A Specific DNA Probe for the Identification of *Campylobacter jejuni*

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A 6.1 kb DNA probe for the human enteric pathogen *Campylobacter jejuni* has been isolated from a genomic library constructed in the plasmid vector pBR322 in *Escherichia coli*. The DNA sequence used as a probe was identified from recombinant plasmids following immunological screening of transformants using polyclonal antisera to whole cells and to membrane antigens of *C. jejuni*. Restriction endonuclease fragment mapping of *C. jejuni* DNA inserts from three of the recombinant plasmids showed an overlapping DNA fragment. One of these recombinant plasmids, when used as a DNA probe in Southern hybridization, specifically hybridized with chromosomal DNA from all of the *C. jejuni* strains tested. Hybridization was not detected at high stringency between the DNA probe and chromosomal DNA from any other *Campylobacter* species tested except weakly with the chromosomal DNA of strains of *Campylobacter coli*. Hybridization was also not detected with chromosomal DNA from a range of other enteric bacteria likely to be encountered in faecal material. The intensity of hybridization with *C. coli* could be increased by reducing the stringency of hybridization.

INTRODUCTION

Acute gastroenteritis due to *Campylobacter jejuni* infections has become a major cause of human diarrhoeal disease worldwide (Blaser & Reller, 1981; Skirrow, 1982). The isolation and cultivation of *C. jejuni* from faeces require specialized media containing antibiotics for selection and supplements for enrichment, strict microaerophily and specialized media for their transport to the laboratory (Skirrow *et al.*, 1982). The identification of *C. jejuni* and its characterization are based on a number of different criteria including biochemical tests, serological analysis, restriction endonuclease DNA fingerprinting, membrane protein profiling and DNA-DNA hybridization (Skirrow *et al.*, 1982; Hébert *et al.*, 1982; Blaser *et al.*, 1983; Penner *et al.*, 1983; Bradbury *et al.*, 1984; Owen & Dawson, 1986). Thus its cultivation and identification are time-consuming.

In the context of instituting immediate chemotherapeutic measures in acute cases of infection it would be highly desirable to have a technique which can rapidly and reliably identify *C. jejuni* directly from clinical material (faeces) without recourse to cultivation and subsequent identification by one or more of the conventional methods. Potentially, this may be provided by the use of specific DNA probes for the rapid and reliable identification of *C. jejuni*. DNA probes are now being developed for the diagnosis and epidemiology of a range of bacterial infections including those caused by *Legionella pneumophila* and *Salmonella typhi* (Grimont *et al.*, 1985; Rubin *et al.*, 1985).

A strategy for *Campylobacter* probes has been reported (Rashtchian *et al.*, 1986). However, of the two synthetic oligonucleotide probes based on *C. jejuni* 16S rRNA sequences, one was unable

Abbreviations: Ap, ampicillin; Tc, tetracycline.

to distinguish between *C. jejuni*, *C. coli* and *C. lariidis* whereas the second hybridized to all *Campylobacter* species tested. Nick-translated random chromosomal DNA fragments from *Campylobacter* species have also been used in their epidemiology (Steele *et al.*, 1985). This paper reports the construction of a specific DNA probe for *C. jejuni* by the cloning of a *C. jejuni* DNA sequence into the plasmid vector pBR322 as the first step in the development of rapid and reliable probes for *C. jejuni*. The feasibility of constructing such probes has been reported in a preliminary communication (Claus *et al.*, 1982).

METHODS

Bacterial strains and plasmids. These are shown in Table 1.

Cultural conditions. *Campylobacter* species were grown on Oxoid Columbia Agar supplemented with 7% (v/v) defibrinated horse blood, for 48–72 h at 37 °C in a gaseous atmosphere of 90% N₂, 5% CO₂ and 5% O₂. All other bacteria were grown on Oxoid Blood Agar Base supplemented with 0.5% Oxoid yeast extract or in Oxoid Nutrient Broth no. 2 supplemented with 0.5% Oxoid yeast extract for 18 h at 37 °C.

