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Molecular Cloning and Expression of *Campylobacter pylori* Species-Specific Antigens in *Escherichia coli* K-12

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A gene bank of *Campylobacter pylori* DNA in *Escherichia coli* was constructed by cloning *Sau*3A-cleaved DNA fragments into the bacteriophage vector λEMBL3. The expression of *C. pylori* antigens was determined by screening the gene library with adsorbed *C. pylori* whole-cell rabbit antisera. One recombinant clone which reacted positively (λCP2) was studied further. Immunoblot analysis with λCP2 showed a polypeptide band of 66 kilodaltons (kDa) reacting antigenically with the adsorbed antiserum. Extraction of DNA from λCP2 and digestion with *Sal*I revealed a DNA insert of 17 kilobases (kb). Subcloning with *Sal*I and the *E. coli* vector pUC18 showed that the DNA also encoded a 31-kDa antigen. The cloned antigens were shown by immunoblotting to have the same molecular weight in *E. coli* as in *C. pylori* and to be present in all *C. pylori* strains. Antiserum was raised against the cloned polypeptides and found to react only with *C. pylori* when analyzed by dot blotting and indirect immunofluorescence. The cloned antigens were determined to be expressed from the pUC18 *lac* promoter. The DNA encoding these antigens was radiolabeled with ^{32}P and found to hybridize only to *C. pylori* strains. Immunoblotting with affinity-purified polyclonal antibody to the urease enzyme of *C. pylori* revealed that the cloned antigens may be part of the urease enzyme.

In 1983, gram-negative spiral microaerophilic bacteria were cultured from the human gastric mucosa (37) and later named *Campylobacter pylori* (27). Their presence was found to be linked to histologically confirmed gastritis (37). This organism has since been implicated in the pathogenesis of gastritis and duodenal and peptic ulceration in humans by various studies (12, 13, 18, 22, 26, 28, 31). Samples from patients colonized with *C. pylori* have been found to elicit a specific antibody response (18, 19, 25), and noninvasive serological assays have been developed for the diagnosis of *C. pylori* infection (18) and used in numerous epidemiological studies (1, 4, 16, 18, 19). However, enzyme-linked immunosorbent assay (ELISA) systems that have been developed have poor specificity due to antigenic cross-reactivity with other *Campylobacter* species (9, 32).

Relatively little is known about the antigenic properties of *C. pylori* and the host immune response to infection. Although *C. pylori* infection is prevalent, the pathogenic determinants of this organism have not been defined. Molecular cloning and expression of *C. pylori* DNA will provide purified antigens for studies on their role in the immunological and pathological events of infection.

In this study we used recombinant DNA techniques to clone *C. pylori* immunogenic proteins, and this is, to our knowledge, the first report of cloning and expression of *C. pylori* DNA in *Escherichia coli*. These antigens were used to raise specific antisera that can be used in immunoassays to aid in the diagnosis of *C. pylori* infection, and the cloned antigens may be useful for the development of more specific serological assays.

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MATERIALS AND METHODS

Bacterial strains. The *C. pylori* strains mainly used in this study were isolated routinely from human biopsy samples collected from patients attending the gastroenterology clinic at St. Bartholomew's Hospital. The samples were microaerophilically cultured on *Campylobacter*-selective medium containing blood agar base (5% [wt/vol]; Oxoid, Basingstoke, UK), lysed horse blood (8%, vol/vol), vancomycin (10 μg/ml), colomycin (10 U/ml), trimethoprim (5 μg/ml), and amphotericin B (2 μg/ml). They were identified on the basis of morphology on Gram stain and by the oxidase, catalase, and rapid urease tests (22). All of the strains were maintained frozen at −70°C in Oxoid no. 2 broth containing 15% (vol/vol) glycerol. Working stocks of strains were obtained by culturing on Columbia blood agar (Gibco, Uxbridge, UK) at 37°C in a microaerophilic atmosphere for 96 h. *Campylobacter jejuni* strains were isolated routinely from fecal samples on *Campylobacter*-selective medium and identified by morphology on Gram stain and by oxidase and hippurate hydrolysis tests (6). The relevant details of other bacterial strains used in this study are summarized in Table 1. *E. coli* K-12 strains were grown in Luria-Bertani (LB) broth or on LB agar (24).

Antisera preparation. Antibody was prepared against *C. pylori* CPM630 by washing the cultured bacteria in phosphate-buffered saline (PBS; Dulbecco A Oxoid) and immunizing a 3-kg adult New Zealand White rabbit with 10^8 *C. pylori* cells per ml in the form of a Formalin-killed suspension by the injection schedule of Heard et al. (7). Preimmune and immune sera were tested for antibodies to *C. pylori* by dot-blotting ELISA. Antiserum to the cloned antigens was raised in a rabbit by lysing *E. coli* K-12 strain 392 with the recombinant phage λCP2 in liquid culture (24) and repeated injections of the supernatant.

