 2 mM $MgCl_2$, 0.625 U *rTaq* (Takara) DNA polymerase, a 0.4 mM concentration of each of the four dNTPs, and the universal primer mixture consisting of a 10 nM concentration of each primer. The cycling conditions consisted of an initial denaturation at 95°C for 10 min, followed by 50 cycles of denaturation (95°C for 15 s), annealing (65°C for 1 min), and extension (72°C for 1 min), with a final 7-min extension at 72°C.

Computational restriction fragment length analyses of the 960-bp amplified region predicted that the DdeI and XspI enzymes would generate species-specific digestion patterns. For RFLP analysis, the purified PCR products were digested in a total volume of 20 μl with either 5 U of DdeI (Toyobo, Osaka, Japan) or 10 U of XspI (Takara). The resulting fragments were size separated using 3.0% agarose prepared in 1× Tris-acetate-EDTA buffer and stained with Sybr green I dye (Invitrogen, Carlsbad, CA). PCR-RFLP results using DdeI and XspI are shown in Fig. 1A and B, respectively. All *Campylobacter* species studied had species-specific DdeI and XspI digestion patterns. Furthermore, computer analysis of the sequences using the DNASIS program predicted the unambiguous identification of the 12 species of *Campy-*

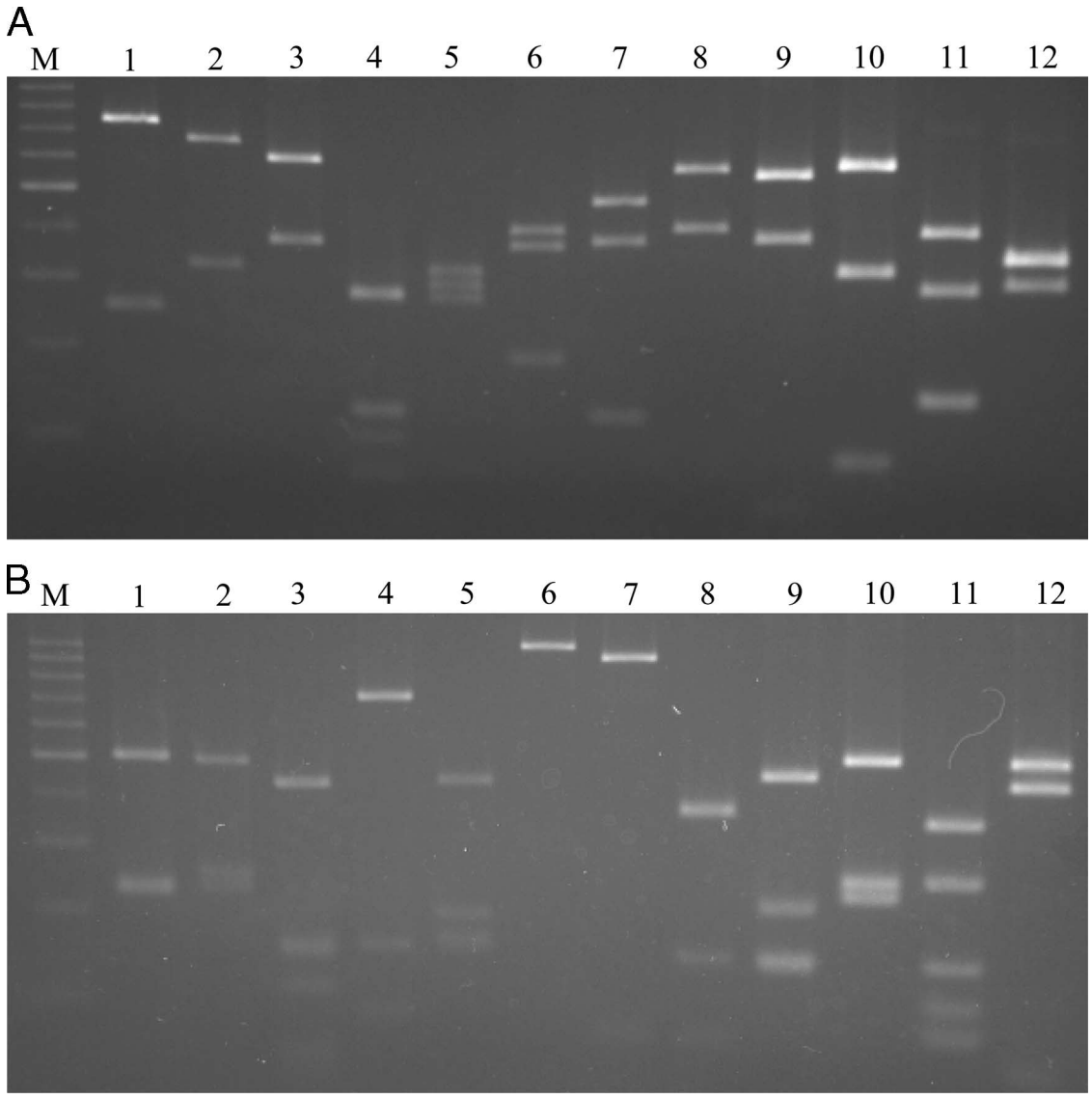

FIG. 1. Patterns from PCR-RFLP with DdeI (A) and XspI (B) for *C. jejuni* NADC 5096 (lane 1), *C. coli* NADC 5095 (lane 2), *C. concisus* ATCC 33237 (lane 3), *C. curvus* ATCC 35224 (lane 4), *C. showae* ATCC 51146 (lane 5), *C. mucosalis* ATCC 49352 (lane 6), *C. fetus* subsp. *fetus* ATCC 15296 (lane 7), *C. hyointestinalis* ATCC 35217 (lane 8), *C. sputorum* subsp. *sputorum* ATCC 33562 (lane 9), *C. helveticus* ATCC 51210 (lane 10), *C. upsaliensis* ATCC 49816 (lane 11), and *C. lari* ATCC 35221 (lane 12). Lane M, 100-bp molecular size markers.

lobacter by double digestion of the 960-bp *gyrB* region with MboI and HindIII. This was also confirmed experimentally with the 960-bp PCR product (data not shown). Kärenlampi et al. (7) demonstrated that partial *groEL* sequencing and the resultant PCR-RFLP analyses were more discriminating than *Campylobacter* species identification based on 16S rRNA. A similar conclusion was made when the *rpoO* gene sequences of 16 *Campylobacter* species were compared with their 16S rRNA sequences (9).

