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# Identification of *Campylobacter jejuni* on the Basis of a Species-Specific Gene That Encodes a Membrane Protein

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**To facilitate discrimination between the closely related enteropathogens *Campylobacter jejuni* and *C. coli*, unique differences in antigenic surface structure were examined. A genomic library of *C. jejuni* 81116 was constructed in plasmid pBluescriptIISK$^-$ and expressed in *Escherichia coli* K-12. Rabbit hyperimmune serum raised against *C. jejuni* ATCC 29428 recognized a clone expressing a *C. jejuni* 24-kDa membrane-associated protein. Antiserum raised against sonicated recombinant *E. coli* expressing the 24-kDa protein reacted with *C. jejuni*, whereas *C. coli* did not react specifically. Determination of the nucleotide sequence of the DNA insert of this recombinant plasmid revealed an open reading frame encoding 214 amino acids; the gene was designated *mapA*; and its gene product was designated MAPA. The 18 N-terminal amino acid residues constitute a signal sequence characteristic of prokaryotic membrane lipoproteins. In a dot blot hybridization assay with a *mapA* probe, 120 clinical isolates of *C. jejuni* were unequivocally discriminated from 126 other campylobacters, including 34 *C. coli* isolates. A PCR test based on the *mapA* sequence was developed for identification of *C. jejuni*. A PCR product was obtained with all of the clinical isolates of *C. jejuni* tested from human, dog, cat, bovine calf, and chicken sources. Recombinant MAPA with an added C-terminal six-histidine tail was affinity purified and used to immunize rabbits. The rabbit anti-MAPA serum specifically recognized the protein in whole cells of *C. jejuni* on Western blots (immunoblots). The MAPA protein was present in all of the *C. jejuni* strains tested and was absent in *C. coli* and related campylobacters.**

Thermophilic *Campylobacter* species, particularly *Campylobacter jejuni* and *C. coli*, are among the most frequently isolated bacteria that cause diarrheal disease in humans (25). The organisms can occur, often as commensals, in cattle, pigs, sheep, poultry, various domestic animals, birds, apes, rodents, and even insects (3, 15, 28). *C. coli* is often isolated from feces of pigs, and both enteropathogens are considered to share many clinical and epidemiological characteristics. Detailed knowledge is dependent on accurate differentiation between *C. jejuni* and *C. coli*. Most *C. jejuni* infections are associated with consumption of poultry, raw milk, or untreated surface water and contact with pets (25).

Isolation of campylobacters from clinical samples requires long incubation times with special growth conditions (9). Discrimination between the closely related species *C. jejuni* and *C. coli* is based only on the hippurate hydrolysis test (2). However, this phenotypic test does not always distinguish between *C. jejuni* and *C. coli*. A minority of isolates do not conform to the pattern exhibited by the majority (11, 18, 26). To complement the hippurate hydrolysis test and improve discrimination between *C. jejuni* and *C. coli*, a number of new genetic or phenotypic identification and discriminatory assays have recently been developed by many investigators.

Genetic identification methods exploit the species specificity of cloned arbitrary DNA sequences (7, 8, 16), specific PCR amplification of defined DNA fragments of 16S or 23S rRNA genes and their intervening sequences (6, 7, 27), or amplification of parts of regions of the flagellin genes (17). Immunological identification tests, on the other hand, have been used for many groups of bacteria, including the enteropathogens *Shigella flexneri*, *Vibrio cholerae*, salmonellae, and *Escherichia coli* (4). The use of species-specific antibodies raised against surface antigens for identification of *C. jejuni* and *C. coli* has also been reported (10). The method requires preparation of partially purified membrane protein, immunization of rabbits, and absorption of the resulting antisera with cross-reacting *Campylobacter* species. A simplification of serological identification of campylobacters based on recombinant species-specific antigens would therefore be very helpful. In the present report, we describe the cloning and characterization of a species-specific 24-kDa membrane-associated protein of *C. jejuni* 81116, named MAPA for membrane-associated protein A. Rabbit antisera raised against the *C. jejuni* MAPA protein did not cross-react with proteins of *C. coli* or other related bacteria. The *mapA* gene has been found to be useful for genetic identification of *C. jejuni* by PCR or hybridization, and the MAPA protein is also a good candidate for serological discrimination between *C. jejuni* and *C. coli.*

