

Molecular Identification of *Campylobacter concisus*

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Received 6 March 2001/Returned for modification 2 May 2001/Accepted 15 July 2001

A 1.6-kb DNA fragment isolated from a *Campylobacter concisus* genomic library gave *C. concisus*-specific restriction fragment length patterns when it was used as a probe in hybridization studies. All of the strains tested, including type strains and clinical isolates, contained a 0.5-kb *Hind*III fragment that hybridized to the probe. DNA sequencing of the 1.6-kb fragment identified three open reading frames (ORFs). One of the ORFs encodes the carboxy terminus of GyrB, and the translational products of ORF2 and ORF3 showed similarity to hypothetical proteins, previously identified in *Campylobacter jejuni*. DNA-DNA hybridization studies with a fragment internal to ORF3 showed that this sequence was responsible for the signal observed with the 0.5-kb *Hind*III fragment. A rapid PCR assay was developed and evaluated. Primers that annealed to the extremities of the 1.6-kb fragment were used to obtain an amplicon of the correct size from both reference and clinical strains of *C. concisus*.

Traditionally *Campylobacter concisus* has been recovered exclusively from and considered part of the bacterial flora of the human oral cavity (22). This species has been isolated predominantly from gingival crevices associated with the onset of periodontal disease (15, 22, 24). Subsequently, strains from outside the oral cavity have been isolated; however, these were initially misidentified or not classified (25).

Recent studies have increasingly reported on the isolation of *C. concisus* from the stools of patients with diarrhea (6, 12, 27). This has largely been attributed to an improvement in culture techniques, where the adoption of the membrane filter technique has improved isolation rates (13). Following the introduction of the Cape Town protocol (13), which utilizes the membrane filter technique in combination with a hydrogen-enriched microaerobic environment, the prevalence of *C. concisus* has increased to 31% of the total *Campylobacter* isolates from children at the Red Cross War Memorial Children's Hospital, Cape Town, South Africa. Of all the *Campylobacter* spp., only *Campylobacter jejuni* subsp. *jejuni* is more frequently isolated from children at this hospital. During a reevaluation of conventional isolation methods, similar results were obtained in a study in Denmark (6).

In Cape Town, South Africa, *C. concisus* displays clinical and seasonal characteristics similar to those of *C. jejuni*, an established gastrointestinal pathogen (A. J. Lastovica and M. E. Engel, Abstr. 100th Gen. Meet. Am. Soc. Microbiol. 2000, abstr. D250, 2000). Monthly isolation frequencies showed that both *C. concisus* and *C. jejuni* were notably higher in the 3-month period towards the end of summer (February to April). Clinical symptoms of diarrhea, such as severity and stool consistency, were similar to those associated with *C. jejuni*; however, *C. concisus* was predominantly isolated from children 1 year old or older, whereas *C. jejuni* was mostly found in children younger than 12 months. In addition, *C. concisus*

was more likely to be associated with children of mixed descent than with indigenous black children. This observation could not be explained by age, gender differences, genetic susceptibility, eating habits or geographical location (Lastovica and Engel, Abstr. 100th Gen. Meet. Am. Soc. Microbiol.). Although some studies have suggested that *C. concisus* is not a primary pathogen (6, 27), the role of *C. concisus* and its association with diarrhea have yet to be clearly defined.

An understanding of the epidemiology of *C. concisus* has been hampered by the lack of a suitable identification tool. Like other members of the genus, *C. concisus* is fastidious and is characterized by low biochemical activity, properties that make the identification of *C. concisus* problematic, using conventional phenotypic techniques (7, 10, 17). DNA-DNA hybridization, immunotyping and whole-cell protein electrophoresis have been used to identify *C. concisus* (11, 23, 25). As these techniques are time-consuming and require considerable expertise, they are not suitable for large epidemiological studies. A limited number of molecular approaches for the identification of *C. concisus* have been described (3, 14). A PCR of the 23S ribosomal DNA (rDNA) (3) did not consistently identify *C. concisus* (6), probably because of the genotypic heterogeneity within the species (3, 25). A similar approach is based on the restriction profile of 16S rDNA PCR products (14). During the course of the study described in this report, a fragment of *C. concisus* genomic DNA was cloned and characterized. DNA-DNA hybridization studies and PCR assays indicate that this fragment could be used to identify *C. concisus*.