Antibiotics. Ampicillin (Ap; Beecham) and oxytetracycline hydrochloride (Tc; Sigma) were used at 200 µg ml⁻¹ and 60 µg ml⁻¹, respectively.

Antisera. Bacteria for antigen preparations were prepared by harvesting the confluent growth of 72 h *C. jejuni* cultures grown on 20 horse blood agar plates under appropriate gaseous conditions. Cells were washed from the plate with 0.1 M-Tris/HCl buffer (pH 8.3). After washing twice with 0.1 M-Tris/HCl buffer, the bacteria were suspended in a final volume of 25 ml 0.1 M-Tris/HCl buffer. For whole cell antigens, 5 ml of the cell suspension was lightly sonicated and stored at -20 °C. For membrane antigens the membranes were prepared using the lysozyme-EDTA extraction procedure as described by Mills & Bradbury (1984) and stored at -20 °C. They were checked for purity by SDS-PAGE (Laemmli, 1970). Both whole cell and membrane antisera were tested and confirmed to possess antibodies that reacted with membrane antigens in the Western immunoblotting procedure (Towbin *et al.*, 1979).

For production of antisera adult New Zealand White rabbits were immunized with 1 ml whole cell or membrane antigens mixed with an equal volume of Freund's complete adjuvant (Commonwealth Serum Laboratories) and injected into two sites in the hindquarter muscles. Booster doses of the antigens in Freund's incomplete adjuvant were administered in a similar manner on days 14, 28 and 56. Sera were collected on day 70 and stored. Prior to use, antisera diluted 1/50 in 10 mM-Tris/HCl (pH 7.5), were absorbed with viable cells of *E. coli* strain DH1 and grown confluent on three agar plates to remove *E. coli*-specific antibodies. The absorptions were done three times for 24 h at 4 °C.

Chromosomal DNA extraction. Bacteria harvested from agar plates into 10 mM-Tris/HCl (pH 7.5) were used and DNA was extracted by the method described by Collins & Ross (1984).

Plasmid DNA extraction, purification, restriction endonuclease digestion and agarose gel electrophoresis. These were done as described previously (Krishnapillai *et al.*, 1984). Conditions of restriction endonuclease digestion were as recommended by the manufacturer except that with *Campylobacter* DNA, 20–30 units of enzyme were used to digest 1 µg DNA. *Bgl*II was from Promega Biotec; *Eco*RV and *Hind*III were from New England Biolabs; *Cla*I, *Pvu*II, *Sau*3A and *Bam*HI were from Boehringer Mannheim.

Construction of a *C. jejuni* genomic library. This was done as described by Maniatis *et al.* (1982).

Immunological screening of the *C. jejuni* library. Transformants of *E. coli* strain DH1 were screened for recombinant plasmids expressing *C. jejuni* antigens by colony blotting with *C. jejuni* antisera. Transformants were transferred by replica-plating to nitrocellulose filters (0.45 µm; Biorad), lysed *in situ* in 0.2 M-NaOH for 30 min, washed sequentially in 50%, 70% and 95% (v/v) ethanol and dried. Non-reactive sites were blocked with 5% (w/v) skim milk in 10 mM-Tris/HCl buffer (pH 7.5) and 0.05% (w/v) Tween 20 for 30 min at 37 °C. Filters were then incubated with antisera (either to whole cells or to membranes) diluted 1/200 in 10 mM-Tris/HCl buffer (pH 7.5) and 0.05% (w/v) Tween 20 for 60 min at 37 °C. They were washed and incubated with goat anti-rabbit IgG conjugated with horse-radish peroxidase (Biorad) for 60 min at 37 °C. Positive signals were identified using 4-chloro-1-naphthol (Biorad) as a chromogenic substrate.

Preparation of nick-translated probe DNA. DNA was radio-labelled *in vitro* using [α -³²P]dATP (Bresa) as described by Maniatis *et al.* (1982), to a specific activity of 10⁶–10⁷ c.p.m. (µg DNA)⁻¹, except that labelled DNA was collected by ethanol precipitation and unincorporated nucleotides were removed by washing the pellet sequentially with 70% and 100% ethanol.

