JOURNAL OF BACTERIOLOGY, Nov. 1992, p. 6800–6806
0021-9193/92/216800-07$02.00/0

Vol. 174, No. 21

Construction of a *Helicobacter pylori* Genome Map and Demonstration of Diversity at the Genome Level

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Received 4 June 1992/Accepted 2 September 1992

Genomic DNA from 30 strains of *Helicobacter pylori* was subjected to pulsed-field gel electrophoresis (PFGE) after digestion with *Not*I and *Nru*I. The genome sizes of the strains ranged from 1.6 to 1.73 Mb, with an average size of 1.67 Mb. By using *Not*I and *Nru*I, a circular map of *H. pylori* UA802 (1.7 Mb) which contained three copies of 16S and 23S rRNA genes was constructed. An unusual feature of the *H. pylori* genome was the separate location of at least two copies of 16S and 23S rRNA genes. Almost all strains had different PFGE patterns after *Not*I and *Nru*I digestion, suggesting that the *H. pylori* genome possesses a considerable degree of genetic variability. However, three strains from different sites (the fundus, antrum, and body of the stomach) within the same patient gave identical PFGE patterns. The genomic pattern of individual isolates remained constant during multiple subcultures in vitro. The reason for the genetic diversity observed among *H. pylori* strains remains to be explained.

Helicobacter pylori is frequently visible in and can usually be isolated from the gastric mucosa of patients with histological gastritis (for a review, see references 5 and 9). The organism was first successfully isolated in 1982 (23) as a result of work by Warren and Marshall (44). Originally called *Campylobacter pyloridis*, the bacterium was subsequently named *Campylobacter pylori* and more recently was transferred to the new genus *Helicobacter* on the basis of comparison of 16S rRNA sequences (36), fatty acid profiles, biochemical reactions, and morphological characteristics (11). Members of the genus *Helicobacter* are microaerophilic, with a spiral shape and four to six sheathed flagella at one or both poles.

H. pylori plays a role in the pathogenesis of gastritis and in the development of duodenal and possibly gastric ulcers (5, 9) as well as gastric cancer (28, 32). An unusual feature of all *H. pylori* isolates is the production of a large quantity of urease responsible for hydrolysis of urea to ammonia and carbon dioxide (6, 24). The urease is believed to be an important factor in the colonization of the gastric mucosa by *H. pylori* and may play a role in its ability to cause damage to mucosal tissue (12, 39). Genes responsible for catalase (27) and urease production (8, 17), for flagella (19), and for a 26,000-Da surface protein of unknown function (30) have been cloned.

Although mounting evidence indicates that *H. pylori* is pathogenic and that it appears to participate in the production of symptomatic disease in the human gastric mucosa, its origin and mode of transmission remain unexplained. To study these factors, it will be necessary to compare strains from family members and individuals in close contact with one another and to examine *H. pylori* isolates from patients before and after treatment. We have recently used pulsed-field gel electrophoresis (PFGE) to determine the genome sizes of *Campylobacter jejuni* and *Campylobacter coli* chromosomes (7) and found the technique to be helpful for the comparison of strains from outbreaks of *Campylobacter* gastroenteritis (46). Our goals in this study were to identify restriction endonucleases for PFGE analysis of *H. pylori* genome DNA which would be useful for subsequent epidemiological studies, to determine the genome size of *H. pylori*, and to construct a genome map.

MATERIALS AND METHODS

Bacterial strains and culture techniques. *H. pylori* strains used in this study were obtained from H. Lior (Lab Centre for Disease Control, Ottawa, Ontario, Canada), viz., UA763 (NCTC 11639) and UA765 (LCDC 5790), or were isolated from endoscopic biopsy specimens obtained at the University of Alberta Hospital. Biopsy material was immediately placed in broth (brain heart infusion [Oxoid Ltd., Basingstoke, United Kingdom] containing 5% bovine serum and 0.25% yeast extract) and transported to the laboratory as described previously (41). The sample was crushed with a sterile glass rod, and aliquots were streaked onto *Helicobacter* medium (brain heart infusion agar containing 5% bovine serum and 0.25% yeast extract), which was incubated at 37°C for 2 to 5 days under microaerobic conditions (5% H_2, 5% CO_2, and 84% N_2). Colonies of *H. pylori* were small, pale, and translucent. Their identity was verified by a gram-negative staining reaction; corkscrew-like motility under phase-contrast microscopy; and positive urease, oxidase, and catalase tests.