Isolation of chromosomal DNA and construction of *C. pylori*

TABLE 1. Bacterial strains

Strain	Source[a]
Campylobacter pylori	
CPM 630	Clinical isolate; this laboratory
CPM 51	Clinical isolate; this laboratory
CPM 192	Clinical isolate; this laboratory
CPM 270	Clinical isolate; this laboratory
CPM 271	Clinical isolate; this laboratory
CPM 02	Clinical isolate; this laboratory
CPM 432	Clinical isolate; this laboratory
CPM 489	Clinical isolate; this laboratory
CPM 03	Clinical isolate; this laboratory
CPM 493	Clinical isolate; this laboratory
CPM 738	Clinical isolate; this laboratory
CPC 2	S. Gray, PHLS, Cardiff
CPC 34	S. Gray, PHLS, Cardiff
CPC 29	S. Gray, PHLS, Cardiff
CPC 999	S. Gray, PHLS, Cardiff
CPC 6	S. Gray, PHLS, Cardiff
CPC 4	S. Gray, PHLS, Cardiff
Campylobacter jejuni	
CJ 1	Clinical isolate; this laboratory
CJ 2	Clinical isolate; this laboratory
CJ 6	Clinical isolate; this laboratory
CJ 7	Clinical isolate; this laboratory
CJ 8	Clinical isolate; this laboratory
CJ 9	Clinical isolate; this laboratory
NCTC 11950[b]	PHLS, Colindale, London
NCTC 11848[b]	PHLS, Colindale, London
NCTC 11924 (nitrate negative)[b]	PHLS, Colindale, London
NCTC 11925 (nitrate negative)[b]	PHLS, Colindale, London
Gastric campylobacter	
NCTC 11847[b]	PHLS, Colindale, London
NCTC 11849[b]	PHLS, Colindale, London
Campylobacter coli NCTC 11366	PHLS, Colindale, London
Campylobacter fetus subsp *fetus* NCTC 10842	PHLS, Colindale, London
Campylobacter fetus subsp *veneralis* NCTC 10354	PHLS, Colindale, London
Campylobacter concisus NCTC 11485	PHLS, Colindale, London
Campylobacter faecalis NCTC 11415	PHLS, Colindale, London
Campylobacter sputorum subsp *bubulus* NCTC 11528	PHLS, Colindale, London
Campylobacter cinaedi NCTC 11611	PHLS, Colindale, London
Campylobacter lardis NCTC 11352	PHLS, Colindale, London
Canine catalase-negative/weak *Campylobacter* group NCTC 11540	PHLS, Colindale, London
Urease-positive thermophilic *Campylobacter*	
83/12830/1	F. Bolton, PHLS, Preston
83/10231/9	F. Bolton, PHLS, Preston
82A1/1	F. Bolton, PHLS, Preston
Catalase-negative *Campylobacter* sp. strain P27819	F. Bolton, PHLS, Preston
GCLO[c]	
P1	D. Jones, PHLS, Manchester
B3	D. Jones, PHLS, Manchester
F6	D. Jones, PHLS, Manchester
WO831	D. Buckley, Beechams Pharmaceuticals, Epsom
WO834	D. Buckley, Beechams Pharmaceuticals, Epsom
Escherichia coli K-12	
392	
JM109	
JM105	
HB101	

[a] PHLS, Public Health Laboratory Service.
[b] Strains isolated from human antral mucosa biopsy specimens originally described as GCLO type II (20).
[c] Campylobacterlike organisms isolated from the gastric mucosa of a pig (P1), baboon (B3), and ferrets (F6, WO831, and WO834) (11, 15).

gene libraries. *C. pylori* CPM630 was cultured in broth, and the genomic DNA was isolated by a modification of a method described by Langenberg et al. (23). The strain was grown on Columbia blood agar to give a confluent lawn after 96 h and was then transferred to 100 ml of nutrient broth (no. 2; Oxoid) supplemented with horse serum (10% [vol/vol]; Wellcome, Dartford, UK), vancomycin (10 μg/ml), colomycin (10 U/ml), trimethoprim (5 μg/ml), and amphotericin B (5 μg/ml). This was incubated for 36 h at 37°C under microaerophilic conditions with constant shaking. The bacteria were harvested by centrifugation, washed once with 50 mM Tris (pH 8.0)–5 mM EDTA–50 mM NaCl (TES), and suspended