Southern hybridization. Chromosomal DNA (1–2 µg) was digested with appropriate restriction endonucleases and DNA fragments separated by horizontal agarose gel electrophoresis. The fragments were then transferred bidirectionally to Hybond-N nylon filters (0.45 µm; Amersham) for 2–4 h as described by Smith & Summers (1980). The filters were then used in hybridizations as described by Maniatis *et al.* (1982) with the following modifications. Pre-hybridizations were done for 2–4 h at 65 °C in SSCP solution, pH 7.2 (600 mM-NaCl, 60 mM-trisodium citrate, 50 mM-KH₂PO₄, 4 mM-EDTA) supplemented with 5 × Denhardt's solution (0.1% Ficoll, 0.1%

Table 1. Bacterial strains and plasmids

Bacteria	Strain no.	Country of origin	Source or reference*
<i>Campylobacter jejuni</i>	FF1	Australia	A
<i>Campylobacter jejuni</i>	FF2		A
<i>Campylobacter jejuni</i>	FF3		A
<i>Campylobacter jejuni</i>	FF6		A
<i>Campylobacter jejuni</i>	CSIRO 18		A
<i>Campylobacter jejuni</i>	NCTC 11351		B
<i>Campylobacter jejuni</i>	D114	Japan	C
<i>Campylobacter jejuni</i>	D117	USA	C
<i>Campylobacter jejuni</i>	D123	Canada	C
<i>Campylobacter jejuni</i>	396	Australia	D
<i>Campylobacter jejuni</i> †	80-104	USA	E
<i>Campylobacter coli</i>	NCTC 11366		B
<i>Campylobacter coli</i>	900	Australia	D
<i>Campylobacter laridis</i>	NCTC 11352		B
<i>Campylobacter laridis</i>	011	Australia	D
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	NCTC 10842		B
<i>Campylobacter fetus</i> subsp. <i>fetus</i>	80-256	USA	E
* <i>Campylobacter fetus</i> subsp. <i>venerealis</i>	NCTC 10354		B
* <i>Campylobacter fetus</i> subsp. <i>venerealis</i>	D1133	USA	C
* <i>Campylobacter fecalis</i>	NCTC 11415		B
<i>Campylobacter pyloridis</i>	P2	Australia	A
<i>Salmonella enteritidis</i>	G516		F
<i>Salmonella typhimurium</i>	G505		F
<i>Escherichia coli</i> O111:K58(B4)	G108		F
<i>Shigella sonnei</i>	G604		F
<i>Enterobacter cloacae</i>	G402		F
<i>Citrobacter freundii</i>	G201		F
<i>Klebsiella pneumoniae</i>	G309		F
<i>Proteus vulgaris</i>	G701		F
<i>Vibrio parahaemolyticus</i>	L102		F
<i>Serratia marcescens</i>	G802		F
<i>Pseudomonas aeruginosa</i>	PAO1		G
<i>Escherichia coli</i>	DH1‡		Frey <i>et al.</i> (1983)
Plasmids	Characteristics		Source or reference
pBR322	Ap ^R Tc ^R		Bolivar <i>et al.</i> (1977)
pMO1999	Ap ^R pBR322Ω [<i>C. jejuni</i> DNA, <i>Sau</i> 3A:7.4 kb]		This study
pMO2000	Ap ^R pBR322Ω [<i>C. jejuni</i> DNA, <i>Sau</i> 3A:6.5 kb]		This study
pMO2005	Ap ^R pBR322Ω [<i>C. jejuni</i> DNA, <i>Sau</i> 3A:6.1 kb]		This study

* The source of the strains were as follows: A, Strain collection, Department of Applied Biology, Royal Melbourne Institute of Technology, Melbourne, Victoria 3000, Australia; B, type strains from the National Collection of Type Cultures, London, United Kingdom received from W. Tee, Department of Clinical Pathology, Fairfield Infectious Diseases Hospital, Fairfield, Victoria 3078, Australia; C, C. M. Patton, Hospital Infectious Program, Centres for Disease Control, Atlanta, Georgia 30333, USA; D, T. W. Steele, Institute of Medical and Veterinary Science, Adelaide, South Australia 5000, Australia; E, M. J. Blaser, Infectious Disease Section, Veterans Administration, Denver, Colorado 80220, USA; F, Strain collection, Department of Microbiology, Monash University, Clayton, Victoria 3168, Australia; G, strain collection, Department of Genetics, Monash University, Clayton, Victoria 3168, Australia.