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** The bacterial strains and type strains used in this study are listed in Table 1. *Campylobacter*, *Bacteroides*, *Arcobacter*, and *Helicobacter* spp. were cultured on Columbia agar supplemented with 5% sheep blood (bioMérieux, Geneva, Switzerland) in a microaerophilic atmosphere of 6% $O_2$, 7% $CO_2$, and 7% $H_2$ in $N_2$ at 37°C for 20 h. For all gene cloning and expression experiments, *E. coli* Sure (Stratagene, La Jolla, Calif.) or *E. coli* BL21 (DE3) (24; gift from Stan Tabor, Harvard Medical School, Boston, Mass.) was used. Plasmids pBluescriptIISK$^-$ (Stratagene), pT7-7 (24; gift from Stan Tabor), and pQE16 (Qiagen Inc.) were used for cloning of genomic DNA fragments of *C. jejuni*. Genomic DNA of reference strain *C. jejuni* 81116 was partially digested with *Hin*dIII. Selected DNA fragments in the size range of 1 to 7 kb were isolated from agarose gels and ligated into the *Hin*dIII site of pBluescriptIISK$^-$. The ligation mixture was transformed into *E. coli* Sure cells by the calcium chloride procedure (22). For screening of recombinant *E. coli* colonies, growth was transferred to nitrocellulose filters (Schleicher & Schuell, Dassel, Germany) by replica blotting. The filters were then placed on new Luria-Bertani agar plates supplemented with 25 mg of ampicillin per ml, 25 mg of tetracycline per ml, and 166 μM isopropyl-β-D-thiogalactopyranoside (IPTG; 22) and incubated at 37°C. After 4 h, the recombinants were lysed in situ by soaking the filters in lysis buffer (100 mM Tris-HCl [pH 7.8], 150 mM NaCl, 5 mM $MgCl_2$, 1.5%

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TABLE 1. Bacterial strains of the *Campylobacter-Helicobacter-Arcobacter* group used in this study

| Bacterium | Strain no.[a] | Explanation (reference) |
|---|---|---|
| *C. jejuni* | ATCC 29428 | Reference strain |
| *C. jejuni* | NCTC 11351 | Type strain |
| *C. jejuni* | NCTC 11828 | Strain 81116 (20) |
| *C. jejuni* | CCUG 12085 | Reference strain for serogroup Lior 5 |
| *C. jejuni* | CCUG 15114 | Reference strain for serogroup Lior 7 |
| *C. jejuni* | CCUG 12082 | Reference strain for serogroup Lior 11 |
| *C. jejuni* | CCUG 12072 | Reference strain for serogroup Lior 17 |
| *C. coli* | LMG 6440 | Type strain |
| *C. coli* | CCUG 12073 | Reference strain for serogroup Lior 12 |
| *C. coli* | CCUG 12079 | Reference strain for serogroup Lior 20 |
| *C. coli* | CCUG 12080 | Reference strain for serogroup Lior 21 |
| *C. coli* | CCUG 15029 | Reference strain for serogroup Lior 29 |
| *C. lari* | NCTC 11352 | Type strain |
| *C. upsaliensis* | LMG 8850 | Reference strain |
| *C. helveticus* | NCTC 12470 | Type strain |
| *C. fetus* subsp. *fetus* | ATCC 25936 | Type strain |
| *C. hyointestinalis* | ATCC 35217 | Type strain |
| *C. mucosalis* | ATCC 43264 | Type strain |
| *C. sputorum* biovar *sputorum* | ATCC 35980 | Type strain |
| *C. rectus* | ATCC 33238 | Type strain |
| "*Bacteroides*" *ureolyticus* | ATCC 33387 | Type strain |
| *A. butzleri* | ATCC 49616 | Type strain |
| *H. pylori* | ATCC 43504 | Type strain |
| *H. fennelliae* | NCTC 11612 | Type strain |
| *H. cinaedi* | CCUG 18818 | Type strain |
| *H. canis* | NCTC 12739 | Type strain |
| *H. pullorum* | NCTC 12824 | Type strain |

[a] ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, United Kingdom; CCUG, Culture Collection of the University of Göteborg, Göteborg, Sweden; LMG, Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium.