in 2 ml of 50 mM Tris (pH 8.0)–25% sucrose. Lysozyme (1 ml of a 10-mg/ml solution) in 0.25 M EDTA (pH 8.0) was then added, and the mixture was incubated for 20 min at 0°C. Lysis solution (0.25 ml of sodium lauroyl sarcosine [5%, wt/vol] in 50 mM Tris–EDTA, pH 8.0), 0.75 ml of 10 mM Tris–1 mM EDTA, and 0.5 ml of pronase (20 mg/ml) were then added, and the solution was incubated for 1 h at 56°C. The resulting lysate was treated with RNase (final concentration, 50 μg/ml) for 30 min at 37°C. The DNA was then extracted three times with equal volumes of phenol-chloroform. After ether extraction, the DNA was precipitated by mixing with 2 volumes of absolute ethanol (−20°C) and incubated for 1 h at −20°C. It was harvested by centrifugation (13,000 × *g*), washed once with 70% ethanol, dried, and dissolved in water to give 100 μg of DNA per ml. A bacteriophage library of *C. pylori* DNA from CPM630 was constructed by partially digesting the DNA with *Sau*3A to produce fragments of 9 to 20 kb, and this was ligated into *Bam*HI-digested λEMBL3 DNA. The gene library was then packaged (Gigapack kit; Northumbria Biologicals Ltd. [NBL], Cramlington, UK), and the recombinant phage were transduced into *E. coli* K-12 strain 392 (24). A cosmid library was prepared from *C. pylori* CPM51; the DNA was partially digested with *Sau*3A to give fragments between 30 and 50 kb, and this was ligated into *Bam*HI-digested cosmid pHC79 (Pharmacia, Milton Keynes, UK). The library was packaged and transduced into *E. coli* K-12 strain HB101 (24).

Screening of *C. pylori* gene libraries. The bacteriophage library was plated on *E. coli* 392 to give approximately 300 plaques per 90-mm-diameter plate (24). *C. pylori* antiserum was adsorbed three times with whole-cell and λEMBL3-lysed *E. coli* 392 cells to remove nonspecific antibodies. The library was then screened for antigen recombinants by in situ ELISA of plaques replicated onto nitrocellulose filters (38). Positive plaques were localized, picked, and stored in 0.5 ml of SM buffer (0.1 M NaCl, 10 mM $MgSO_4$, 50 mM Tris [pH 7.5], 0.01% gelatin) and 20 μl of chloroform. Liquid lysates of the recombinant phages were prepared (24), centrifuged, and used for immunoblot analysis. The cosmid library of *C. pylori* CPM51 was plated to give approximately 100 colonies per plate. These were replicated onto nylon membranes, lysed, and screened for hybridization to a radiolabeled recombinant DNA probe as detailed by New England Nuclear (NEN) Research Products.

SDS-PAGE and immunoblotting. *Campylobacter* strains were harvested from plates and *E. coli* K-12 clones were centrifuged from LB broth cultures for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The cells were washed in PBS and suspended by vortexing in sample-cracking buffer (49 mM Tris hydrochloride [pH 6.7] containing 2.5% SDS, 1.3% 2-mercaptoethanol, 5% [vol/vol] glycerol, and 0.002% [wt/vol] bromophenol blue). Recombinant phage lysates were mixed with an equal volume of sample buffer. Samples were boiled for 5 min, and 1 to 2 μg of protein was applied to the gels. SDS-PAGE gels, electrophoretic transfer (immunoblotting), and the development of immunoreactive protein products were performed essentially as described by Heard et al. (7). Preimmune rabbit sera were used as controls for immunoblotting. Gels for Coomassie blue staining were fixed in 20% (vol/vol) acetic acid–20% (vol/vol) isopropanol, stained with 0.05% (wt/vol) Coomassie blue–40% (vol/vol) methanol–10% (vol/vol) acetic acid, destained in 12.5% (vol/vol) isopropanol–10% acetic acid, and dried.

Isolation of recombinant DNA and subcloning. The plate lysate method (24) was used for isolation of DNA from the recombinant λEMBL3 clone. Plasmid DNA from the recombinant subclones was isolated in bulk amounts by an alkaline lysis method (24). Restriction endonuclease digestion of recombinant DNA was performed as recommended by the manufacturers (NBL). The vector plasmid pUC18 (39) was used for subcloning the recombinant inserts, and they were transformed into *E. coli* K-12 strains JM109 and JM105 by the calcium chloride procedure (24). All recombinant *E. coli* clones were grown on LB broth or agar supplemented with ampicillin (100 μg/ml).