† *C. jejuni* strain 80-104 was received by us as *C. coli* strain 80-104. However, in Southern hybridization with the probe pMO2005 the band specificity was identical to that shown by the other *C. jejuni* strains (data not shown). It was also unable to hydrolyse hippurate like typical *C. jejuni* strains and unlike *C. coli* strains. For these reasons we refer to strain 80-104 as *C. jejuni*.

‡ *recA1 endA1 gyrA96 thi-1 hsdR17 (r⁻m⁺) supE44 λ⁻* [gene symbols according to Bachmann (1983)].

polyvinylpyrrolidone, 0.1% bovine serum albumin fraction V) and 0.1% SDS, 0.002% tRNA and 100 µg denatured salmon sperm DNA ml⁻¹. Hybridizations were done for 24 h at 65 °C using 1–1.5 µg labelled DNA in a fresh volume of the same solution. The filters were washed to remove non-hybridized probe as follows: in 2 × SSC (0.3 M-NaCl, 0.03 M-trisodium citrate for 15 min at 65 °C, twice; in 2 × SSC with 0.1% SDS for 30 min at 65 °C;

and finally in $0.1 \times \text{SSC}$ for 15 min at 65°C to maintain high stringency conditions. The filters were air-dried and autoradiograms were exposed to X-ray film (Fuji) for 6–72 h at -70°C with a regular intensifying screen.

Dot-blot hybridization. Total chromosomal DNA was diluted appropriately, heated for 5 min at 95°C and chilled for 2 min in ice prior to application on to Hybond-N nylon filter using a dot-blot apparatus (Schleicher & Schuell, Minifold™). The filters were then treated for 1 min with 0.5 M-NaOH containing 1.5 M-NaCl , neutralized with 0.5 M Tris/HCl buffer (pH 7.2) containing 1.5 M-NaCl and 0.001 M EDTA and washed in $2 \times \text{SSC}$. The DNA was then bound to the filter using UV. Hybridizations were carried out as described under Southern hybridization (see above).

