Rapid Identification of Thermotolerant \textit{Campylobacter jejuni}, \textit{Campylobacter coli}, \textit{Campylobacter lari}, and \textit{Campylobacter upsaliensis} from Various Geographic Locations by a GTPase-Based PCR-Reverse Hybridization Assay

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Received 3 November 1998/Returned for modification 2 February 1999/Accepted 4 March 1999

Recently, a gene from Campylobacter jejuni encoding a putative GTPase was identified. Based on two semiconserved GTP-binding sites encoded within this gene, PCR primers were selected that allow amplification of a 153-bp fragment from C. jejuni, C. coli, C. lari, and C. upsaliensis. Sequence analysis of these PCR products revealed consistent interspecies variation, which allowed the definition of species-specific probes for each of the four thermotolerant Campylobacter species. Multiple probes were used to develop a line probe assay (LiPA) that permits analysis of PCR products by a single reverse hybridization step. A total of 320 reference strains and clinical isolates from various geographic origins were tested by the GTP-based PCR-LiPA. The PCR-LiPA is highly specific in comparison with conventional identification methods, including biochemical and whole-cell protein analyses. In conclusion, a simple method has been developed for rapid and highly specific identification of thermotolerant Campylobacter species.

Campylobacter bacteria are common foodborne pathogens and cause enteric disease in humans worldwide. The thermotolerant (growth at 42°C) species Campylobacter jejuni and C. coli are especially frequent causes of acute bacterial enteritis due to consumption of contaminated food, primarily chicken (2). Outbreaks of C. upsaliensis infection also have been reported (4, 14). The clinical relevance of C. lari, another thermotolerant Campylobacter species, is not yet fully understood. C. jejuni infections also are important antecedents of the Guillain-Barré syndrome (1). Therefore, accurate methods of species identification are essential for clinical and epidemiological purposes.

Rapid and reliable identification of the different Campylobacter species is not always possible by conventional procedures that are based on selective plating, biochemical identification, and serotyping (17, 23). Recently, several DNA-based methods have been developed for the detection and identification of Campylobacter species in food and in clinical and environmental samples (7, 10, 11, 16, 19, 21). However, these methods also have several limitations, as they require amplification with multiple primer sets or digestion with multiple restriction enzymes, yield complex electrophoretic patterns, or offer only limited discrimination between species.

Recently, we identified a Campylobacter gene that encodes a putative GTPase (26). As with all GTPases, this protein contains several semiconserved GTP-binding sites designated G-1, G-3, and G-4 (5). The Campylobacter GTPase represents a special family of GTPases with two adjacent GTP-binding domains, each comprising a full set of GTP-binding sites. Homologs appeared to be present in Bacillus subtilis, Haemophilus influenzae, Mycoplasma genitalium, and Mycobacterium leprae (26). Degenerate PCR primers based on the G-1 and G-3 sites of the first GTPase domain of the C. jejuni GTPase gene allow amplification of a 153-bp fragment from C. jejuni, C. coli, C. lari, and C. upsaliensis. This report describes further analysis of the 153-bp PCR products and development of a reverse hybridization format that allows rapid identification of the four thermotolerant Campylobacter species. The performance of this reverse hybridization test was evaluated by analysis of a large number of Campylobacter reference strains and well-characterized clinical isolates from various geographic locations.

MATERIALS AND METHODS

Bacterial strains. Five different panels were analyzed, comprising a total of 320 bacterial strains. The first panel contained 49 reference strains obtained from the BCCM/LMG Culture Collection (Ghent, Belgium). For most strains, the country of origin is given. The panel comprised C. jejuni (n = 7; LMG 6629 [United States], ATCC 33250 [United States], LMG 9972 [Sweden], LMG 9885, LMG 8870 [South Africa], LMG 9878, and LMG 10386), C. coli (n = 7; LMG 6440 [Belgium], LMG 8530 [Sweden], LMG 9799 [Belgium], LMG 9839, LMG 8848 [United Kingdom], LMG 9220 [Belgium], and LMG 12109 [Belgium]), C. lari (n = 9; LMG 8846 [United Kingdom], LMG 7929 [United Kingdom], LMG 8844 [United Kingdom], LMG 14338 [Belgium], LMG 7607 [United Kingdom], LMG 9132 [Sweden], LMG 9253 [Belgium], LMG 11251 [Belgium], and LMG 11760 [Canada]), C. upsaliensis (n = 10; LMG 9127, LMG 9128, LMG 9227, LMG 9274, LMG 9129, LMG 9223, LMG 9144, LMG 9109, LMG 9239, and LMG 13475 [all from Belgium]), and other Campylobacter species (n = 16: C. fetus LMG 6727 [Belgium], LMG 6442, LMG 6569 [Belgium], and LMG 6571 [Belgium], C. helveticus LMG 12638 [Switzerland] and LMG 14103 [Switzerland]; C. mucosalis LMG 8806 [United Kingdom] and LMG 8499 [United Kingdom]; C. hyointestinalis LMG 7538 [Sweden] and LMG 9276 [Belgium], C. rectus
LMG 7614 [United States]; C. concisus LMG 7967 [Sweden]; C. spurtorum LMG 6447 [Belgium] and ATCC 33491; C. curvus LMG 769 [United States]; and C. showae LMG8543 [Sweden]. Identification of the different species was based on multiple methods, including conventional biochemical assays and whole-cell protein analysis as previously reported (8, 22).