MATERIALS AND METHODS

Bacterial strains and plasmids. Reference *Campylobacter* strains used in this study included *C. concisus* NCTC 11485, *C. concisus* NCTC 11684, *C. concisus* CCUG 13144, *C. concisus* CCUG 19995, *C. curvus* NCTC 11649, *C. rectus* NCTC 11489, *C. sputorum* bv. *fecalis* NCTC 11415, *C. coli* CCUG 11283, *C. lari* NCTC 11352, *C. upsaliensis* NCTC 12183, *C. helveticus* NCTC 12470, *C. jejuni* subsp. *doylei* NCTC 11487, *C. jejuni* subsp. *jejuni* NCTC 11168, *C. mucosalis* NCTC 11000, and *C. fetus* subsp. *fetus* NCTC 10842. *Bacteroides ureolyticus* NCTC 10941 was also used. Local clinical isolates of *C. concisus* ($n = 104$) included in this study were collected during the period 1992 to 1999. One of the strains was a dental isolate from an adult; the remainder were obtained from children at the Red Cross War Memorial Children's Hospital. S. L. W. On (Veterinary Research Laboratory, Copenhagen, Denmark) kindly provided two Danish clinical

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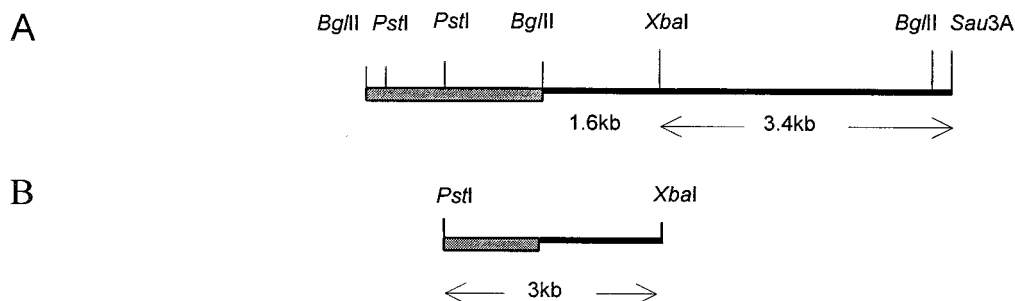


FIG. 1. Restriction map of DNA fragment from *C. concisus*. (A) The shaded block represents pEco251, and the bold line (5.0 kb) represents the 5.0-kb fragment from *C. concisus* cloned in the vector, generating pB3C. (B) Shown is the 3.0-kb fragment that was cloned into pUC19, generating pNeo.

isolates (SSI 63936 and SSI 11286) from children with diarrhea. *Escherichia coli* LK111 (30) was used as a recipient in transformation studies. The vectors, pEco251 (provided by M. Zabeau, Plant Genetics Systems N. V., Ghent, Belgium) and pUC19 (16) were used in cloning experiments.

Preparation and analysis of DNA. Genomic DNA was extracted using guanidinium thiocyanate (19). Plasmid DNA was isolated using either the alkaline lysis method (8) or a Nucleobond kit (Macherey-Nagel). DNA was digested with various restriction enzymes, and the fragments were separated in horizontal gels of 0.8 to 1.2% (wt/vol) agarose dissolved in 0.4 M Tris-acetate and 0.01 M EDTA. Gels were stained with ethidium bromide, and DNA was visualized by UV transillumination.

Ligations. Ligations were carried out as described (20), in 20- μ l reaction mixtures containing 1 \times T4 DNA ligase buffer and 10 U of T4 DNA ligase (Boehringer Mannheim). Typically, an approximate ratio of 3 to 1 molar concentrations of insert and vector were used in ligations, and the reaction mixtures were incubated at 16°C for 4 to 16 h.

Transformation studies. Competent *E. coli* LKIII cells were prepared as described (4). Ligations were added to competent cells, and transformants were selected on 2 \times YT agar containing appropriate antibiotics.

PCR assays. Based on the DNA sequence (GenBank accession number AY026942) of the 1.6-kb *BglII-XbaI* fragment (Fig. 1), three pairs of oligonucleotide primers were used to amplify sequences internal to the three open reading frames (ORFs) identified. pcisus1 (5'-GAGCTTGTTGGTAAAGA-3') and pcisus2 (5'-GCGTGGTCTTGGATATAG-3') were used to amplify ORF1. pcisus3 (5'-TTGACGTAGAGAGTGACCTTG-3') and pcisus4 (5'-ATCAGAGCTGATCTCGATAAC-3') were used to amplify ORF2, and pcisus5 (5'-AGCA TCATCTATATCACGTT-3') and pcisus6 (5'-CCCGTTTGATAGGCGATAG-3') were used to amplify ORF3. PCRs were carried out with a Perkin-Elmer thermocycler (Gene Amp 2400) in a final volume of 50 μ l containing 50 ng of campylobacter DNA, a 50 pM concentration of each primer, a 2.5 mM concentration of each deoxynucleoside triphosphate, and 2.5 U of *Taq* DNA polymerase (TaKaRa Biochemicals) in the prescribed buffer. Initial denaturation (94°C for 120 s) was followed by 30 cycles of amplification. Each cycle consisted of 94°C for 60 s, 45°C for 60 s, and 72°C for 120 s. A final extension step (72°C for 10 min) completed the amplification. Aliquots of PCR products were separated by electrophoresis.