Immunoassay and immunofluorescence tests. Dot-blotting ELISA was used for detecting immunoreactions to proteins of *Campylobacter* strains and *E. coli* K-12 clones. The bacterial cells were harvested from plates, lysed in water by repeated freezing and thawing, spotted onto gridded nitrocellulose filters (Schleicher and Schuell, London, UK) and screened for immunoreaction (7). *E. coli* K-12 clones were also screened for reactions with *C. pylori* antiserum by chloroform lysis of colonies on nitrocellulose filters as described by Holmans et al. (8). Immunofluorescence studies were performed on *Campylobacter* strains and *E. coli* K-12 clones. The cells were suspended in PBS, and 5-μl drops were placed onto multispot PTFE slides (Hendley-Essex, Loughton, UK). After drying, the cells were fixed to the slides by immersion in acetone for 15 min. Antiserum (10 μl) at a 1/10 dilution was added, and the slides were incubated for 30 min at 37°C in a moist chamber. They were rinsed in PBS, anti-rabbit IgG fluorescein conjugate (5 μl) at a 1/10 dilution was added, and the slides were incubated for 30 min at 37°C. The slides were then washed, dried, and viewed for fluorescence.

DNA radiolabeling and hybridization tests. Recombinant DNA was excised from low-melting-point agarose gels and radiolabeled with ^{32}P by using [^{32}P]dCTP (Amersham, Amersham, UK) and the random sequence hexamer method described by Feinberg and Vogelstein (3). *Campylobacter* strains were harvested, and suspensions (3 μl) were applied to nitrocellulose filters. After drying, the bacterial cells were lysed by the method of Grunstein and Hogness (5). Hybridization with the DNA probe was performed with dextran sulfate enhancer with 50% formamide, and the filters were washed as described by NEN Research Products. The filters were exposed to X-ray film overnight and developed.

RESULTS

A total of 3,000 λEMBL3 plaques were screened for *C. pylori* antigen-producing recombinants, and only one positive clone was detected (λCP2). This positive recombinant clone was purified and studied further. Immunoblot analysis with liquid lysate from λCP2 showed a single polypeptide band of 66 kDa reacting antigenically with the *E. coli*-adsorbed antiserum (Fig. 1). A 66-kDa band was not seen for the negative control λEMBL3 recombinant lysate. The *C. pylori* whole-cell antiserum reacted with numerous bands of the *C. pylori* strains of 78, 66, 54, 50, 48, 31, 25, and 19 kDa and also cross-reacted with a 62-kDa band present in six *C. jejuni* strains. The major immunogenic polypeptides of *C. pylori* were the bands at 78, 66, 54, and 31 kDa, as determined by immunoblotting various strains.

Extraction of DNA from λCP2 and digestion with *Sal*I revealed a DNA insert of 17 kb. Subcloning was performed with the high-copy-number *E. coli* vector pUC18 and *E. coli* JM109. A positive recombinant, pSCP1, was detected by colony blot ELISA and found to contain the entire 17-kb *Sal*I fragment. Immunoblot analysis of pSCP1 revealed that

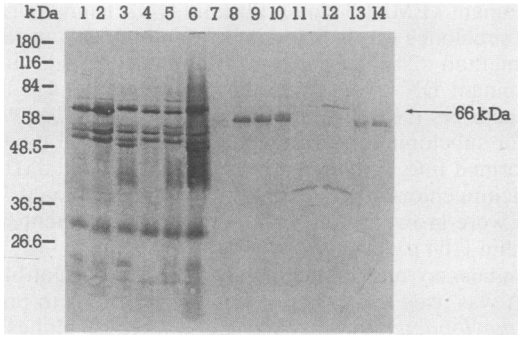

FIG. 1. Immunoblot of SDS-PAGE gel (12.5% acrylamide) of the following: total protein profiles of *C. pylori* strains CPM271 (lane 1), CPM270 (lane 2), CPM630 (lanes 3 and 6), CPM192 (lane 4), and CPM 51 (lane 5); total protein profiles of *C. jejuni* strains CJ9 (lane 7), CJ8 (lane 8), CJ7 (lane 9), CJ6 (lane 10), CJ2 (lane 13), and CJ1 (lane 14); control liquid lysate of random λEMBL3 recombinant (lane 11); and lysate of λCP2 (lane 12). All reactions were against adsorbed *C. pylori* antisera. Arrow indicates position of 66-kDa cloned antigen. Prestained molecular weight standards used were subunits of α_2-macroglobulin (180 kDa), β-galactosidase (116 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (58 kDa), fumarase (48.5 kDa), lactate dehydrogenase (36.5 kDa), and triose-phosphate isomerase (26.6 kDa) (Sigma, Poole, UK).

the DNA encoded a 31-kDa antigen in addition to the 66-kDa band (Fig. 2, immunoblot A, lane 2). The 66- and 31-kDa bands were not present in JM109 with pUC18 only. These polypeptides were found to be present in 17 of 17 *C. pylori* strains so far examined by immunoblot analysis, and their presence in strain CPC2 is shown in Fig. 2, immunoblot B, lane 1. They were also found in gastric campylobacterlike organisms (GCLOs) isolated from a pig (P1) and baboon (B3) but not in three ferret GCLOs (WO831, WO834, and F6).