DNA preparation and restriction endonuclease digestion. After 24 to 48 h of growth, colonies were suspended in TE buffer (50 mM Tris, 5 mM EDTA, pH 8.0) and embedded in low-melting point (LMP) agarose blocks which were subsequently placed in lysis solution containing 0.25 M EDTA (pH 9.0), 0.5% lauroyl sarcosyl, and 0.5 mg of proteinase K per ml, as described previously (7). Thin slices of the blocks were washed with phenylmethylsulfonyl fluoride solution (0.175 mg/ml) for 15 min, at least three times, and then washed three times with TE buffer. The DNA slices were preincubated with 100 μl of the appropriate restriction buffer before digestion was carried out with 50 U of enzyme in

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fresh buffer. These were incubated overnight at the appropriate temperature.

The restriction endonucleases were obtained from Boehringer GmbH, Mannheim, Germany; Bethesda Research Laboratories, Inc., Gaithersburg, Md.; or Promega Corp., Madison, Wis. Restriction enzymes with hexameric recognition sequences were *Aat*II, *Ban*I, *Bcl*I, *Bgl*II, *Bmy*I, *Bss*HII, *Bst*EII, *Csp*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Mro*I, *Nde*I, *Nru*I, *Sal*I, *Sca*I, *Sma*I, *Sno*I, and *Stu*I. Enzymes with octameric recognition sequences were *Not*I, *Sfi*I, and *Sgr*AI. *Cfo*I and *Mae*I have tetrameric recognition sequences, and *Mva*I has a pentameric recognition sequence.

PFGE. *H. pylori* genome DNA was separated by the contour-clamped homogeneous electric field (CHEF) method of electrophoresis in 1% agarose gels for 24 h at 12°C and 175 to 185 V with the apparatus marketed by LKB Instruments, Inc. (2015 Pulsaphor). The pulse times were varied from 20 to 45 s to examine various-sized fragments. Bacteriophage λ concatamers (Promega) were run alongside the *H. pylori* chromosomal DNA fragments to determine their sizes. After electrophoresis, the gels were stained with ethidium bromide and photographed with a Pentax 35mm camera and Kodak Tri-X Pan film.

DNA probe blotting and hybridizations of DNA probes. The CHEF gels were blotted onto nitrocellulose membranes (BA85; Schleicher & Schuell Co., Keene, N.H.) by following previously described methods (37). Overlapping restriction fragments were isolated from LMP agarose gels, labelled by nick translation with [α-^{32}P]dATP, and hybridized to the blots at 42°C. Various gene probes were labelled with [α-^{32}P]dATP or [α-^{32}P]dCTP by nick translation or random priming (37) and hybridized at 42°C for homologous DNA probes and at 37°C for heterologous DNA probes in 50% formamide–5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate–1 mM EDTA–1× Denhardt solution–250 μg of herring sperm DNA per ml. Filters were then washed at 65°C in 5× SSC for 15 to 60 min. Autoradiograms were made with Kodak XAR-5 (Eastern Kodak, Rochester, N.Y.) by exposing the labelled membranes at −70°C for 3 to 5 days.

PCR probe construction and hybridization. The 16S primers were designed by using data from published 16S rRNA sequences from *Helicobacter felis* and *Helicobacter mustelae* (33). Two consensus regions were chosen, and two oligonucleotide primers, with the sequences 5'TCCTGGCT CAGAGTGAACGCT3' (16S-1) and 5'GGACTACCAGGGT ATCTAATC3' (16S-2), giving an amplified product of 790 bp in a polymerase chain reaction (PCR), were prepared (14). For 23S primers, a consensus sequence constructed from published sequences in the GenBank data base was used. The primers (5'GTCGGGTAAGTTCCGACCT3' [23S-1] and 5'GGCGAACAGCCATACCCTT3' [23S-2]) gave an amplified PCR product of 603 bp. For the *flaA* PCR probe, primer sequences were obtained from the sequence of the *flaA* gene of *H. pylori* (19) as suggested by D. E. Berg (11). These were 5'ATGGCTTTTCAGGTCAATAC3' (flaA-1) and 5'GCCT TAAGATATTTTGTTGAACG3' (flaA-2), which gave an amplified PCR product of 1,527 bp.