The primers pcisus1 and pcisus6 were used in PCR assays for the identification of *C. concisus*. PCR was carried out in a final volume of 50 μ l and contained 50 ng of genomic DNA, a 2.5 nM concentration of each deoxynucleoside triphosphate, a 50 pM concentration of each primer, and 2.5 U of *Taq* DNA polymerase (TaKaRa) in the prescribed buffer. Amplification was carried out with a Perkin-Elmer (Gene Amp 2400) thermocycler, using the parameters described above.

Preparation of probes. DNA of interest was eluted from agarose gels, purified (21), and labeled using an ECL labeling and detection kit (Amersham International).

DNA-DNA hybridization. Probes were hybridized to genomic DNA restricted with *HindIII*, separated by agarose gel electrophoresis, and transferred to Hybond-N+ membranes (Amersham International). Prehybridization, hybridization, and the posthybridization washes were carried out according to the protocol supplied with the ECL gene detection system (Amersham International).

DNA sequencing. DNA for sequencing was cloned into pUC19. Sequencing data were generated by automated laser fluorescence (Pharmacia Biotech AB, Uppsala, Sweden) in the Department of Molecular Biology, University of Cape Town. Internal oligonucleotide primers were used where necessary to ensure that both strands were sequenced. DNA sequences were analyzed with DNAMAN

(version 4.0; Lynnon BioSoft). BLAST (1) was used to search databases in GenBank for nucleic acid and deduced amino acid sequence similarity with existing sequences. Protein alignments were carried out by the fast alignment method (28), using the Blossum matrix with a k-tuple value of 2 and a gap penalty of 4.

Construction of a DNA library. A DNA library of *C. concisus* NCTC 11485 was prepared (2). Genomic DNA from *C. concisus* NCTC 11684 was digested partially with *Sau3AI* (Boehringer Mannheim). Size fractionation was carried out in a sucrose gradient; the 4- to 5-kb DNA fragments were pooled, recovered by ethanol precipitation, cloned into the *BglII* site of pEco251, and introduced into competent *E. coli* LKIII cells.

RESULTS

Identification of *C. concisus* DNA sequence for identification to species level. To select a recombinant clone that could be used to identify *C. concisus*, the pEco251 library was screened using DNA-DNA hybridization studies. DNA inserts were restricted from pEco251 with *BglII*, labeled, and hybridized to *HindIII*-digested DNA from *C. jejuni*, *C. mucosalis*, *C. concisus* type strains, and clinical isolates from Cape Town (404-96 and 406-96) and Denmark (SSI 11286 and SS1 63936). One of the inserts (5.0 kb), from a recombinant plasmid, designated pB3C, hybridized to four bands (3.0, 1.2, 0.5, and 0.3 kb) in *C. concisus* NCTC 11485 and to two bands (1.2 and 0.5 kb) in each of the four clinical isolates (data not shown). In addition, signals were obtained with fragments in *C. mucosalis* and *C. jejuni*, but the banding patterns were entirely different from those observed in *C. concisus*. The presence of common bands, which hybridized to the probe in the *C. concisus* strains, combined with the fact that these bands were not present in *C. jejuni* and *C. mucosalis*, suggested that they could be used as identification markers.

To refine the probe further, a limited restriction map of pB3C containing the *C. concisus* insert was determined. Restriction digestion showed that the insert (5.0 kb) contains an internal *BglII* site 0.4 kb from one of the *Sau3AI* sites (Fig. 1). Digestion of the insert with *BglII* and *XbaI* generated two fragments of 1.6 and 3.0 kb.

When the 3.0-kb *BglII-XbaI* fragment was used to probe the DNA, a strong hybridization signal was obtained with a 3.0-kb band, and weak signals were obtained with 0.5- and 0.3-kb bands in *C. concisus* NCTC 11485 (data not shown). Hybridization signals, albeit weak, were also obtained with 1.2-kb bands in the local and Danish clinical isolates. No signals were obtained with DNA from the *C. jejuni* and *C. mucosalis* type strains.