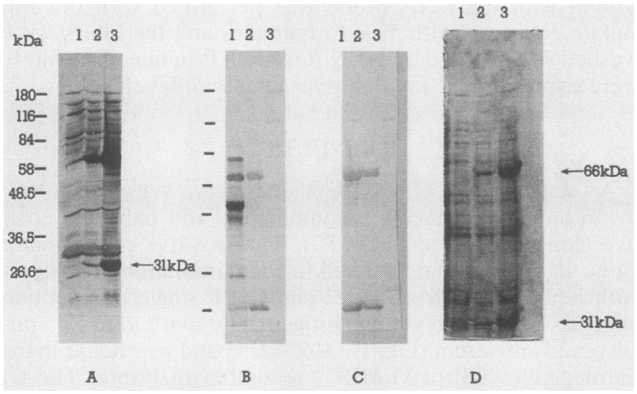

FIG. 2. Immunoblots of SDS-PAGE gels (10% acrylamide) of the following: (A) total protein profiles of control JM109 with pUC18 (lane 1), recombinant subclone pSCP1 in JM109 (lane 2), recombinant subclone pTCP3 in JM109 (lane 3); (B) *C. pylori* strain CPC2 (lane 1), recombinant subclone pTCP3 in JM109 (lane 2), control JM109 with pUC18 (lane 3); (C) recombinant subclone pTCP3 in JM105 grown with 50 mM IPTG (lane 1), pTCP3 in JM105 (lane 2), control JM105 with pUC18 (lane 3) (all reactions in panels A to C were against adsorbed *C. pylori* antiserum); (D) Coomassie blue-stained SDS-PAGE gel (10% acrylamide); total protein profiles of control JM105 with pUC18 (lane 1), recombinant subclone pTCP3 in JM105 (lane 2), and pTCP3 in JM105 grown with 50 mM IPTG (lane 3). Molecular weight standards are indicated (A and B).

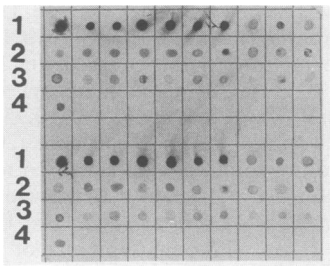

FIG. 3. Dot-blot ELISA analysis of proteins of *Campylobacter* strains and *E. coli* K-12 recombinant clones. The blots were developed with antisera raised to cloned *C. pylori* antigens of recombinant λCP2. In duplicate, from left to right: row 1, *C. pylori* CPM51, CPM192, CPM271, CPM270, CPM630, CPM432, CPM489, and ferret GCLOs WO831, WO834, and F6; row 2, *C. jejuni* 11950, gastric *Campylobacter* sp. strains 11847 and 11849, *C. jejuni* (nitrate negative) 11924, *C. jejuni* 11848, *C. concisus* 11485, *C. jejuni* (nitrate negative) 11925, *C. fetus subsp. fetus* 10842, *C. fetus* subsp. *veneralis* 10345, and *C. faecalis* 11415; row 3, *C. cinaedi* 11611, canine catalase-negative *Campylobacter* sp. strain 11540, *C. coli* 11366, *C. lardis* 11352, *C. sputorum subsp. bubulus* 11528, *E. coli* K-12 carrying pSCP1 and pTCP3, control pUC18, pUCP25, and control pHC79; row 4, *E. coli* K-12(pSCP1).

The DNA encoding these antigens was further subcloned with *Taq*I to give pTCP3 with an insert of 2.6 kb. This subclone yielded higher amounts of the cloned antigens than pSCP1 (Fig. 2, immunoblot A, lane 3). Growth of pTCP3 in the presence of 50 mM isopropyl thiogalactopyranoside (IPTG) was found to further increase the amount of cloned polypeptides, as revealed by both immunoblotting and Coomassie blue staining of proteins (Fig. 2C and D).