RESULTS

Construction of a genomic library for C. jejuni and isolation of the DNA probe

In deciding which particular DNA sequence might be cloned with a view to its use as a specific DNA probe for identification, a DNA sequence encoding a membrane antigen seemed especially suitable because of the characteristic membrane protein profiles displayed by membrane preparations of *C. jejuni* on SDS-PAGE, which are distinct from those shown by other *Campylobacter* species (Logan & Trust, 1982; Blaser *et al.*, 1983; Ferguson & Lambe, 1984). As these proteins were also known to be immunogenic (Logan & Trust, 1983), it was assumed that the products of any cloned gene(s) encoding the membrane antigens could be identified immunologically. A genomic library of *C. jejuni* was constructed as follows. Total chromosomal DNA from *C. jejuni* strain FF3 was isolated and partially digested with the restriction enzyme *Sau*3A to generate DNA fragments in the size range of 5–15 kb. These fragments were fractionated according to size in a sucrose gradient. Suitably sized ones were cloned into the single *Bam*HI site in the Tc resistance gene of the vector plasmid pBR322 (Bolivar *et al.*, 1977), which had been treated with alkaline phosphatase to prevent vector self-ligation. The ligation mixture was used to transform *E. coli* strain DH1 and Ap^R transformants were selected. From 2786 Ap^R transformants, 2597 were $\text{Ap}^R \text{ Tc}^S$. These transformants, which were expected to carry recombinant plasmids with inserts of *C. jejuni* chromosomal DNA, were screened immunologically to detect the presence of recombinant plasmids expressing *C. jejuni* antigens. Heterologous polyclonal antiserum raised to whole cells of *C. jejuni* strain CSIRO 18 was used to screen the genomic library made from *C. jejuni* strain FF3 to exclude the selection of transformants bearing recombinant plasmids expressing strain-specific *C. jejuni* antigens. Transformants reacting with whole-cell antiserum were screened again with antiserum raised against membranes of strain CSIRO 18. This served a twofold purpose. Firstly, to aid identification of positively reacting transformants with both antisera and secondly, to ensure that recombinant plasmids chosen were most likely to encode membrane antigens. Eight clones reacting weakly with both whole cell and membrane antisera were identified by colony immunoblotting. Plasmid DNA extracted from them was purified by ultracentrifugation in caesium chloride gradients containing ethidium bromide. Restriction endonuclease cleavage of three of these recombinant plasmids using a panel of enzymes facilitated the construction of restriction maps (Fig. 1). The plasmids carried a common DNA segment. Thus, the polyclonal antibodies facilitated the isolation of recombinant plasmids carrying a DNA segment presumably encoding one of the surface antigens of *C. jejuni*. Of the three recombinant plasmids, pMO2005 was chosen for use as a probe because it carried the smallest chromosomal insert of *C. jejuni* DNA (6.1 kb; Fig. 1).

Probe specificity of cloned C. jejuni chromosomal DNA

Plasmid pMO2005 was nick-translated and used in Southern hybridization against chromosomal DNA digested with *Bgl*/II of a collection of *C. jejuni* strains isolated from a variety of geographical sources, including the strain used for the cloning of *C. jejuni* chromosomal DNA, namely strain FF3, and the reference strain NCTC 11351 (Fig. 2). Very strong hybridization was observed between pMO2005 and all the *C. jejuni* strains tested. As expected hybridization was not observed between pMO2005 and chromosomal DNA from the *E. coli* cloning host DH1. Neither was hybridization observed between pBR322 and any of the *C. jejuni* strains (data not

Fig. 1. Restriction endonuclease cleavage maps of the *C. jejuni* chromosomal DNA fragments inserted in vector plasmid pBR322 to produce the named recombinant plasmids. Only the physical maps of the inserts are shown. The sizes of the inserts are indicated. B, *Bgl*II; C, *Cla*I; E, *Eco*RV; H, *Hind*III.

Fig. 2. Southern hybridization of plasmid pMO2005 with *Bgl*II-digested chromosomal DNA of *C. jejuni*. An autoradiograph is shown of *C. jejuni* chromosomal DNA after digestion with *Bgl*II, electrophoresis in agarose, transfer to Hybond-N nylon filters and hybridization with 32 P-labelled recombinant plasmid pMO2005. Hybridization was under the stringent condition of 65 °C in SSCP (see Methods) and the filters were washed twice in 2 $\times$ SSC (see Methods) and once in 2 $\times$ SSC with 0.1% SDS and finally in 0.1 $\times$ SSC. Lane 1, M_r standard, 32 P-labelled phage λ DNA digested with *Hind*III; lane 2, *E. coli* strain DH1 chromosomal DNA digested with *Bgl*II; lanes 3-12, *C. jejuni* chromosomal DNA digested with *Bgl*II (lane 3, strain FF3; lane 4, strain FF1; lane 5, strain FF2; lane 6, strain FF6; lane 7, reference strain NCTC 11351; lane 8, strain 396; lane 9, strain D114; lane 10, strain D117; lane 11, strain D123; lane 12, strain 80-104). On the left are λ fragment sizes and on the right are the sizes of *C. jejuni* chromosomal DNA fragments which hybridize with the probe.

shown). Chromosomal DNA from all of the *C. jejuni* strains yielded a common 1.2 kb *Bgl*II fragment. In addition all of the *C. jejuni* strains except strains D117 and D123 revealed two additional *Bgl*II fragments of 2.3 kb and 2.5 kb. The exceptional strains yielded, instead, a single additional *Bgl*II fragment of 4.8 kb. We interpret this result to mean that in these strains the contiguous 2.3 kb and 2.5 kb fragments which add up to 4.8 kb (Fig. 1) have the central *Bgl*II site missing and that this *Bgl*II site appears to be polymorphic in the *C. jejuni* strains tested.