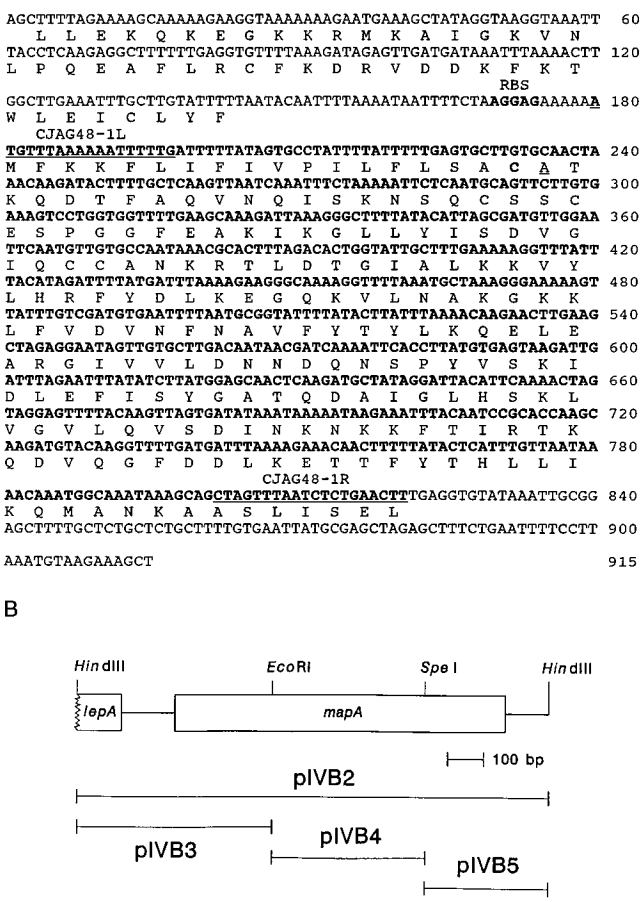


FIG. 1. Nucleotide sequence and restriction enzyme map of the *C. jejuni* 81116 *Hin*dIII insert in pIVB2. (A) Complete nucleotide sequence. The *mapA* gene begins at position 179, and its nucleotide sequence is highlighted. The putative ribosome binding site (RBS) is indicated. The sequences corresponding to the oligonucleotide primer binding sites for CJAG48-1L and CJAG48-1R are underlined. The one-letter amino acid symbols are shown below the nucleotide sequence, and the putative signal sequence cleavage site is in boldface. The alanine residue directing the mature protein to the inner membrane (30) is underlined. The end of a gene showing homology to *lepA* is located upstream of the *mapA* gene. (B) Schematic representation of the insert of pIVB2 together with the restriction enzyme sites used for subcloning. The three subclones were designated pIVB3, pIVB4, and pIVB5. The boxes correspond to the coding regions of *lepA* and *mapA*.

bovine serum albumin, 50 μg of lysozyme per ml) for 4 h and probed with hyperimmune serum raised against live *C. jejuni* ATCC 29428 as previously described (1).

**Nucleic acid methods.** Nucleic acids were extracted by a slightly modified guanidium thiocyanate microprocedure (19). Instead of ammonium acetate, 0.1 volume of a 3 M NaCl solution was added to promote precipitation of DNA. Nucleic acid concentrations were estimated by using agarose gels with lambda DNA as the reference. A dot blot DNA hybridization assay with lysates of campylobacters was performed as described previously (5). To isolate the gene probe for the dot blot and Southern hybridizations, recombinant plasmid pIVB2 was digested with restriction enzymes *Eco*RI and *Hin*dIII in accordance with the instructions of the manufacturer (Boehringer, Mannheim, Germany). The DNA was separated on a 0.7% agarose gel, and the gene probe was eluted by a standard protocol (29) and labeled with [$^{32}$P]dCTP (300 Ci/mmol; Amersham plc) by using a random primed DNA labeling kit (Boehringer). Hybridizations were carried out for 16 h at 37°C in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M trisodium citrate [pH 7.0])–50% formamide–5% polyethylene glycol 6000–0.5% sodium dodecyl sulfate (SDS)–100 μg of denatured and sonicated salmon sperm DNA per ml. Filters were washed in 0.1× SSC–0.1% SDS at room temperature for 30 min. Autoradiography was carried out for 2 days on Fuji RX films with an intensifying screen.