Probing with the 1.6-kb *BglII-XbaI* fragment resulted in signals with bands of 1.2, 0.5, and 0.3 kb in *C. concisus* NCTC

TABLE 1. Hybridization profiles

Profile group	Size (kb) of DNA fragments which hybridized to the 1.6-kb probe	No. of isolates
I	1.2, 0.5, 0.3	12
II	1.6, 0.5, 0.3	9
III	0.8, 0.5	6
IV	1.2, 0.5	36
V	1.6, 1.2, 0.3	1

11485 (data not shown). In addition, a signal was obtained with a 1.2-kb fragment in the Danish and local clinical isolates; a weak hybridization signal was observed with a 0.5-kb band in one of each of the local and Danish clinical isolates. No hybridization signal was obtained with DNA from *C. jejuni*, and in *C. mucosalis*, barely visible signals were detected with fragments (2.7 and 2.2 kb) that had hybridized to the 5.0-kb probe. The fact that similar hybridization profiles were observed in the *C. concisus* type strain and in the Danish and local clinical isolates suggested that the 1.6-kb sequence and its organization in the genome are conserved in *C. concisus*.

The specificity of the 1.6-kb fragment was investigated, using hybridization assays and an extended panel of *Campylobacter* type strains and *B. ureolyticus*, previously considered genotypically similar to campylobacter (26). An expected hybridization banding pattern (1.2, 0.5, and 0.3 kb) was obtained with *C. concisus* NCTC 11485 (data not shown). In addition, strong signals were obtained with DNA from 2 of the 11 *Campylobacter* isolates: bands of 2.2 and 1.7 kb in *C. curvus* and *C. sputorum* bv. fecalis, respectively, hybridized to the probe. A weak signal was obtained with a 2.6-kb fragment in *C. mucosalis*, but signals were not detected with DNA from *C. rectus*, *C. coli*, *C. jejuni* subsp. *jejuni*, *C. lari*, *C. upsaliensis*, *C. helveticus*, *C. fetus* subsp. *fetus*, *C. jejuni* subsp. *doylei*, and *B. ureolyticus*. It is important that when the membrane was hybridized with a 16S rDNA (5) probe, signals were obtained with DNA from these species, indicating that DNA had been transferred to the membrane.

Based on the above results it was concluded that probing with the 1.6-kb *BglII-XbaI* fragment generated a species-specific pattern with DNA from *C. concisus*. To test this further, DNA from 62 clinical isolates, including the dental isolate, and two Swedish reference strains (CCUG 13144 and CCUG 19995), suggested by pulsed-field gel electrophoresis to represent genetically diverse groups (data not shown), was probed with the 1.6-kb fragment. Analysis of the hybridization profiles showed a limited number of restriction fragment length polymorphisms (RFLPs) among the isolates. Five profiles were identified (Table 1). Common to all the profiles was a 0.5-kb fragment that hybridized to the probe, suggesting a genetic marker for the identification of *C. concisus*.

As a first step to gaining insight into the 0.5-kb fragment, which hybridized to the probe, the DNA sequence of the 1.6-kb *BglII-XbaI* fragment was determined. To facilitate sequencing, a 3.0-kb *PstI-XbaI* fragment (Fig. 1), which contained a portion of pEcoR251 (1.375-kb *PstI-BglII* fragment) and the 1.6-kb *BglII-XbaI* fragment from *C. concisus*, was subcloned into the *PstI-XbaI* site of pUC19 and introduced into *E. coli* LKIII. The recombinant plasmid was designated pNeo.

Sequence analysis of the 1.6-kb fragment from *C. concisus* in pNeo. The DNA sequence of the 1.6-kb fragment was determined on both strands (GenBank accession number AY026942). Three major ORFs are contained on the sense strand, while no significant ORFs were identified on the other strand.

Analysis of the sequence upstream of ORF1 did not identify any bacterial transcription signals. This suggested that ORF1 extends upstream of the *XbaI* site and that the 5' end is contained in the 3.4-kb *XbaI-Sau3A* fragment (Fig. 1). A comparison of the deduced amino acid sequence of this ORF with protein databases in GenBank showed 76 and 58% similarity to the carboxy termini of GyrB from *C. jejuni* and *Helicobacter pylori*, respectively.

ORF2 and ORF3 are preceded by ribosome-binding sites upstream of the ATG transcription initiation sites. The sequences (AAGGA) are identical to the *C. jejuni* consensus sequence (29) but shorter than that proposed previously for *C. jejuni* (9). Upstream of ORF2 is the sequence TTTAAAA GGT, which shows similarity to the -35 consensus sequence (TTTAAGTNNTT) for *C. jejuni* promoters (29). Based on the observation that the spacing between the -35 and -10 regions of *C. jejuni* promoters varies from 15 to 19 bp (29), two appropriately spaced putative -10 regions were identified. TTG ACCT and CCTAGTT, which are separated from the putative -35 sequence by 14 and 18 bp, respectively, show similarity to the -10 consensus sequence, TATAATT, for *C. jejuni* promoters (29). Two regions, -35 (TTTAAACA) and -10 (TAAG CTA), separated by 18 bp precede ORF3. The -16 sequence (TTTTTTTG) (29) was not identified in either of the putative promoters.