Fig. 3. Southern hybridization of plasmid pMO2005 with *Cla*I-digested chromosomal DNA of *Campylobacter* species. An autoradiograph is shown of chromosomal DNA from a range of *Campylobacter* species after digestion with *Cla*I, electrophoresis in agarose, transfer to Hybond-N nylon filters and hybridization with 32 P-labelled recombinant plasmid pMO2005. Hybridization was under the stringent conditions of 65 °C (a) and 55 °C (b). Hybridization under the stringent condition of 65 °C was in SSCP (see Methods) and the filters were washed twice in $2 \times$ SSC (see Methods) and once in $2 \times$ SSC with 0.1% SDS and finally in $0.1 \times$ SSC. The low stringency hybridization at 55 °C was done and washed in the same solutions as for 65 °C except that the final wash was omitted. Lane 1, M_r standard, 32 P-labelled phage λ DNA digested with *Hind*III; lane 2, *C. jejuni* strain FF3; lane 3, *C. jejuni* strain 80-104; lane 4, *C. coli* reference strain NCTC 11366; lane 5, *C. coli* strain 900; lane 6, *C. laridis* reference strain NCTC 11352; lane 7, *C. laridis* strain 011; lane 8, *C. fetus* subsp. *fetus* reference strain NCTC 10842; lane 9, *C. fetus* subsp. *fetus* strain 80-256; lane 10, *C. fetus* subsp. *venerealis* reference strain NCTC 10354; lane 11, *C. fetus* subsp. *venerealis* strain D1133; lane 12, '*C. fecalis*' reference strain NCTC 11415; lane 13, *C. pyloridis* strain P2. On the left are λ fragment sizes and on the right are the sizes of *Campylobacter* chromosomal DNA fragments which hybridize with the probe.

To test the specificity of the probe, plasmid pMO2005 was used as a probe in Southern hybridization against *Cla*I-digested chromosomal DNA of strains belonging to a selection of other species of *Campylobacter*, including *C. coli* and *C. laridis*, which are also known to be pathogenic to man (Skirrow, 1982; Tauxe *et al.*, 1985) (Fig. 3). The restriction endonuclease *Cla*I was used rather than *Bgl*II in order to reduce the number of hybridizing bands in the analysis. It can be seen that, as expected, a strong hybridization signal was observed with an 18.5 kb *Cla*I fragment of chromosomal DNA of *C. jejuni* strain FF3, but relatively weak hybridization was also observed with a 9.0 kb *Cla*I fragment from *C. coli* reference strain NCTC 11366 and strain 900. There was no detectable hybridization between the plasmid probe and chromosomal DNA

from any of the other species of *Campylobacter* even after prolonged exposure (72 h) of the autoradiograph. Thus the cloned *C. jejuni* chromosomal DNA was highly specific for *C. jejuni* but with a much weaker level of cross-hybridization with *C. coli*. In addition to the strains shown in Fig. 2, 14 other clinical isolates of *C. jejuni* from the Fairfield Infectious Diseases Hospital, Victoria, Australia were tested using plasmid pMO2005 as probe. These tests were done by dot-blot hybridization using *in situ* lysed cell cultures. By this test as well, all 14 showed positive signals with the probe (data not shown).

When the specificity of the pMO2005 probe was tested against chromosomal DNA of a selection of other bacteria likely to be encountered from the same clinical environment as *C. jejuni*, there was no detectable hybridization under varying stringency conditions of 45, 55 or the standard 65 °C (data not shown). These bacteria were *Salmonella typhimurium*, *Salmonella enteritidis*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Vibrio parahaemolyticus*, enteropathogenic *E. coli* O111:K58(B4), *Shigella sonnei*, *Serratia marcescens*, *Proteus vulgaris* and *Pseudomonas aeruginosa*.