The second panel comprised 56 clinical isolates from The Netherlands (n = 39) and Belgium (n = 17). All isolates of C. jejuni (n = 19), C. coli (n = 35), and C. lari (n = 2) were identified by conventional methods and by whole-cell protein profiling (22).

Panel 3 comprised a total of 100 Campylobacter strains from Australia, including C. jejuni (n = 78), C. coli (n = 19), and C. lari (n = 3), that were analyzed by C. jejuni API (conventional biochemical analysis as described earlier (15)). Some isolates also had been analyzed by protein profiling and DNA-DNA dot blot hybridization.

Panel 4 comprised 25 Campylobacter isolates from humans (blood and stool) and hogs and included C. jejuni (n = 3), C. coli (n = 10), C. lari (n = 3), and C. upsaliensis (n = 9) strains. These isolates were obtained from the United States (n = 18), Thailand (n = 3), France (n = 2), and Bangladesh (n = 2). All isolates had been identified earlier by conventional biochemical methods.

Panel 5 contained a total of 90 strains from a reference laboratory in Switzerland, including C. jejuni (n = 60), C. coli (n = 15), C. helomaculatus (n = 1), C. fetus (n = 6), C. spurtorum (n = 1), Helicobacter sp. (n = 5), and Arcobacter butzleri (n = 2). Most of these strains were unusual in either antimicrobrial resistance pattern or phenotypic identification (e.g., nalidixic acid resistant or nitrate-negative C. jejuni) and were therefore sent to the reference laboratory. These isolates also had been identified earlier by dot blot DNA hybridization with whole genomic DNA as described earlier (6), as well as by conventional biochemical methods.

DNA isolation. Bacterial DNA was isolated from cultures of panels 1 and 2 as described earlier (12, 20). For strains from panels 3, 4, and 5, bacteria were harvested in 2 ml of sterile phosphate-buffered saline, pelleted by centrifugation for 2 min at 12,000 x g, resuspended in 400 μl of 10 mM Tris [pH 8.0]-5 mM EDTA-0.1% sodium dodecyl sulfate-0.1 mg/ml proteinase K, and incubated for at least 2 h at 55°C. Proteinase K was inactivated for 10 min at 95°C, and the lysate was clarified by centrifugation for 5 min at 12,000 x g. The supernatant was diluted 1/100 in sterile water and used directly for PCR.

GTP-based PCR primers. The PCR studies were based on PCR primers that have been deduced from the semiconservative GTP-binding G and G-3 amino acid motifs of the putative GTPase gene first found in C. jejuni (24) (GenBank accession no. U63330). Primer target sites also have been sequenced from C. coli, C. lari, and C. upsaliensis. Forward primers GTP-F1 (5'-biotin-GGAAAATCGATGGGAACATC 3') and GTP-F2 (5'-biotin-GTATCGTATCRA 3') are based on the G-1 GTP-binding site [consensus, (G/T)X(1-5)T; X means any amino acid], and reverse primer GTP-R1 (5'-biotin-GTCTCTACRA GCCGCTCCTAC 3') is based on the G-3 GTP-binding site (consensus, DXXG).

PCRs. PCR mixtures (50 μl) consisted of 10 mM Tris-Cl (pH 8.3), 50 mM KCl, 2 mM MgCl2, 200 μM of each dNTP, 1.5 U of AmpliTaq Gold DNA polymerase, and 70 pmol of each primer. PCR mixtures comprised 40 cycles of 30 s at 94°C, 45 s at 60°C, and 45 s at 74°C. Prior to cycling, the samples were denatured for 2 min at 94°C.