All PCR mixtures contained the following: PCR buffer (50 mM KCl, 10 mM Tris-HCl, 1.5 mM $MgCl_2$, 0.1% Triton X-100, 0.01% [wt/vol] gelatin), 0.2 mM each deoxynucleoside triphosphate, 1 μm of each primer (16S and 23S) or 10 μg of *flaA* per ml and 10 μl of *H. pylori* DNA. A Techne PHC-2 thermocycler was used for the amplifications, with the following cycling profiles: for 16S and 23S, 94°C for 1 min, 48°C for 1 min, and 72°C for 2 min for 30 cycles; for flaA, 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min for 25 cycles.

The amplified PCR products were purified by electrophoresis on a 1% LMP agarose gel, and then DNA was extracted from the agarose gel (37). The amplified products were labelled by random priming (37) by incorporation of [α-^{32}P]dCTP. Hybridization to Southern blots prepared from pulsed field gels was performed as described above except that the blots were washed in 2× SSC instead of 5× SSC.

Assessment of consistency of genomic patterns during in vitro subculture and storage. Five strains of *H. pylori* were inoculated onto plates of *H. pylori* media and were subcultured every 72 h onto fresh plates over a period of 27 days. Streaks from *H. pylori* cultures at days 9, 18, and 27 were used to prepare DNA inserts as described above. The DNA inserts were digested with the restriction endonucleases *Not*I and *Nru*I, and the digestion patterns for each strain from each interval of passaging were compared. Genomic DNA digestion patterns of each of the strains were also compared to a set of DNA inserts obtained from the same strains after a period of 6 months of storage in 20% glycerol-peptone broth at −80°C.

RESULTS

Identification of restriction endonucleases useful for *H. pylori* genome mapping. Chromosomal DNA from two representative *H. pylori* strains, UA763 (NCTC11639) and UA765 (LCDC 5790), was digested with 25 restriction endonucleases to identify restriction patterns suitable for mapping the genome. Most of the enzymes used cut *H. pylori* DNA into many fragments that were too small and too numerous for genome sizing and mapping. *H. pylori* UA763 and UA765 were not cut by *Aat*II, *Bcl*I, or *Bss*HI. In contrast, we identified two enzymes, *Not*I (recognition sequence, 5' . . .GC ↓ GGCCGC . . .3') and *Nru*I (recognition sequence 5' . . .TCG ↓ CGA . . .3'), which gave a small number of DNA fragments that were of higher molecular weight, that were well resolved after PFGE, and that appeared to be suitable for constructing a *H. pylori* genome map. With UA763 and UA765 DNA, both enzymes yielded seven fragments ranging in size from 50 to 500 kb (Fig. 1).

Determination of genomic sizes of *H. pylori* isolates. *H. pylori* genome DNAs obtained from 30 different isolates were digested with *Not*I and *Nru*I. Of the 30 strains tested, 14 were cut by both enzymes, 11 were cut only by *Not*I, and 5 were cut only by *Nru*I. Table 1 contains fragment sizes of 11 different *H. pylori* isolates which were cut into a reasonable number of well-resolved fragments by both *Not*I and *Nru*I. Using these values, the range of genome sizes for *H. pylori* was 1,608 to 1,727 kb, with an average genome size of 1,672 kb (1.67 Mb).

Comparison of different *H. pylori* isolates by PFGE. Digestion of chromosomal DNA from a series of different *H. pylori* isolates with *Not*I and *Nru*I gave widely different restriction patterns. Diagrammatic representations of genome digest patterns from 20 different strains are shown in Fig. 2 for *Not*I and Fig. 3 for *Nru*I. Of the 20 strains examined, only two, UA800 and UA802, gave identical restriction patterns when digested with *Not*I and *Nru*I. These two *H. pylori* strains were isolated from two different patients who appeared to be unrelated to one another. Biopsies were performed by different gastroenterologists on different days. All other *H. pylori* strains possessed unique fingerprints ranging from no cut sites for *Not*I in UA801 and UA825 to multiple cut sites in UA831 (Fig. 2). Similarly,