PCR with *Campylobacter* species-specific primers and specificity testing. Twelve different oligonucleotide primer sets were designed based on regions that were dissimilar among the different species (Table 2). Template DNA (2.5 μl) was amplified in a 25-μl reaction volume containing 1× GeneAmp PCR Gold buffer, 0.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), a 200 μM concentration (each) of the four dNTPs, and a 0.2 μM concentration (each) of the species-specific primers. The cycling conditions for *C. jejuni*, *C. coli*, *C. lari*, *C. concisus*, *C. showae*, *C. curvus*, *C. fetus*, and *C. helveticus* were the following: initial denaturation at 95°C for 10 min and 30 cycles of 95°C for 20 s, 69°C for 20 s, and a final extension at 72°C for 7 min. For *C. upsaliensis*, *C. mucosalis*, and *C. hyointestinalis*, the annealing temperature and time were 68°C for 1 min, and for *C. sputorum*, they were 65°C for 20 s. A specific PCR product was generated for each of the respective target *Campylobacter* species (Fig. 2). No false-positive results were observed when DNA from the nontarget *Campylobacter* species was used, and furthermore, nonspecific bands were not observed with DNA from the non-*Campylobacter* strains tested (see Table S1 in the supplemental

TABLE 2. PCR primer sequences targeting *gyrB* used for *Campylobacter* species-specific identification

Strain	Forward primer	Reverse primer	Amplicon length (bp)	Location
C. jejuni NADC 5096	AGAATGGGTTTAACTCGTGTGATAAGT	TACCACGCAAAGGCAGTATAGCT	493	84–576
C. coli NADC 5095	AAATGCTAGTGCTAGGGAAAAAGACTCT	TGAGGTTCAGGCACTTTTACACTTA CTAC	96	125–220
C. concisus ATCC 33237	AGCGGGCCTAACAAGAGTTATTACA	TGTAAGCACGTCAAAAACCATCTTT	217	86–302
C. curvus ATCC 35224	CTGCCAAAGTAAGGACGCAAGTATA	GGCAAGATCGCCTGAAATACG	108	458–565
C. showae ATCC 51146	AGGGTTTAAGCATAGGAACGCTG	CACCAGATAAAGCTCGCTGATCG	86	415–500
C. mucosalis ATCC 49352	TGCGATTATGAACAAGGCCCTA	TCGCTTGAAACACACGGTCA	224	335–558
C. fetus subsp. *fetus* ATCC 15296	AGAGCTGGGCTTACAAGAGCTATTACA	GGTAAAATCGCTTGAAACGCT CTAT	482	84–565
C. hyointestinalis ATCC 35217	CGGTCAAAAGATGACTTTTGAAGTACTT	GCTTCCCTGCCACGAGCT	108	272–379
C. sputorum subsp. *sputorum* ATCC 33562	AGCTTTACTTGCTGCAAGAGGAAGA	AGGAAGCGTTCCAACAGAAAAGTT	94	350–443
C. helveticus ATCC 51210	CAATAACATACGCACACCAGATGGA	CAGGCACTTTAACGCTCACTATGG	176	38–213
C. upsaliensis ATCC 49816	GCTTACGCGTGTAATTACAAACTATGTC	AATTGCCTTAGCCTCGATAGGG	250	92–341
C. lari ATCC 35221	CTATGTTCGTCCTATAGTTTCTAAGGCTTC	CCAGCACTATCACCCTCAACTAA ATAA	261	257–517