**DNA sequencing and analysis.** With *Eco*RI and *Spe*I, the insert of pIVB2 was cut into three fragments similar in size. The fragments were subcloned into pBluescriptIISK$^-$, resulting in pIVB3, pIVB4, and pIVB5, as shown in Fig. 1B, and used for DNA sequence determination (Fig. 1B). Sequence analysis was accomplished by the dideoxy-chain termination method of Sanger et al. (23) with [$^{35}$S]dATP (Amersham International plc, Amersham, Buckinghamshire, United Kingdom) and T7 DNA polymerase with the Sequenase sequencing kit (United States Biochemicals, Cleveland, Ohio). Standard oligonucleotide primers T3 and T7 (Stratagene) were used for sequencing. The DNA sequences were assembled and analyzed by using the PC-Gene DNA and protein analysis programs (IntelliGenetics Inc., University of Geneva, Geneva, Switzerland).

**PCR.** PCR analysis was performed with a thermal cycler from Perkin Elmer-Cetus (Norwalk, Conn.). A 0.01-μg sample of genomic bacterial DNA was added to 100 μl of a PCR mixture (10 mM Tris-HCl [pH 8.3], 1.5 mM magnesium chloride, 50 mM potassium chloride, 0.005% Tween 20, 0.005% Nonidet P-40), with each deoxynucleoside triphosphate at 170 μM and each oligodeoxyribonucleotide primer at 0.25 μM. Finally, 2.5 U of *Taq* DNA polymerase (Boehringer) was added. The mixture was covered with 2 drops of mineral oil, and samples were subjected to 35 cycles of amplification consisting of denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and chain extension at 74°C for 1 min. An additional extension step at 74°C for 5 min was performed at the end of the reaction. The binding sites of oligodeoxyribonucleotides (Microsynth) CJAG48-1L (5′-cgcggatccATGTTTAAAAAATTTTTG-3′) and CJAG48-1R (5′-cgcggatccAAGTTCAGAGATTAAACTAG-3′) are given in Fig. 1A. They include extensions to generate *Bam*HI restriction enzyme sites (shown in lowercase type). A 20-μl volume of each PCR was loaded onto a 0.7% agarose gel and analyzed after electrophoresis and staining with ethidium bromide.

**Production of antisera.** Hyperimmune serum against whole *C. jejuni* ATCC

29428 cells was produced with bacteria grown on sheep blood agar at 37°C for 36 h. The cells were harvested in 0.9% NaCl to the density of a McFarland no. 1 turbidity standard, and rabbits were immunized five times intravenously at intervals of 2 days. The first immunization was carried out with formalinized cells; for booster injections, live *C. jejuni* cells were used. Titers of antisera were determined by slide agglutination of live cells, and the rabbits were bled by heart puncture. To produce rabbit anti-MAPA serum, recombinant MAPA modified with a six-His tail (MAPA-6×His) was purified by $Ni^{2+}$ chelate affinity chromatography. To obtain recombinant MAPA-6XHis, the *mapA* gene amplified by PCR was digested with *Bam*HI and the fragment was ligated into the cloning site of pQE16 cut with *Bam*HI and *Bgl*II. By this step, a tail of six consecutive triplets encoding histidine was positioned in frame immediately downstream of the *Bgl*II restriction enzyme site. The gene including the six His codons was then recovered from pQE16 by digestion with *Bam*HI and *Hin*dIII. This fragment was ligated into expression vector pT7-7 to achieve a high level of expression in *E. coli* BL21 (DE3). The MAPA-6×His protein accumulated in the insoluble fraction of cell proteins and was purified by $Ni^{2+}$ chelate affinity chromatography (Qiagen Inc.) in the presence of 6 M guanidine hydrochloride. The MAPA-6×His fractions were pooled and repurified by preparative SDS-polyacrylamide gel electrophoresis (13), and the recombinant protein was electroeluted from the gel. Rabbits were immunized subcutaneously with 150 μg of the purified MAPA protein mixed 1:1 with complete Freund adjuvant (Difco Laboratories, Detroit, Mich.). A booster dose containing an equal quantity of MAPA-6×His in complete Freund adjuvant was injected after 4 weeks. The serum was collected 10 days after the booster injection. Mouse sera were produced against recombinant bacteria grown in Luria-Bertani broth and induced by addition of 0.5 mM IPTG. Approximately 50 mg of pelleted cells were suspended in phosphate-buffered saline, sonicated, and mixed 1:1 with complete Freund adjuvant. Mice were immunized subcutaneously with 0.2 ml of the suspension.