Antiserum developed against the cloned antigens was shown by dot blotting to react most strongly with the seven *C. pylori* strains. The *E. coli* K-12 clones pSCP1 and pTCP3 also reacted weakly but more strongly than the *E. coli* strains carrying the vector alone (Fig. 3). There was no reaction with other *Campylobacter* species. This antiserum was shown by immunofluorescence to react with 17 of 17 *C. pylori* strains and with the GCLO strains P1 and B3, but gave no reaction with the ferret GCLOs or other *Campylobacter* species. It was often found that, on preparation for immunofluorescence, the *C. pylori* strains underwent transformation to coccoid forms. However, they were still positive by immunofluorescence, and fluorescent coccoid *C. pylori* are shown in Fig. 4. The *E. coli* K-12 clones pSCP1 and pTCP3 were found not to react with either the whole-cell or cloned polypeptide antiserum in the immunofluorescence assay.

The 2.6-kb *Taq*I DNA fragment of the clone pTCP3, which encodes the 66- and 31-kDa antigens, was radiolabeled with ^{32}P for hybridization studies. The probe hybridized to seven *C. pylori* strains and to GCLOs P1 and B3, but not to the ferret GCLOs or to various other *Campylobacter* species (Fig. 5).

The urease enzyme of *C. pylori* constituted a major protein produced by this organism, and the cloned antigens were tested for reactivity with antibody to the *C. pylori* urease enzyme. Affinity-purified polyclonal antibody to the purified *C. pylori* urease enzyme, kindly provided by T. Itoh (10), was found by immunoblotting to react with the 66- and 31-kDa antigens present in both *C. pylori* and the recombinant clone pTCP3 (Fig. 6, immunoblot A). The antibody reacted with a high-molecular-weight band of nonboiled *C. pylori* antigens determined to be the active urease enzyme by the method of Mobley et al. (29) and also to a number of