material). Thus, the species-specific primer sets based on *gyrB* sequences can be used for the rapid detection and identification of *Campylobacter* species.

To determine if *gyrB* gene sequences were sufficiently unique to distinguish the *Campylobacter* species of public health significance, we sequenced a 1,020-bp region of the *gyrB* gene of 12 *Campylobacter* species and demonstrated that PCR-RFLP and direct PCR analyses with species-specific primer sets unambiguously distinguished the 12 species. DNA sequence analyses showed that the resultant PCR-RFLP and PCR assays were more discriminating for *Campylobacter* species identification than similar analyses based on the 16S rRNA gene. In addition to accelerating the identification of currently recognized species, *gyrB* gene sequence information will facilitate taxonomic studies of novel *Campylobacter* species. As new species of *Campylobacter* emerge, their *gyrB* gene can be sequenced, and high-fidelity PCR primers can be designed for the new taxa.

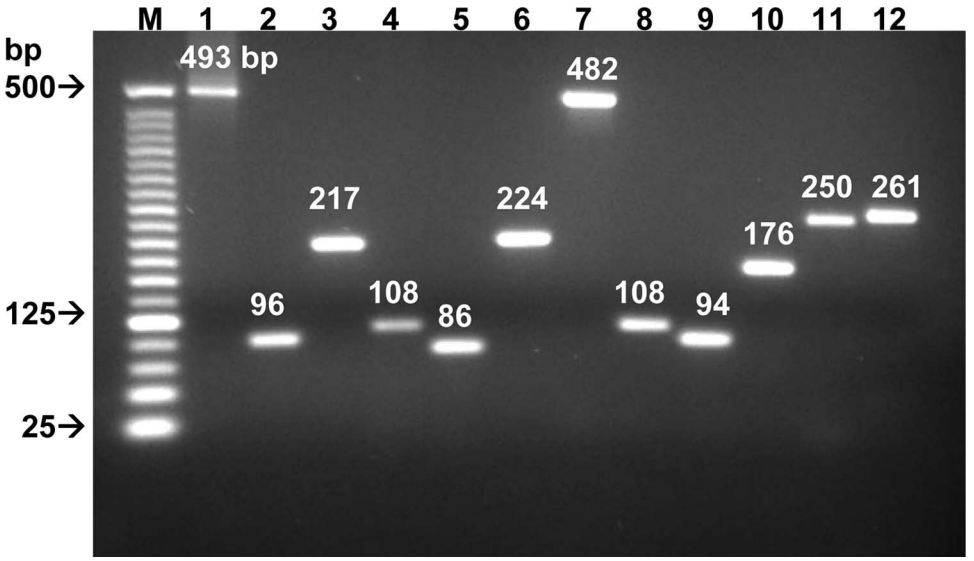

FIG. 2. Species-specific identification of *Campylobacter* species. Each lane represents the results of PCR assays using one set of primers and DNA from each of the following 12 *Campylobacter* spp.: *C. jejuni* primers (lane 1), *C. coli* (lane 2), *C. concisus* (lane 3), *C. curvus* (lane 4), *C. showae* (lane 5), *C. mucosalis* (lane 6), *C. fetus* subsp. *fetus* (lane 7), *C. hyointestinalis* (lane 8), *C. sputorum* (lane 9), *C. helveticus* (lane 10), *C. upsaliensis* (lane 11), and *C. lari* (lane 12). Lane M, 25-bp molecular size markers.

Nucleotide sequence accession numbers. The *gyrB* gene sequences determined in this study and accession numbers have been deposited in the DDBJ (DNA Data Bank of Japan) nucleotide sequence database (see Table S1 in the supplemental material).