**Nucleotide sequence accession number.** The nucleotide sequence reported here has been submitted to GenBank and assigned accession number X80135.

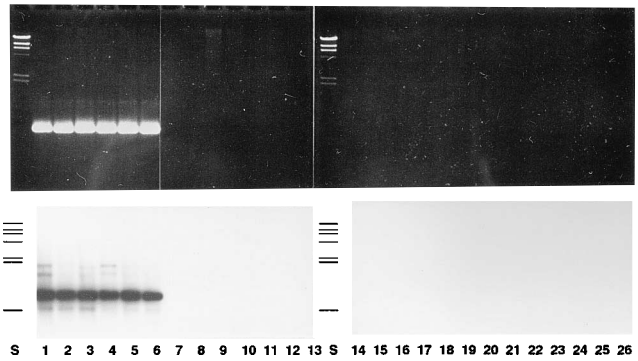


FIG. 2. Species specificity of the *mapA* gene by Southern blot hybridization and PCR amplification. (Top) PCR products obtained with *mapA* oligonucleotide primers by using genomic DNA as the template. (Bottom) Southern blot using the *Eco*RI-*Hin*dIII fragment of pIVB2 as the probe. The lanes represent the following strains: 1, *C. jejuni* ATCC 29428; 2, *C. jejuni* 81116; 3, *C. jejuni* CCUG 12085; 4, *C. jejuni* CCUG 14114; 5, *C. jejuni* CCUG 12082; 6, *C. jejuni* CCUG 12072; 7, *C. coli* LMG 6440; 8, *C. coli* CCUG 12073; 9, *C. coli* CCUG 12079; 10, *C. coli* CCUG 12080; 11, *C. coli* CCUG 15029; 12, *C. lari* NCTC 11352; 13, *C. upsaliensis* LMG 8850; 14, *C. helveticus* NCTC 12740; 15, *C. fetus* subsp. *fetus* ATCC 25936; 16, *C. hyointestinalis* ATCC 35217; 17, *C. mucosalis* ATCC 43264; 18, *C. sputorum* biotype *faecalis* ATCC 35980; 19, *C. curvus* ATCC 35224; 20, *Bacteroides ureolyticus* ATCC 33387; 21, *Arcobacter butzleri* ATCC 49616; 22, *Helicobacter pylori* ATCC 43504; 23, *H. fennelliae* NCTC 11612; 24, *E. coli* XL1 blue; 25, *Haemophilus influenzae* type b; 26, negative (no-DNA) control. Lanes S contained molecular weight standards (lambda DNA digested with *Hin*dIII).

## RESULTS

Five recombinant *E. coli* clones, carrying *Hin*dIII inserts of genomic DNA of *C. jejuni* 81116 in pBluescriptIISK$^-$, expressed proteins recognized by hyperimmune serum against *C. jejuni* ATCC 29428. All five clones were cultured in liquid medium, and their proteins were analyzed by SDS-polyacrylamide gel electrophoresis and immunoblotting with the same serum. Two of the recombinant *E. coli* clones expressed a 15-kDa protein, one clone expressed a 24-kDa protein, and the two remaining clones each expressed a 30-kDa protein recognized by the rabbit anti-*C. jejuni* serum. For further investigations, mouse antisera were produced against each of the recombinants. Antisera raised against all of the 15- or 30-kDa protein-expressing clones recognized proteins similar in size in all of the thermophilic campylobacters tested. The mouse serum raised against the recombinant clone expressing the 24-kDa antigen recognized a 22-kDa protein together with a somewhat weaker band at 20 kDa in *C. jejuni*, and no reaction was observed with other *Campylobacter* species. We therefore focused our interest on this recombinant *E. coli* clone carrying a plasmid, designated pIVB2, with a 915-bp *Hin*dIII insert of *C. jejuni* 81116 genomic DNA.