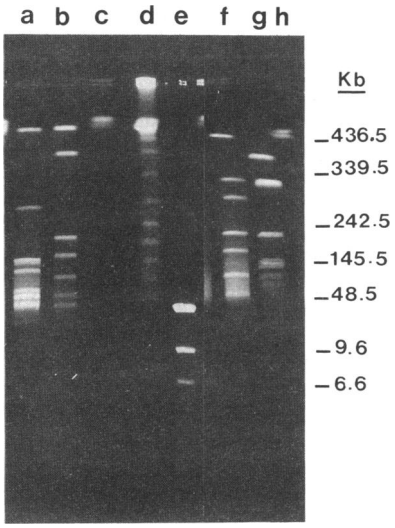

FIG. 1. PFGE (CHEF method) of *H. pylori* DNAs from strains UA763 and UA765 digested with *Nru*I and *Not*I. Lanes a (UA763) and b (UA765) contain DNAs digested with *Nru*I, whereas lanes f (UA763) and g (UA765) contain DNAs digested with *Not*I. Undigested *H. pylori* DNA is shown in lanes c and h. λ DNA concatamers (lane d) and λ DNA digested with *Hin*dIII (lane c) were used as size markers. This gel was subjected to electrophoresis for 24 h at 185 V and 12°C, with a pulse time of 35 s in a 1% agarose gel.

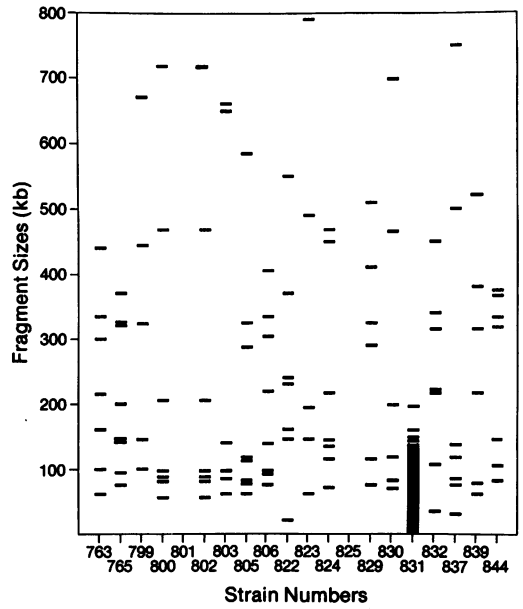

FIG. 2. Diagrammatic representation of genome DNA from 20 *H. pylori* strains restricted with *Not*I. Strains were isolated from gastric biopsy material obtained from different patients. Strains UA801 and UA825 were not cut by *Not*I. Strain UA831 produced many unresolved fragments.

with *Nru*I (Fig. 3), UA806, UA824, UA831, UA837, and UA839 are not digested, whereas UA799 and UA822 have multiple cut sites for this enzyme.

From one patient we were able to isolate *H. pylori* from biopsies taken from three different sites (i.e., the antrum, fundus, and body of the stomach). DNAs from these three isolates possessed identical PFGE patterns with both *Not*I and *Nru*I, indicating that their genomes were identical (data not shown).

Individual isolates of *H. pylori* maintained their characteristic *Not*I and *Nru*I restriction fragments after long-term storage (6 months) at −80°C. Multiple passages of the strains in the laboratory (see Materials and Methods) resulted in no change in *Not*I and *Nru*I digest patterns.

Genome map of *H. pylori* UA802. *Not*I and *Nru*I cut UA802 into seven and eight distinct fragments, respectively (Table 2 and Fig. 4). A genome map (Fig. 5) was constructed from partial digestion patterns and by hybridization of radiolabelled DNA fragments extracted from LMP agarose gels after PFGE to Southern blots prepared from CHEF gels.

Positions of various genes on the map which had been constructed were determined by hybridization of DNA probes and PCR probes. A 16S rRNA probe (pAR140) from

TABLE 1. Genome sizes of *H. pylori* isolates determined by PFGE

Strain	*Not*I digest no. of fragments	Size (kb)	*Nru*I digest no. of fragments	Size (kb)	Avg genome size (kb)
763	7	1,610	9	1,605	1,608
765	8	1,674	7	1,639	1,657
800	7	1,710	8	1,710	1,710
802	7	1,710	8	1,710	1,710
803	6	1,692	8	1,640	1,666
805	8	1,643	6	1,710	1,676
823	5	1,690	7	1,695	1,693
829	6	1,727	7	1,655	1,651
830	6	1,635	9	1,643	1,639
832	7	1,679	6	1,585	1,640
844	7	1,722	7	1,699	1,711
Avg size					1,672 ± 64