Analysis of amplified fragments. PCR products were separated and visualized on 2% agarose gels. PCR products were directly sequenced by using cycle sequencing (Amersham) and assayed on an ALF-Express electrophoresis unit. The PCR products were sequenced using a combination of forward and reverse primers that were designed from conserved GTPase regions.

RESULTS

Recently, a novel GTPase-encoding gene of C. jejuni was identified (26). This gene consists of two complete GTPase domains, each comprising a set of semiconserved GTP-binding motifs, in contrast to typical GTPases, which contain only a single GTP-binding domain. Homologs of the C. jejuni GTPase, also containing two separate GTPase domains, had been found earlier in B. subtilis, H. influenzae, M. genitalium, and M. leprae (26). In addition, the most recent database search (August 1998) revealed that Helicobacter pylori, Synechocystis sp., Buchnera aphidicola, Borrelia burgdorferi, Escherichia coli, Aquifex aeolicus, Treponema pallidum, and Mycobacterium tuberculosis also contain homologs.

Based on the semiconservated GTP-binding sites, PCR primers were selected that permit general amplification of a 153-bp fragment from C. jejuni, C. coli, C. lari, and C. upsaliensis. Degenerate primers were used to amplify the GTPase fragments from the four thermotolerant species, since these show limited but consistent species-specific heterogeneity in their G-1 and G-3 GTP-binding site sequences (data not shown). In the present study, we further analyzed the heterogeneity of the GTPase-derived sequences flanked by these semiconservated GTP-binding sites. The entire PCR-LiPA system based on the species-specific sequence variation was evaluated extensively by analysis of reference and clinical isolates.

Phylogenetic analysis of the 153-bp amplicon. The specificity of the GTPase-based PCR with respect to other Campylobacter species was determined by performing an uncharacterized gene of C. jejuni by Aul, Del, and Drul. The analysis was performed as previously described (16).

FIG. 1. Alignment of 117-bp sequences from 17 representative C. jejuni, C. coli, C. lari, and C. upsaliensis strains. Sequences were derived from 153-bp PCR fragments with the primer sites excluded. For each species, a number of representative sequences are shown, and the number corresponds to the position on the phylogenetic tree (see Fig. 2). For C. coli, the sequences from isolates C30-8 and C30-22, containing two separate GTPase domains, were shown. The positions of the LiPA probes are boxed. The strains used for sequences are as follows: C. jejuni 1, C31-720 (13); C. jejuni 2, C36-1880 (13); C. jejuni 3, 21 (27); C. jejuni 4, C463288 (13); C. jejuni 5, C563255 (13); C. coli 1, 27 (27); C. coli 2, C32-1213 (13); C. lari 1, C32-1213 (13); C. lari 2, C32-1213 (13); C. upsaliensis 1, C68725 (13); C. upsaliensis 2, C32-1213 (13); C. upsaliensis 3, C68725 (13); and C. upsaliensis 4, C68725 (13).
FIG. 2. Phylogenetic analysis of the 117-bp sequences (153-bp PCR fragments without primer sequences) from 53 thermotolerant Campylobacter species. *C. jejuni*, n = 11; *C. coli*, n = 9; *C. lari*, n = 27; *C. upsaliensis*, n = 6. Bootstrap values of >50 are shown.
lobacter species and non-Campylobacter bacterial isolates has been described previously (26). To assess the heterogeneity of the GTPase fragments from various Campylobacter species, 153-bp PCR products amplified from a total of 53 isolates, comprising C. jejuni (n = 11), C. coli (n = 9), C. lari (n = 27), and C. upsaliensis (n = 6), described earlier (26) were directly sequenced (Fig. 1), and phylogenetic analysis was performed. C. lari sequences also were obtained from strains that were cultured from mussels and oysters (9, 27). All sequences remain in frame, and no stop codons were found. The phylogenetic tree shows four distinct clusters, representing C. jejuni, C. coli, C. lari, and C. upsaliensis (Fig. 2). Intraspecies phylogenetic distances (using Jukes and Cantor parameters) are limited in C. jejuni (0.017 ± 0.011), C. coli (0.038 ± 0.019), and C. upsaliensis (0.011 ± 0.005) but are considerably greater among C. lari strains (0.126 ± 0.154).