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REFERENCES

1. **Broczyk, A., S. Thompson, D. Smith, and H. Lior.** 1987. Water-borne outbreak of *Campylobacter laridis*-associated gastroenteritis. Lancet **i:**164–165.
2. **Damborg, P., K. E. P. Olsen, E. Møller Nielsen, and L. Guardabassi.** 2004. Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. J. Clin. Microbiol. **42:**1363–1364.
3. **Foster, G., B. Holmes, A. G. Steigerwalt, P. A. Lawson, P. Thorne, D. E. Byrer, H. M. Ross, J. Xerry, P. M. Thompson, and M. D. Collins.** 2004. *Campylobacter insulaenigrae* sp. nov., isolated from marine mammals. Int. J. Syst. Evol. Microbiol. **54:**2369–2373.
4. **Goossens, H., B. A. J. Giesendorf, P. Vandamme, L. Vlaes, C. van den Borre, A. Koeken, W. G. Quint, W. Blomme, P. Hanicq, D. S. Koster, H. Hofstra, J.-P. Butzler, and J. van der Plas.** 1995. Investigation of an outbreak of *Campylobacter upsaliensis* in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotypic typing methods. J. Infect. Dis. **172:**1298–1305.
5. **Gorkiewicz, G., G. Feierl, C. Schober, F. Dieber, J. Köfer, R. Zechner, and E. L. Zechner.** 2003. Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. J. Clin. Microbiol. **41:**2537–2546.
6. **Jacobs-Reitsma, W.** 2000. *Campylobacter* in the food supply, p. 467–481. *In* I. Nachamkin and M. J. Blaser (ed.), *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
7. **Kärenlampi, R. I., T. P. Tolvanen, and M.-L. Hänninen.** 2004. Phylogenetic analysis and PCR-restriction fragment length polymorphism identification of *Campylobacter* species based on partial *groEL* gene sequences. J. Clin. Microbiol. **42:**5731–5738.
8. **Klein, B. S., J. M. Vergeront, M. J. Blaser, P. Edmonds, D. J. Brenner, D. Janssen, and J. P. Davies.** 1986. *Campylobacter* infection associated with raw milk. An outbreak of gastroenteritis due to *Campylobacter jejuni* and thermotolerant *Campylobacter fetus subsp. fetus*. JAMA **255:**361–364.
9. **Korczak, B. M., R. Stieber, S. Emler, A. P. Burnens, J. Frey, and P. Kuhnert.** 2006. Genetic relatedness within the genus *Campylobacter* inferred from the *rpoB* sequences. Int. J. Syst. Evol. Microbiol. **56:**937–945.
10. **Lastovica, A. J., and M. B. Skirrow.** 2000. Clinical significance of *Campylobacter* and related species other than *Campylobacter jejuni* and *C. coli*, p. 89–120. *In* I. Nachamkin and M. J. Blaser, *Campylobacter*, 2nd ed. ASM Press, Washington, DC.
11. **Manning, G., C. G. Dowson, M. C. Bagnall, I. H. Ahmed, M. West, and D. C. Newell.** 2003. Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni*. Appl. Environ. Microbiol. **69:**6370–6379.
12. **On, S. L. W.** 2001. Taxonomy of *Campylobacter*, *Arcobacter*, *Helicobacter* and related bacteria: current status, future prospects and immediate concerns. J. Appl. Microbiol. **90:**1S–15S.
13. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
14. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
15. **Venkateswaran, K., N. Dohmoto, and S. Harayama.** 1998. Cloning and nucleotide sequence of the *gyrB* gene of *Vibrio parahaemolyticus* and its application in detection of this pathogen in shrimp. Appl. Environ. Microbiol. **64:**681–687.
16. **Wesley, I. V., and J. H. Bryner.** 1989. Antigenic and restriction enzyme analysis of isolates of *Campylobacter fetus* subsp. *venerealis* recovered from persistently infected cattle. Am. J. Vet. Res. **50:**807–813.
17. **Wesley, I. V., R. D. Wesley, M. Cardella, F. E. Dewhirst, and B. J. Paster.** 1991. Oligodeoxynucleotide probes for *Campylobacter fetus* and *Campylobacter hyointestinalis* based on 16S rRNA sequences. J. Clin. Microbiol. **29:**1812–1817.
18. **Yamada, S., E. Ohashi, N. Agata, and K. Venkateswaran.** 1999. Cloning and nucleotide sequence analysis of *gyrB* of *Bacillus cereus*, *B. thuringiensis*, *B. mycoides*, and *B. anthracis* and their application to the detection of *B. cereus* in rice. Appl. Environ. Microbiol. **65:**1483–1490.
19. **Yamamoto, S., and S. Harayama.** 1995. PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. Appl. Environ. Microbiol. **61:**1104–1109.