The translational product of ORF2 showed 70% similarity to a conserved hypothetical protein of 127 amino acids in *C. jejuni* (CDS Cj 1724c), while that of ORF3 showed 70% similarity with the NH₂-terminal of a conserved hypothetical protein (408 amino acids) in *C. jejuni* (CDS Cj 0015c).

DNA-DNA hybridization studies with sequences internal to ORF1, ORF2, and ORF3 as probes. Internal portions of each ORF were amplified, labeled, and hybridized to DNA from *Campylobacter* spp. When the fragment internal to ORF1 (*gyrB*) was used to probe the DNA, a strong signal was obtained with the 1.2-kb band in *C. concisus* (Fig. 2A, lane 1). In addition, signals were obtained with a 2.2-kb fragment in *C. curvus* (Fig. 2A, lane 3), a 1.7-kb fragment in *C. sputorum* bv. fecalis (Fig. 2A, lane 5) and a 0.5-kb fragment in *C. helveticus* (Fig. 2A, lane 10). Weak hybridization signals were obtained with DNA fragments in *C. mucosalis*, *C. rectus*, *C. coli*, *C. upsaliensis*, *B. ureolyticus*, and *C. jejuni* subsp. *doylei* (Fig. 2A, lanes 2, 4, 6, 9, 11, and 12). No signals were obtained with the DNA from *C. jejuni* subsp. *jejuni*, *C. lari*, and *C. fetus* subsp. *fetus*.

Similar studies using the probe for ORF2 resulted in strong signals with fragments of 1.2 and 0.3 kb in *C. concisus* (Fig. 2B, lane 1). That signals were obtained with two *HindIII* fragments was not unexpected, since this restriction site is present in ORF2. A strong signal was also obtained with a 2.2-kb fragment in *C. curvus* (Fig. 2B, lane 3). Weak signals were obtained with DNA from *C. rectus*, *C. sputorum* bv. fecalis, and *B. ureolyticus* (Fig. 2B, lanes 4, 5, and 11).

Probing with the PCR product of ORF3 resulted in a strong signal with the 500-bp fragment in *C. concisus* (Fig. 2C, lane 1).

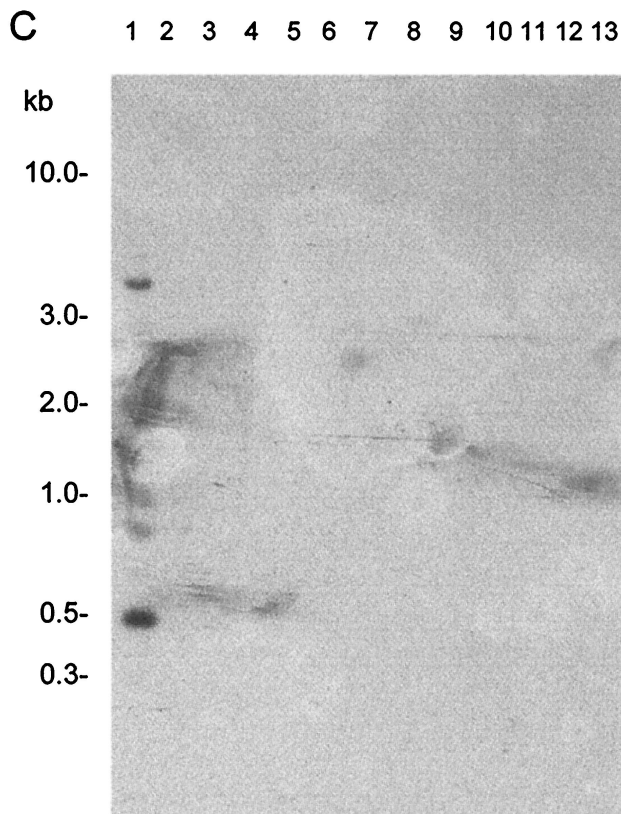
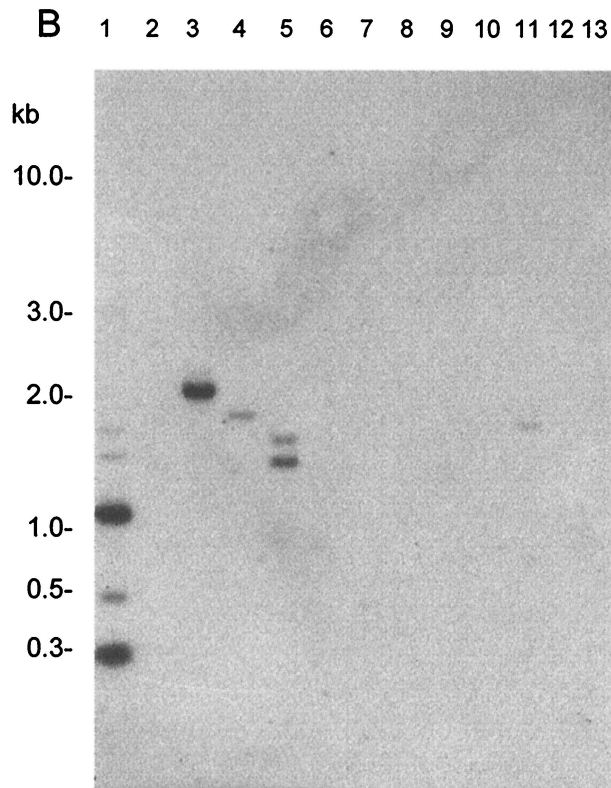
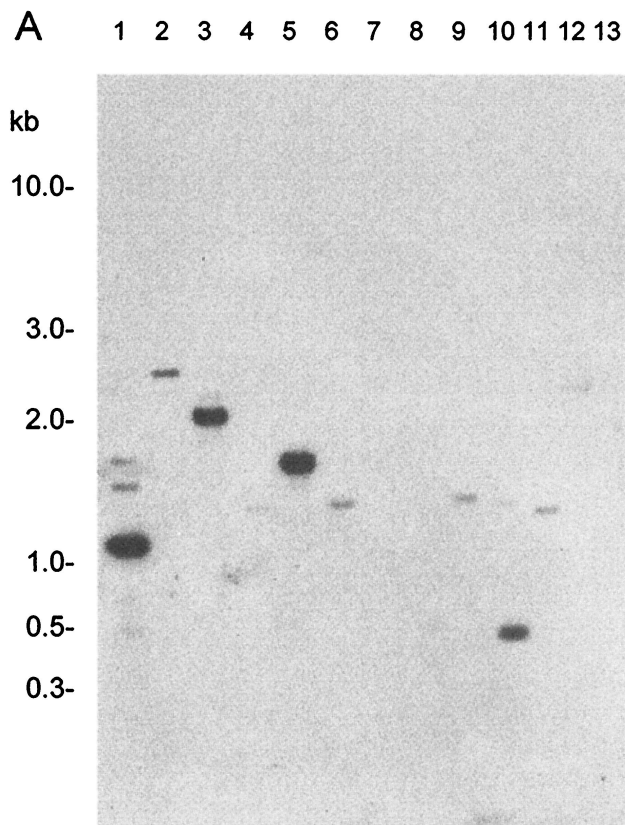