Development of a LiPA. Based on the apparent sequence diversity among the 153-bp PCR products from C. jejuni, C. coli, C. lari, and C. upsaliensis, species-specific probes were developed. Because of the limited intraspecies heterogeneity, two probes were selected from different parts of the amplified sequence for each species. To permit analysis by a single-step reverse hybridization assay, probes that had the same hybridization characteristics were developed. The outline of the LiPA strip and representative examples are shown in Fig. 3.

Evaluation of the PCR-LiPA with reference strains (panel 1). To evaluate the performance of the Campylobacter LiPA, five different panels of Campylobacter isolates were tested. All results are summarized in Table 1.

In panel 1, a total of 49 reference strains from Western Europe were tested by the Campylobacter LiPA. PCR products from each of the strain reacted exclusively with the corresponding probes on the strip. PCR products from C. hyointestinalis, C. helveticus, C. fetus, and C. mucosalis did not show cross-reactivity to any of the probes on the strip (Fig. 3). No PCR products were obtained from C. concisus, C. sputorum, C. showae, and C. curvus. These findings indicated the utility of the methods based on the use of known reference strains.

Evaluation of the PCR-LiPA with clinical isolates from The Netherlands. Panel 2 comprised 56 clinical isolates, and all strains were unequivocally identified by PCR-LiPA. Results, except in three cases, were in complete agreement with results from whole-cell protein electrophoresis. LiPA analysis of three isolates, identified as C. coli by conventional methods, reproducibly showed hybridization with both the C. jejuni and C. coli probes, suggesting the presence of both species in the culture. To exclude cross-reactivity between the different probes on the LiPA, single colonies were recultured from the original isolates. LiPA analysis of individual colonies resulted in the exclusive identification of either C. jejuni or C. coli. Two C. coli isolates (strains C30-8 and C30-22) showed weaker reactivity with one of the two C. coli-specific probes on the LiPA (probe Cc473). Sequence analysis revealed several point mutations in the target sequence compared to sequences from reference isolates (Fig. 2). Thus, for panel 2, the PCR-LiPA analysis correctly identified all of the strains.

Evaluation of the PCR-LiPA with Australian isolates. In panel 3, a total of 100 thermotolerant Campylobacter strains from Australia were analyzed by the PCR-LiPA (Table 1). The strains had been analyzed earlier by conventional methods, including catalase and oxidase reactions, growth at 42°C, and API-Campy (15). Isolates were identified as C. jejuni or C. coli if they were resistant to cephalothin and susceptible to nalidixic acid. C. jejuni was distinguished from C. coli on the basis of positive hippurate hydrolysis. Isolates resistant to nalidixic acid and negative for hippurate hydrolysis were presumptively identified as C. lari. PCR-LiPA yielded completely concordant identification results in 95 (95%) of the 100 strains. Two strains were originally identified as C. coli by API-Campy, but PCR-LiPA identified them as C. jejuni. Conversely, two other strains were initially identified as C. jejuni, whereas PCR-LiPA classified them as C. coli. Another strain was originally identified as C. coli, but PCR-LiPA yielded C. lari-specific hybridization. These five discrepant strains were retested by conventional methods (15), as well as by PCR-LiPA, and the results remained the same. Subsequently, the strains were subjected to the independent DNA-based method of Jackson et al. (16), and the results were in complete agreement with the PCR-LiPA results for all five strains. Although the method of Jackson was not described for identification of C. lari, analysis of a number of C. lari reference strains and clinical isolates yielded discrete RFLP patterns. The single strain identified as C. lari by PCR-LiPA but as C. coli by conventional methods yielded an RFLP pattern matching the patterns of the reference C. lari strains but clearly different from the pattern expected for C. coli (data not shown).