The nucleotide sequence of the entire *Hin*dIII fragment cloned in pIVB2 is shown in Fig. 1A. The insert is 915 bp long and contains two open reading frames (ORF), which are shown as boxes in Fig. 1B. One ORF begins at position 179 and is 642 bp long. It is preceded by a putative ribosome binding site and can encode a 214-amino-acid protein with a molecular mass of 24.0 kDa. Analysis of the deduced amino acid sequence revealed that the N terminus contains a signal sequence with a putative signal peptidase II cleavage site cysteine residue (20) and a sorting signal alanine residue (30). The gene product may be posttranslationally processed to a 197-amino-acid peptide with a molecular mass of 22.0 kDa. We designated the gene *mapA* (for membrane-associated protein A) and the corresponding protein, MAPA. Computation of the amino acid sequence of protein MAPA performed at the National Center for Biotechnology Information with the BLAST program revealed no homology to any known proteins. Upstream of *mapA*, the end of a possible ORF containing 80 nucleotides of the coding region of a gene was found. This partial ORF showed homology to the end of a *lepA* gene of *E. coli* over its entire length. The amino acid sequence homology with *lepA* genes of *E. coli* and *Salmonella typhimurium* was 84%. No other significant ORF was found in either direction.

Southern blots of the strains listed in Table 1, including all of the type strains of the thermophilic *Campylobacter* group, as well as type strains and reference strains of closely related species and genera, were hybridized with subclones pIVB3, pIVB4, and pIVB5. All three probes hybridized strongly with the same conserved 915-bp *Hin*dIII fragment in all of the strains of *C. jejuni* tested (Fig. 2). A weak hybridization signal was observed in *C. coli* with pIVB3, indicating that genes homologous to *lepA* are probably also present in *C. coli*. To further corroborate the specificity of *mapA* for *C. jejuni*, a *mapA* probe was used in a colony blot hybridization assay. The probe consisted of the labeled *Eco*RI-*Hin*dIII fragment of pIVB2, which includes most of the coding part of *mapA*, as well as 91 bp of the downstream flanking sequence (Fig. 1B). Except for *C. jejuni*, no *Campylobacter* strains or related organisms reacted with the probe (results not shown). To further examine the sensitivity and specificity of the *mapA* probe, 246 fresh clinical isolates were analyzed and the results were compared with identification obtained by total genomic DNA hybridization. All 120 isolates of *C. jejuni* tested showed positive hybridization with the *mapA* probe, whereas none of 126 other *Campylobacter*, *Arcobacter*, and *Helicobacter* species tested (34 *C. coli*, 63 *C. upsaliensis*, 8 *C. helveticus*, 3 *C. concisus*, 3 *Arcobacter butzleri*, 4 *Helicobacter canis*, 7 *H. cinaedi*-like, and 4 *H. pullorum* strains) gave a positive result (data not shown). Thus, both the sensitivity and specificity of the assay were 100%. A *mapA*-specific PCR was developed for identification of *C. jejuni* by using oligonucleotides CJAG48-1L and CJAG48-1R, located at both ends of *mapA*. Genomic DNAs of the type and reference strains listed in Table 1 were subjected to PCR. The result of the *C. jejuni*-specific amplification of the *mapA* gene