FIG. 2. Genomic DNA from *Campylobacter* type strains digested with *Hind*III and hybridized to internal fragments of ORF1, ORF2, and ORF3. Autoradiographs of DNA probed with a 0.35-kb fragment internal to ORF1 (A), a 0.338-kb fragment internal to ORF2 (B), and a 0.441-kb fragment internal to ORF3 (C) are shown. Lanes: 1, *C. concisus* NCTC 11485; 2, *C. mucosalis* NCTC 11000; 3, *C. curvus* NCTC 11649; 4, *C. rectus* NCTC 11489; 5, *C. sputorum* bv. *fecalis* NCTC 11415; 6, *C. coli* CCUG 11283; 7, *C. jejuni* NCTC 11168; 8, *C. lari* NCTC 11352; 9, *C. upsaliensis* NCTC 12183; 10, *C. helveticus* NCTC 12470; 11, *B. ureolyticus* NCTC 10941; 12, *C. jejuni* subsp. *doylei* NCTC 10847; 13, *C. fetus* subsp. *fetus* NCTC 10842.

A weak signal was obtained with a fragment of 5 kb in this strain (probably undigested DNA). Notwithstanding the background on the autoradiograph, there is a suggestion of a hybridization signal with a fragment of 2.6 kb in *C. mucosalis* (Fig. 2C, lane 2). No signals were obtained with DNA from any of the other *Campylobacter* spp. In another hybridization experiment, the internal fragment from ORF3 was used to probe DNA from clinical isolates representing three of the RFLP profile groups (II, III, and IV), previously identified (Table 1). A strong signal was obtained with a 500-bp fragment in each of the *C. concisus* isolates, further evidence that this sequence is common to and conserved in *C. concisus*.