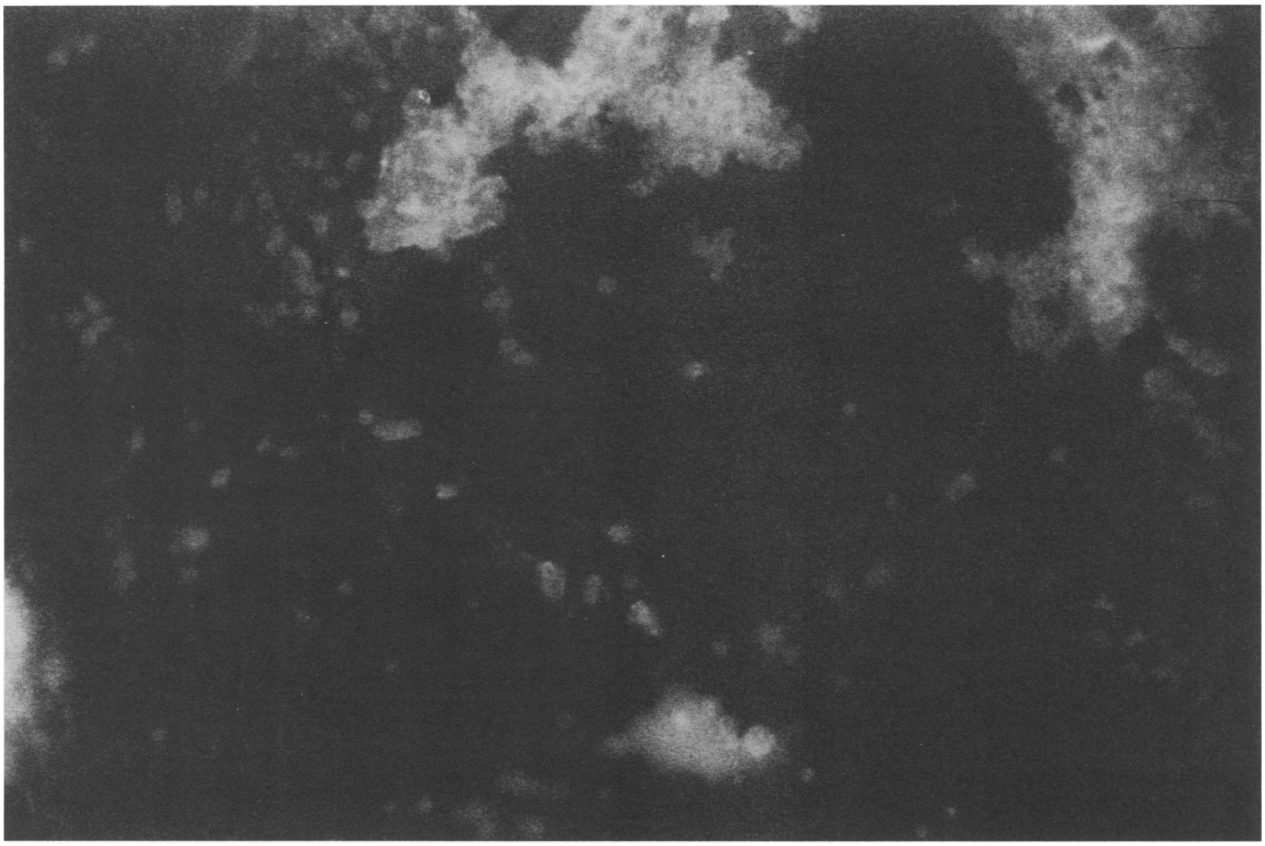

FIG. 4. Immunofluorescence of *C. pylori* CPC34 developed with antiserum raised to cloned *C. pylori* antigens of recombinant λCP2.

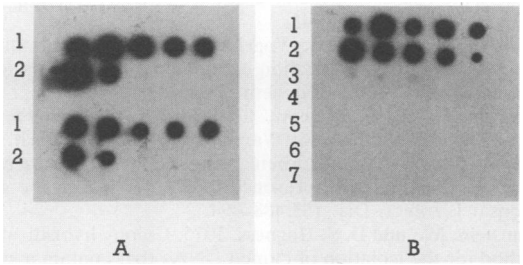

FIG. 5. Autoradiographs of hybridization of ^{32}P-labeled 2.6-kb *TaqI* DNA probe from recombinant subclone pTCP3 to bacterial strains. (A) In duplicate, from left to right: row 1, *C. pylori* CPC34, CPC2, CPC6, CPM02, and CPC999; row 2, pig GCLO P1, baboon GCLO B3, and ferret GCLOs F6, WO831, and WO834. (B) From left to right: row 1, *C. pylori* CPC29, CPC34, CPC2, CPC6, and CPM02; row 2, *C. pylori* CPM03, CPC999, CPC2, baboon GCLO B3, and pig GCLO P1; row 3, ferret GCLOs F6, WO831, and WO834, *C. jejuni* (nitrate negative) 11925, gastric *Campylobacter* sp. strain 11847; row 4, gastric *Campylobacter* sp. strain 11849, *C. jejuni* 11848, *C. fetus subsp. veneralis* 10354, *C. lardis* 11352, *C. coli* 11366; row 5, *C. jejuni* 11950, *C. fetus subsp. fetus* 10842, *C. faecalis* 11415, *C. cinaedi* 11611, *C. jejuni* (nitrate negative) 11924; row 6, *C. sputorum subsp. bubulus* 11528, *C. concisus* 11485, and urease-positive thermophilic *Campylobacter* sp. strains 88/12830/1, 83/10231/9, and 82A1/1; row 7, catalase-negative *Campylobacter* sp. strain P27819.

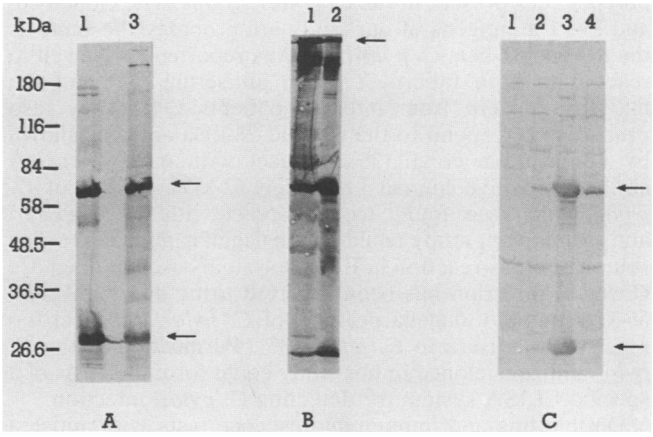

FIG. 6. Immunoblots of SDS-PAGE gels (10% acrylamide) of the following. (A) Total proteins of recombinant subclone pTCP3 in *E. coli* JM109 (lane 1) and *C. pylori* CPC34 (lane 3); arrows indicate position of 66- and 31-kDa antigens. (B) Proteins of nonboiled *C. pylori* CPC34 (lane 1) and CPM51 (lane 2); arrow indicates position of active urease enzyme. Reactions were with polyclonal antibody to purified *C. pylori* urease. (C) Total proteins of control cosmid HB101 with pHC79 (lane 1), cosmid clone pUCP25 in HB101 (lane 2), pTCP3 in JM109 (lane 3), and pSCP1 in JM109 (lane 4). Reactions were with adsorbed *C. pylori* antiserum. Arrows indicate positions of 66- and 31-kDa antigens.