Evaluation of the PCR-LiPA with isolates from the United States and other countries. The fourth panel comprised 25 Campylobacter isolates. Most were obtained in the United States, but others originated from several other countries. All C. jejuni, C. coli, and C. lari strains yielded the expected result by PCR-LiPA. However, one of nine C. upsaliensis strains was identified as C. jejuni by PCR-LiPA and was further analyzed by the method of Jackson et al. (16), but the results were inconclusive. Complex RFLP patterns with multiple restriction fragments were observed that were compatible with the presence of both C. jejuni, C. coli, C. upsaliensis, and/or other Campylobacter species. This culture probably contains a mix-
Identification by PCR-LiPA | No. of strains identified by conventional methodsa | Other | Total
---|---|---|---
Panel 1 | | | |
C. jejuni | 7 | 7 | 7
C. coli | 7 | 7 | 7
C. lari | 9 | 10 | 9
C. upsaliensis | 10 | 16 | 10
Negative | 7 | 9 | 10
Subtotal | 7 | 7 | 9 | 10 | 16b | 16 | 49
Panel 2 | | | |
C. jejuni | 19 | 32 | 19
C. coli | 22 | 2 | 22
C. lari + C. coli | 3c | 2 | 3
Subtotal | 19 | 35 | 2 | 3 | 56
Panel 3 | | | |
C. jejuni | 76 | 2d | 78
C. coli | 2e | 16 | 18
C. lari | 1f | 3 | 4
C. upsaliensis | 3g | 8 | 8
Subtotal | 78 | 19 | 3 | 9 | 100
Panel 4 | | | |
C. jejuni | 3 | 1g | 4
C. coli | 10 | 10 | 10
C. lari | 3 | 3 | 3
C. upsaliensis | 8 | 8 | 8
Subtotal | 3 | 10 | 3 | 9 | 25
Panel 5 | | | |
C. jejuni | 60 | 60 | 60
C. coli | 15 | 1h | 16
Negative | 14 | 14
Subtotal | 60 | 15 | 15i | 31 | 90
Total | 167 | 86 | 17 | 19 | 31 | 320

a The conventional identification methods used for the different panels of cultures are described in Materials and Methods.

b Includes C. fetus (n = 4), C. helveticus (n = 2), C. mucosalis (n = 2), C. hyointestinalis (n = 2), C. rectus (n = 1), C. concisus (n = 1), C. spororum (n = 2), C. curvus (n = 1), and C. showae (n = 1).

c PCR-LiPA analysis of purified single colonies from the primary culture identified both C. jejuni and C. coli, indicating the presence of both species in the original isolate.

d Subsequently identified as C. jejuni by the method of Jackson et al. (16).

e Subsequently identified as C. coli by the method of Jackson et al. (16).

f Subsequently identified as C. lari by the method of Jackson et al. (16).

g Not unequivocally identified by the method of Jackson et al. (16); possibly contains a mixture of strains.

h Identified as C. coli by the method of Jackson et al. (16).

i Includes C. hyointestinalis (n = 1), C. fetus (n = 6), C. spororum (n = 1), Helicobacter sp. (n = 5), and A. butzleri (n = 2).

Evaluation of the PCR-LiPA with Swiss isolates. The fifth panel consisted of 90 strains from Switzerland. PCR-LiPA results were in complete agreement with conventional identification results, except for one C. fetus isolate, which was identified as C. coli by PCR-LiPA. This C. fetus strain also was analyzed by the method of Jackson et al. (16) and yielded an RFLP pattern compatible with C. coli. The PCR primers of Jackson et al. had a very low yield, and since these PCR primers were not intended to amplify C. fetus DNA, this finding may indicate that C. coli DNA was present at a low concentration. Since the other five C. fetus strains in this panel were completely negative (as were the four reference C. fetus strains of panel 1), it is most likely that this C. fetus strain was not completely pure and also contained traces of C. coli.

Taken together, of the 167 strains identified as C. jejuni by conventional methods, 165 (99%) also were identified as C. jejuni by PCR-LiPA. The two discrepant strains also were identified as C. jejuni by the method of Jackson et al. (16). Similarly, 80 (93%) of the 86 strains originally identified as C. coli were correctly identified by PCR-LiPA. Three isolates contained a mixture of C. jejuni and C. coli. Two strains were identified as C. jejuni and one was identified as C. lari by PCR-LiPA, and these findings were confirmed by the method of Jackson et al. All (100%) of the 17 C. lari strains were correctly identified. Of the 19 C. upsaliensis strains, 18 (95%) were identified accordingly by PCR-LiPA, whereas 1 isolate was identified as C. jejuni, which was not unequivocally identified by the method of Jackson et al. and probably contained a mixture of strains. Of the 31 isolates that were not identified as either C. jejuni, C. coli, C. lari, or C. upsaliensis by conventional methods but represented clinically closely related species, 30 remained negative by PCR-LiPA, whereas 1 was identified as C. coli and possibly contained a mixture of strains.
DISCUSSION

Conventional methods for identification of Campylobacter spp. are based on specific enzymatic activities, antibiotic susceptibilities, and growth performance (17). However, these phenotyping methods often provide inadequate or incorrect discrimination and can be difficult to perform and interpret. Recently, alternative methods based on genetic differences have been developed. Discrimination between various Campylobacter species has been demonstrated by species-specific PCR and/or RFLP analysis (7, 10, 16, 19, 21). However, these methods require multiple PCRs per sample, and digestion with multiple restriction enzymes can yield electrophoresis patterns that are sometimes difficult to interpret and standardize.