PCR assay for the identification of *C. concisus*. As hybridization studies are time consuming and not the preferred approach in a diagnostic laboratory, a PCR assay based on the DNA sequence of the 1.6-kb fragment was developed and evaluated. Using *pcisus1* and *pcisus6*, which anneal to DNA sequences in ORF1 and ORF3, respectively, a product of the expected size (1.5 kb) was obtained from *C. concisus* NCTC 11485 (Fig. 3). No PCR products were obtained from *C. jejuni*, *C. lari*, *C. upsaliensis*, *C. helveticus*, *B. ureolyticus*, *C. sputorum*

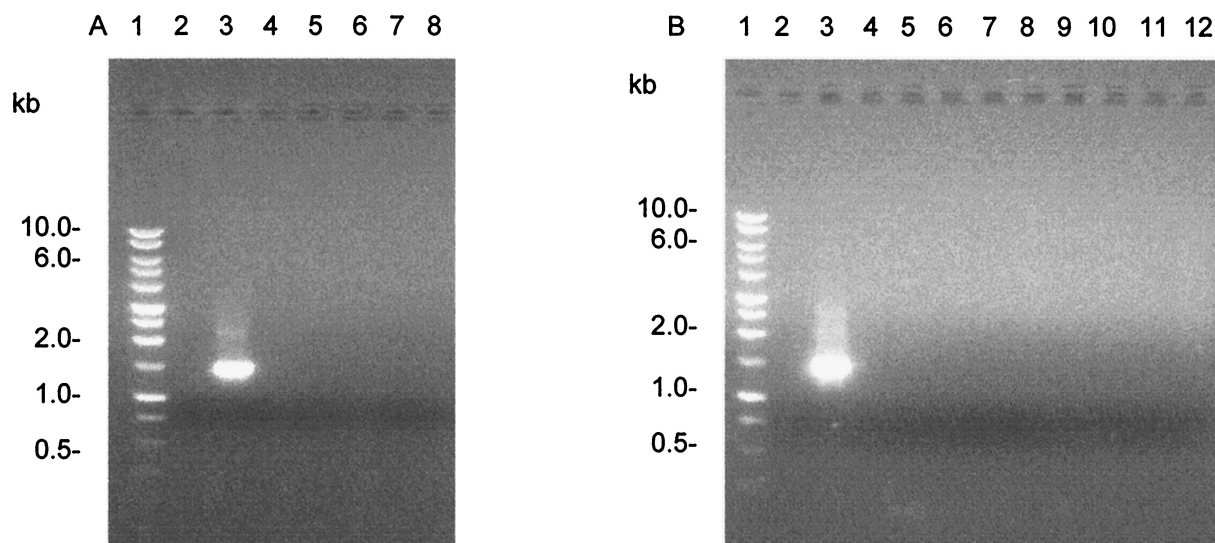


FIG. 3. PCR analysis of DNA. (A) Lanes: 1, molecular weight marker (Promega, Madison, Wis.); 2, negative control (no DNA); 3, *C. concisus* NCTC 11485; 4, *C. jejuni* NCTC 11168; 5, *C. lari* NCTC 11352; 6, *C. upsaliensis* NCTC 2183; 7, *C. helveticus* NCTC 12470; 8, *B. ureolyticus* NCTC 10941. (B) Lanes: 1, molecular weight marker (Promega); 2, no DNA; 3, *C. concisus*; 4, *C. sputorum* bv. fecalis NCTC 11415; 5, *C. curvus* NCTC 116490; 6, *C. mucosalis* NCTC 11000; 7, *C. rectus* NCTC 11489; 8, *Helicobacter pylori*; 9, *Acinetobacter baumannii*; 10, *Mycobacterium* BCG; 11, *Escherichia coli*; 12, *Salmonella* sp.

bv. fecalis, *C. curvus*, *C. mucosalis*, *C. rectus* and super-family member *Helicobacter pylori*. Amplicons were not obtained with DNA from unrelated bacteria *Acinetobacter baumannii*, *Mycobacterium bovis* BCG, *E. coli*, and *Salmonella* sp. It is noteworthy that, although hybridization signals were obtained with the DNA from *C. curvus* and *C. sputorum* bv. fecalis when it was probed with the 1.6-kb fragment, no PCR products were obtained from these species. It must also be noted that in a PCR assay using universal primers for 16S rDNA (5), a product of the appropriate size was obtained from all of the species tested, indicating the suitability of the DNA for PCR and the absence of inhibitors in the reaction mixture.

Primers were used to carry out PCR assays of 91 clinical isolates, including the 49 clinical isolates used in the hybridization studies. A single product of the expected size was obtained from 88 of the isolates. Three products, of which one was the correct size, were obtained from one isolate. No amplicons were obtained from two of the isolates. As these two strains had not been previously characterized by hybridization studies, *Hind*III-digested DNA from the two PCR-negative isolates was probed with the 1.6-kb *Bgl*III-*Xba*I fragment. The hybridization profiles obtained were not consistent with those for *C. concisus* isolates (data not shown). In addition, when these strains were investigated using the 16S rDNA method (14), the resulting *Dde*I restriction profiles of the amplicons were different from the *Campylobacter* profiles described, including that of *C. concisus*. Taken together, these data suggested that the two strains are not *C. concisus*.