To test whether strong hybridization between the *C. jejuni* probe pMO2005 and *C. coli* strains could be demonstrated, which might be useful in diagnosis and in epidemiology, the stringency of hybridization was relaxed by doing the hybridizations at 55 instead of 65 °C. Under these conditions hybridization signals in the heterologous combination with *C. coli* reference strain NCTC 11366 and strain 900 were observed (Fig. 3b), which were as strong as those in the homologous combination with *C. jejuni* strain FF3 (Fig. 3a). The pMO2005 probe hybridized to 9.0 kb *Cla*I fragments of *C. coli* chromosomal DNA instead of to 18.5 kb *Cla*I fragments as in the homologous combination. Even under this reduced stringency condition of 55 °C there was no detectable hybridization between the probe and the chromosomal DNA from the other *Campylobacter* species.

DISCUSSION

The important outcome of this investigation was the construction of a highly specific DNA probe for *C. jejuni* by recombinant DNA techniques. The probe reported herein is more specific than those obtained previously (Rashtchian *et al.*, 1986; Steele *et al.*, 1985) and may aid the rapid identification of campylobacteriosis in man. The fact that a much weaker hybridization signal was detectable between the *C. jejuni* probe and chromosomal DNA from *C. coli* strains under the standard high stringency condition of 65 °C was expected in view of the close similarity between these two species by conventional methods of identification (Hébert *et al.*, 1982, 1984). As both *C. jejuni* and *C. coli* are frequently encountered in human campylobacteriosis, the probe might be useful in the identification of *Campylobacter* pathogenic to man especially by reducing the stringency condition to 55 °C (Fig. 3b). However, the probe pMO2005 that has been constructed, in addition to readily distinguishing *C. jejuni* and *C. coli* from other *Campylobacter* species and the other Gram-negative bacteria tested here, can additionally be used to distinguish between *C. jejuni* and *C. coli* by the size of chromosomal fragments that hybridize to the probe (Fig. 3b) and by varying the stringency condition of hybridization (Fig. 3a, b). Thus pMO2005 can be used in epidemiology, particularly where other methods are unable to distinguish between these two species.

Although pMO2005 did not hybridize with genomic DNA of the enteric bacteria tested here, it has to be recognized that the vector plasmid pBR322, being a ColE1-like plasmid, will be expected to hybridize with genomic DNA of some enteric bacteria such as *Salmonella* because of the ubiquity of this type of plasmid (Tompkins *et al.*, 1986). Therefore, in diagnostic and epidemiological studies for the direct and rapid identification of *C. jejuni* from faecal material the *C. jejuni* chromosomal insert in pMO2005 would need to be freed of the vector and used directly as a probe. In addition, although the Southern hybridization procedure using the probe as shown here may be valuable for the molecular epidemiology of strains which are especially difficult to classify by conventional methods, it would not be feasible to use this procedure for routine diagnostic purposes. For the latter it will be necessary to use dot-blot hybridizations on cells lysed *in situ*. Current experiments are in progress towards this goal. Furthermore, for safety and ease of use in a routine diagnostic laboratory radioactively labelled probes would not be practical and probes based on non-radioactive reporter groups need to be developed.

Attempts to determine which of the membrane antigens are encoded by the recombinant plasmid pMO2005 (Fig. 1) by Western immunoblotting (Towbin *et al.*, 1979) and SDS-PAGE (Laemmli, 1970) using protein denaturing and non-denaturing conditions were unsuccessful (data not shown). This was probably due to inefficient expression of *C. jejuni* membrane antigen(s) in *E. coli*. Furthermore, the 6.1 kb insert in pMO2005 is likely to encode information for more than a single gene product and one or more of these are being detected by the antisera used in screening the transformants. Other methods of gene-product identification are currently under investigation such as by sub-cloning into *E. coli* expression vectors and maxicell analysis or by expression in Gram-negative bacterial hosts other than *E. coli*.

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