This report describes the development of a simple and rapid method to discriminate among four important thermotolerant Campylobacter species, i.e., C. jejuni, C. coli, C. lari, and C. upsaliensis. The method is based on the amplification of a fragment of a gene that encodes a putative GTPase. The nucleotide sequences of GTPases are highly diverse, except for the GTP-binding motifs, which are conserved at the amino acid level in all GTPases (5). The strategy of our identification method is the use of PCR primers deduced from these relatively conserved sites that allow amplification of a 153-bp fragment from the four thermotolerant Campylobacter species. Limited degeneracy of primers resulted in amplification of a single fragment from these Campylobacter species with apparently equal efficiency. Moreover, the location and spacing of these GTP-binding sites within GTPase genes are highly conserved, yielding PCR products that are uniform in size. That homologs of the GTPase sequences were found in a number of other bacteria suggests that similar genes may be more widely conserved than previously appreciated. The function of such GTPase-encoding genes, comprising two complete GTPase domains, remains unknown.

Sequences encoding the 16S rRNA are virtually identical in C. jejuni, C. coli, and C. lari and therefore are not adequate for species identification (12). Phylogenetic analysis of the 117-bp intervening sequence between the GTP-binding sites revealed limited but consistent interspecies sequence variability among C. jejuni, C. coli, and C. upsaliensis. C. lari appears to be the most heterogeneous group, which is consistent with earlier reports of phenotypic and genotypic heterogeneity among strains of this species (9, 18).

Based on the consistent interspecies nucleotide sequence heterogeneity, highly specific probes were designed for each of the four species studied. The reverse hybridization principle allows hybridization analysis of amplified DNA fragments with multiple probes in a single step (25). An important requirement of such an assay is the design and selection of probes with highly similar hybridization characteristics. Stringent hybridization resulted in highly specific signals with no detectable cross-reactivity. Two clinical isolates of C. coli from panel 2 showed weaker reactivity to one of the species-specific probes, indicating limited sequence variation, which was confirmed by sequence analysis (Fig. 1). These findings illustrate the usefulness of using two different probes for each species on this LiPA strip.

The performance of the test was evaluated with a panel of reference strains that were identified to the species level by various methods. Importantly, the PCR-LiPA method does not require extensive DNA purification or the transport of viable strains. Treatment with proteinase K yielded stable bacterial lysates that could be shipped at ambient temperature and were of sufficient quality for performance of the assay. Also, the PCR-LiPA method only requires a single PCR, followed by a single-step reverse hybridization. The entire procedure, starting with cultured bacteria, can be easily completed within a day.

All of the isolates of C. jejuni, C. coli, C. lari, or C. upsaliensis tested could be identified by the LiPA. For only 9 (2.8%) of 320 isolates tested did the LiPA yield results discordant with those of conventional methods. Three of these samples contained C. jejuni as well as C. coli. In contrast to conventional identification methods, the LiPA allows sensitive detection of cultures, and thus infections, with multiple species. Purification of single colonies from several primary cultures resulted in unequivocal detection of only a single Campylobacter species, indicating that initial detection of two species was not due to cross-reactivity of the probes on the strip. In five of the remaining six discrepant cases, the PCR-LiPA results were confirmed by an alternative molecular method, and in one case, the isolate probably contained a mixture of strains. Our findings strongly confirm previous observations (3, 9) that conventional biochemical identification methods, such as hippurate hydrolysis and resistance to cephalothin and nalidixic acid, are not always accurate for identification of Campylobacter species. Taken together, this study indicates that the specificity of the PCR-LiPA method is virtually 100%.

In conclusion, the present study describes a novel tool that allows simple, rapid, and reliable identification of thermotolerant Campylobacter species. Further experiments will focus on the direct application of the PCR system to clinical samples, such as human feces. This would allow rapid detection of infection with thermotolerant Campylobacter species followed by species identification by reverse hybridization.

ACKNOWLEDGMENTS

P.V. is indebted to the Fund for Scientific Research-Flanders (Belgium) for a position as post-doctoral research fellow. This study was supported in part by the Medical Research Service of the Department of Veterans Affairs.

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