smaller high-molecular-weight bands (Fig. 6, immunoblot B). When the *C. pylori* antigens were boiled, there was only a reaction to the 66- and 31-kDa polypeptides. The 2.6-kb DNA insert encoding these antigens was radiolabeled with ^{32}P and used to screen a cosmid library of *C. pylori* CPM51 by colony hybridization. A number of positive clones were detected, and these were tested by colony blot ELISA for reaction with the urease antiserum. One clone, pUCP25, was found to react positively with the urease antiserum and with the *C. pylori* antiserum. This clone contained a DNA insert of 35 kb and was found by immunoblotting to encode the 66- and 31-kDa *C. pylori* antigens (Fig. 6, immunoblot C). The cosmid clone did not, however, encode any higher-molecular-weight antigens or exhibit any urease enzyme activity.

DISCUSSION

A higher number of clones were expected to be detected on screening the *C. pylori* gene library, as the number of recombinants screened represented a number of genomes of *C. pylori*. The results obtained indicate that the expression of *C. pylori* DNA in *E. coli* K-12 is probably poor, as has been reported for *C. jejuni* DNA (21). A more sensitive assay for screening the *C. pylori* gene library, such as immunoblotting of recombinant lysates, may detect more clones.

Subcloning of the DNA from the initial clone detected was found to increase the yield of the cloned antigens, and this was probably due to the high copy number of the recombinant plasmids per *E. coli* cell and their subsequent increase in number as the DNA insert was reduced in size. As IPTG increased expression of the cloned antigens, they are probably expressed from the vector *lac* promoter rather than the *C. pylori* promoter.

Immunoblotting of *C. pylori* cells with rabbit *C. pylori* antiserum demonstrated the presence of both common and strain-specific immunogenic polypeptides. Major immunogenic polypeptides detected included ones of 78, 66, 54, and 31 kDa. The 66- and 31-kDa antigens cloned in *E. coli* were found to correspond to the highly conserved 66- and 31-kDa polypeptides present in the *C. pylori* strains. The cloned 66- and 31-kDa antigens of our study are probably the same as the 61- and 31-kDa *C. pylori* proteins reported by Newell to react with both rabbit *C. pylori* antiserum (33) and the majority of sera from infected patients (34). They also probably correspond to the 63- and 28-kDa antigens shown by von-Wulffen et al. (35) to react with most *C. pylori* antibody-positive human sera. The 62-kDa antigen of *C. jejuni* which was found to cross-react with the *C. pylori* antiserum in our study could be the flagella antigen (33). The source of cross-reaction in ELISA systems used to diagnose *C. pylori* infection has been reported to be due to 54- and 56-kDa putative flagella proteins of *C. pylori* which cross-react with antisera to *C. jejuni* (33). Purification of the *C. pylori* antigens cloned in our study could form the basis of a specific ELISA system for detecting *C. pylori* infection.

Dot-blotting and immunofluorescence tests with antisera developed against the cloned antigens demonstrated that these antigens were species specific. However, these antigens were probably not expressed on the surface of *E. coli* K-12 strains, as they were not detected by immunofluorescence. The DNA probe encoding these antigens was also species specific. This probe will be useful for detecting nonculturable *C. pylori* that may be present in human saliva and fecal material and in identifying possible sources of this organism. The GCLOs isolated from a pig and baboon were demonstrated by immunoblotting, immunofluorescence, and DNA hybridization to have *C. pylori*-related antigen-encoding genes. They were also found by microscopy, total protein profiles, and biochemical tests to be very similar to *C. pylori* (data not shown). The ferret GCLOs, however, were significantly different from *C. pylori*, confirming previous structural and biochemical studies (14, 15).

The positive reaction of the affinity-purified polyclonal *C. pylori* urease antibody to the cloned 66- and 31-kDa antigens suggests that they may be part of the 625-kDa urease enzyme that has recently been characterized (30). The 66- and 31-kDa antigens are indeed analogous to proteins of similar size reported to be major components of bacterial ureases (2, 29, 36). The clone pSCP1 and the cosmid clone pUCP25 contained DNA inserts of 17 and 35 kb, respectively, which should have been enough to encode the active urease enzyme. However, neither clone exhibited any urease activity. We are currently cloning these large DNA inserts into shuttle vectors that replicate in both *E. coli* and *C. jejuni* (21) in order to determine whether the *C. pylori* DNA is better expressed in *C. jejuni* and encodes urease. Further characterization of the cloned antigens and immunoblot studies with human sera are currently in progress.

In conclusion, the cloned species-specific immunogenic proteins and their monospecific antisera may be useful in the investigation of pathogenic mechanisms of *C. pylori* to localize antigen in infected tissue and in serodiagnostic assays.

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