DISCUSSION

Hybridization studies and PCR assays carried out in this study indicate that a 1.6-kb *Bgl*III-*Xba*I fragment, which was cloned from *C. concisus* NCTC 11485, could be used to identify this species. When this fragment was used to probe DNA from

Campylobacter spp., a *C. concisus*-specific *Hind*III hybridization profile was generated. Strong hybridization signals were obtained with the DNA from only two other species, *C. sputorum* bv. fecalis and *C. curvus*, suggesting that these species are more closely related to *C. concisus* than are the other species, a finding that is supported by phylogenetic rRNA studies (26). A weak signal was obtained with the DNA from *C. mucosalis*, but signals were not obtained with DNA from *C. rectus*, *C. coli*, *C. jejuni*, *C. lari*, *C. upsaliensis*, *C. helveticus*, *B. ureolyticus*, *C. jejuni* subsp. *doylei*, and *C. fetus* subsp. *fetus*.

Five RFLP profiles were observed in the *C. concisus* strains ($n = 106$) probed with the 1.6-kb fragment. All of the strains contained a 0.5-kb *Hind*III fragment that hybridized to the probe. The profiles obtained were consistent in both local and Danish clinical isolates. In addition, profiles indicative of *C. concisus* were observed in the two Swedish reference strains (CCUG 13144 and CCUG 19995), belonging to two separate DNA-DNA hybridization homology groups (25). These data suggest that the observed hybridization pattern is not confined to strains from a specific geographic region. Moreover, the stability of the profile is suggested by the fact that the strains were collected over a period of 7 years (1992 to 1999).

An analysis of the DNA sequencing data of the 1.6-kb fragment identified three ORFs. ORF1 contains the 3' end of *gyrB*. It is assumed that the 5' end of this gene is contained in the 3.4-kb *Xba*I-*Sau*3A portion of the 5.0-kb insert in pB3C (Fig. 1). As *gyrB* is contiguous with *gyrA* in *C. jejuni* it is probable that the latter gene is also contained in the 3.4-kb *Xba*I-*Sau*3A portion. The strong signals obtained with DNA from *C. curvus*, *C. sputorum* bv. fecalis, and *C. helveticus* when probed with a portion of ORF1 (Fig. 2) suggest that the 3' ends of the *gyrB* genes in these species are closely related to their counterpart in *C. concisus*.

The fragment internal to ORF2 hybridized to DNA from *C. concisus* and *C. curvus*. As ORF2 contains a *Hind*III site,

two fragments (1.2 and 0.3 kb) in *C. concisus* hybridized to the probe. On the other hand, only one band (2.2 kb) in the DNA from *C. curvus* hybridized to the probe. Since ORF1 also hybridized to a fragment of 2.2 kb in this strain, it is likely that as in *C. concisus*, ORF1 and ORF2 are contiguous in *C. curvus*.

Hybridization studies using a portion of ORF3 showed that this sequence was responsible for the signal obtained with the 0.5-kb *Hind*III fragment in all of the *C. concisus* strains investigated. The translational product of this ORF showed 70% similarity with the amino terminus of a hypothetical protein (408 amino acids) in *C. jejuni* (CDS Cj 0015c).

A rapid PCR assay for the identification of *C. concisus* was evaluated, using primers that annealed to the extremities of the 1.6-kb fragment. The assay was specific for *C. concisus*, and PCR products were not obtained from any of the other *Campylobacter* spp. tested. Amplicons were obtained from local (87 of 89) and Danish ($n = 2$) clinical isolates. Importantly, amplicons were also obtained from the two Swedish reference strains shown to be genetically diverse (25). It was not necessary to obtain a restriction enzyme profile of the amplicons, making the method rapid and suitable for epidemiological studies. It was not the purpose of this study to evaluate the use of the PCR assay to detect *C. concisus* directly from stools. In other studies, PCR assays have not always detected *Campylobacter* spp. in stools, suggesting the presence of inhibitors in the specimen (18).

The two clinical isolates from which PCR products were not obtained were investigated further. Hybridization studies, using the 1.6-kb fragment as a probe, supported the results of the PCR assay (data not shown). Using the 16S rDNA method (14), the resulting *Dde*I restriction profiles of the amplicons obtained from these two strains were different from the *Campylobacter* profiles described, including that of *C. concisus*. These data suggest that the two strains might have been misidentified as *C. concisus* and underscore the necessity for an unequivocal identification tool.

ACKNOWLEDGMENTS

We thank Harold Zappe for his help in the preparation of the *C. concisus* DNA library.

M. I. Matsheka and this study were funded by the Rockefeller and Carnegie Foundations through the University Science, Humanities, and Engineering Partnerships in Africa (USHEPiA) program. M. I. Matsheka thanks the University of Botswana for financial assistance received during the course of his studies. A. J. Lastovica thanks the South African Medical Research Council for financial assistance.

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