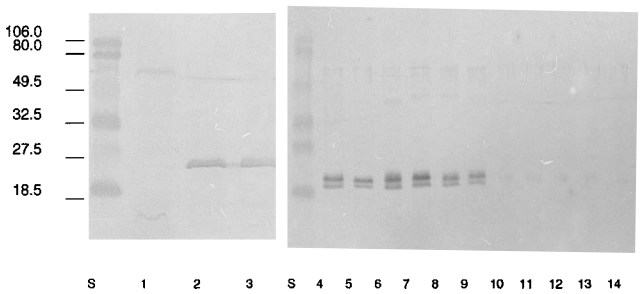


FIG. 3. Immunoblots of *E. coli* clones expressing recombinant MAPA and representative strains of *C. jejuni* and *C. coli* with rabbit anti-MAPA serum. The lanes contained the following samples: 1, *E. coli* carrying the pBluescript plasmid with no insert; 2 and 3, two individual clones of *E. coli* expressing recombinant MAPA; 4, *C. jejuni* ATCC 29428; 5, *C. jejuni* 81116; 6, *C. jejuni* CCUG 12085; 7, *C. jejuni* CCUG 14114; 8, *C. jejuni* CCUG 12082; 9, *C. jejuni* CCUG 12072; 10, *C. coli* LMG 6440; 11, *C. coli* CCUG 12073; 12, *C. coli* CCUG 12079; 13, *C. coli* CCUG 12080; 14, *C. coli* CCUG 15029. Recombinant *E. coli* expressed the unprocessed 24-kDa protein, whereas the processed 22-kDa mature MAPA product and a smaller protein were recognized in *C. jejuni*, and a weak cross-reaction was observed in *C. coli*. The sizes of molecular mass marker proteins are given in kilodaltons on the left.

is shown in Fig. 2. To test the sensitivity of this detection assay, 10-fold dilutions containing 50 ng to 5 fg of genomic DNA of *C. jejuni* 81116 were used as templates for PCR. The 5-fg amount gave an amplification reaction with a product sufficient for visualization on agarose gels stained with ethidium bromide. Template DNA for PCR was also obtained by boiling a cell suspension. Tenfold dilutions of suspensions of *C. jejuni* 81116, containing $2.6 \times 10^7$ to 26 cells were made in water. The suspensions were boiled for 5 min prior to use of the supernatant as a PCR template. Amplification products were reliably obtained from 26 *C. jejuni* cells. In addition, we were able to amplify *mapA* in 41 strains of *C. jejuni* isolated from sources as different as humans, dogs, cats, bovine calves, and chickens. All amplification products showed identical restriction patterns with restriction enzymes *Eco*RI and *Alu*I (data not shown).

Finally, serological identification of *C. jejuni* was attempted with monospecific, polyclonal anti-MAPA hyperimmune serum. For this purpose, we genetically modified the *mapA* gene to obtain a MAPA-6×His fusion protein (see Materials and Methods) which was purified by $Ni^{2+}$ chelate affinity chromatography. Sera of rabbits immunized with the recombinant MAPA-6×His protein recognized the recombinant, nonmodified MAPA protein in *E. coli* at a dilution of 1:8,000 as a 24-kDa protein band (Fig. 3). *C. jejuni* cells were weakly labeled by anti-MAPA antibodies coupled to fluorescein isothiocyanate. However, the anti-MAPA sera strongly reacted in immunoblots with a 22-kDa protein of *C. jejuni*. A weaker band reacting at 20 kDa was also observed in *C. jejuni*, whereas some strains of *C. coli* showed a very weak reaction (Fig. 3). The remaining bacterial species included in Table 1 showed no reaction (results not shown).

## DISCUSSION

Discrimination between the closely related human enteropathogens *C. jejuni* and *C. coli* (26) is based solely on one phenotypic characteristic, hippurate hydrolysis (2, 18). This test is not entirely reliable because hippurate-negative *C. jejuni* can be encountered, which leads to misidentifications (11, 18). Because the precise differentiation between *C. jejuni* and *C. coli* is of epidemiological significance, additional methods are needed. Therefore, several investigators have developed alternative means of identification and many of the methods are based on genetic approaches (6–8, 12, 17, 27). However, most of the procedures involved are complex and require specialized reagents and equipment. Therefore, genetic methods are confined to specialized research establishments; for routine use in clinical laboratories, they would have to be more rapid and involve fewer steps (12).

On the other hand, serological means of bacterial identification have been traditionally used for many groups of bacteria (4) and have been suggested to differentiate between *C. jejuni* and *C. coli* (10). We therefore cloned and expressed in *E. coli* an antigen which is specific for *C. jejuni* from a gene bank made with genomic DNA of strain 81116.

Among several clones expressing *C. jejuni* antigens, one, mediating the expression of a 24-kDa protein designated MAPA, was chosen since mouse sera raised to MAPA specifically reacted with *C. jejuni*. The deduced protein sequence of MAPA showed a consensus with prokaryote signal sequence peptidase II recognition sequences for the N-terminal 18 amino acids. The highly conserved amino acid cysteine at position 18 (Fig. 1A) is known to be the cleavage site for the peptidase (20). This suggests that MAPA is posttranslationally modified by signal cleavage into a 22-kDa peptide. The signal sequence also indicates that MAPA probably belongs to the group of lipoproteins which contains fatty acid residues linked to the glyceryl cysteine which provides the site for cleavage. The amino acid adjacent to the signal peptidase cleavage site is known to be the sorting signal for the mature protein, i.e., that which determines its localization to the inner or outer membrane. This amino acid is alanine for MAPA, suggesting that MAPA is an inner membrane protein (30). Indirect experimental evidence of the localization of the mature MAPA protein in the inner membrane of *C. jejuni* was as follows: (i) MAPA was absent in outer membrane preparations of *C. jejuni*, (ii) *C. jejuni* was not agglutinated by anti-MAPA sera, and (iii) the surface of *C. jejuni* was not labeled by rabbit anti-MAPA antibodies conjugated to fluorescein isothiocyanate. On immunoblots, anti-MAPA antibodies reacted with a 24-kDa protein in the recombinant *E. coli* strain containing pIVB2 and with 22- and 20-kDa proteins in all of the *C. jejuni* strains tested (Fig. 3). This indicates that recombinant MAPA expressed in *E. coli* remains unprocessed, while the 22-kDa protein in *C. jejuni* would represent the processed MAPA protein after cleavage of the signal sequence. Available evidence does not give clues as to the nature of the weakly reacting band at 20 kDa, which may indicate further processing or degradation. A protein band slightly smaller in size than 22 kDa giving very weak serological cross-reactions in some strains of *C. coli* may indicate the presence of a distantly related protein in this species.

The specificity of the *mapA* gene for *C. jejuni* was shown by colony blot hybridization. All 120 isolates of *C. jejuni* tested hybridized with a *mapA* probe, whereas none of 126 other *Campylobacter*, *Arcobacter*, and *Helicobacter* isolates, including 34 *C. coli* isolates, did. In addition, the *mapA* gene was amplified by PCR in 41 clinical *C. jejuni* isolates originating from sources as different as humans, dogs, cats, bovine calves, and chickens. The amplification product showed no restriction fragment polymorphisms when digested with multiple restriction enzymes, further indicating that the gene is well conserved among isolates of *C. jejuni*. Thus, a simple PCR amplification allows specific identification of *C. jejuni* on the basis of *mapA* sequences.

Our sequence data on *C. jejuni* show the end of an ORF upstream of the *mapA* gene that shares significant homology with the 3′ end of the *E. coli lepA* gene. In *E. coli*, *lepA* encodes a membrane-associated GTP binding protein (14, 21). Se-

quences hybridizing with pIVB3, which carries the *lepA*-homologous sequences of *C. jejuni*, were also found among other campylobacters, indicating that this function is widespread among phylogenetically distant gram-negative bacteria. Whether the *lepA* homolog and *mapA* form part of an operon in *C. jejuni* cannot be ascertained.

The high degree of conservation of the *mapA* gene in *C. jejuni* and its apparent specificity for the species make MAPA a good candidate for serological identification of *C. jejuni*. Hyperimmune rabbit sera raised against purified recombinant MAPA-6×His were shown to be able to differentiate *C. jejuni* from a broad variety of other campylobacters and related organisms, including *C. coli*. The use of a defined antigen, which has been cloned and expressed, offers many advantages over sera raised against whole campylobacter cells for the immunodiagnosis of *C. jejuni*. The main advantage over existing methods is the specificity and reproducibility of reagents, as well as the fact that lengthy and cumbersome absorptions are not needed (10). In summary, we have shown that specific identification of *C. jejuni* on the basis of *mapA* can be achieved both by PCR and serologically.

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