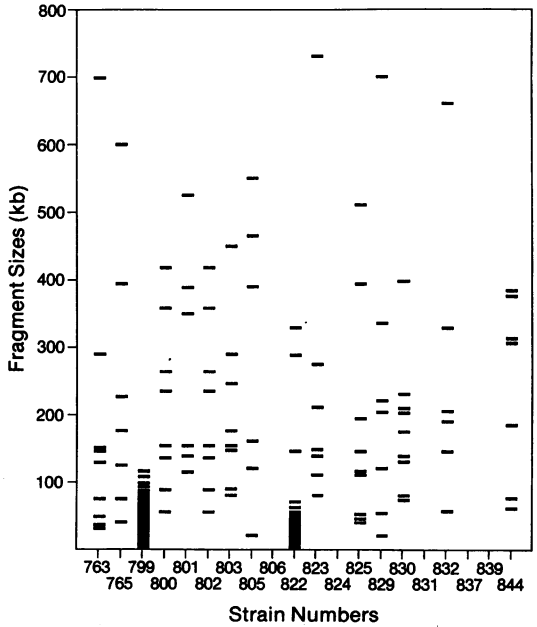

FIG. 3. Diagrammatic representation of genome DNA from 20 *H. pylori* strains restricted with *Nru*I. Strains are arranged in the same order as those in Fig. 2. Strains UA806, UA824, UA831, UA837, and UA839 were not cut by *Nru*I.

TABLE 2. *Not*I- and *Nru*I-digested fragments of *H. pylori* UA802 DNA

Fragment no.	Size (kb) of fragments digested by:	
	*Not*I	*Nru*I
1	718 ± 5	419 ± 4
2	468 ± 3	358 ± 2
3	206 ± 2	263 ± 6
4	97 ± 3	234 ± 3
5	87 ± 2	155 ± 5
6	80 ± 2	137 ± 5
7	54 ± 3	89 ± 4
8		55 ± 2
Total size	1,710 ± 12	1,710 ± 12

C. jejuni hybridized with *Not*I-1 and two *Nru*I fragments (Table 3). This would place one copy of 16S rRNA within *Nru*I-2 and a second copy at the overlap between *Not*I-1 and *Nru*I-1 (Fig. 5). However, a PCR probe prepared by using *H. pylori* DNA with primers selected from *H. felis* and *H. mustelae* 16S rRNA, which have 94% homology with *H. pylori* 16S rRNA (33), hybridized with these fragments and also with *Not*I-2 and *Nru*I-3 (Table 3). These data indicate that *H. pylori* contains three rRNA gene copies, two of which are highly homologous to the *C. jejuni* 16S rRNA gene probe and the third of which has a lower degree of homology and hybridizes only with the PCR probe.

The 23S rRNA gene probe from *Escherichia coli* (pCW1) hybridized with two *Not*I and two *Nru*I fragments (Table 3), demonstrating that two of the copies of 23S rRNA genes map in *Not*I-3 and *Nru*I-8 (Fig. 5). However, a PCR probe for 23S rRNA prepared from *H. pylori* by using primers chosen from conserved sequences from various bacteria hybridized with an additional *Not*I and *Nru*I fragment (Table 3 and Fig. 5). Therefore, *H. pylori* contains three copies of 23S rRNA, one of which cannot be detected with a heterologous 23S rRNA gene probe.

At least two of the three copies of rRNA genes show a separate arrangement of 16S and 23S genes (Fig. 5). The third copy located within the *Nru*I-1 fragment 1 and at the overlap between *Not*I-1 and -3 may be contiguous.

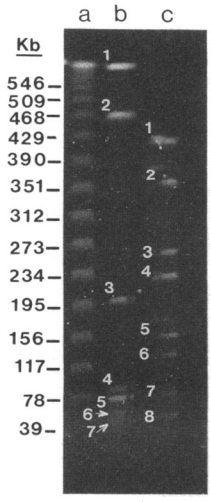

FIG. 4. PFGE of *H. pylori* UA802 DNA digested with *Not*I (lane b) and *Nru*I (lane c). This gel was subjected to electrophoresis as described for Fig. 1. Bacteriophage λ Δ39 DNA concatamers were used to determine the fragment sizes (lane a). For positions of the digest fragments shown on the gel, see Fig. 5.

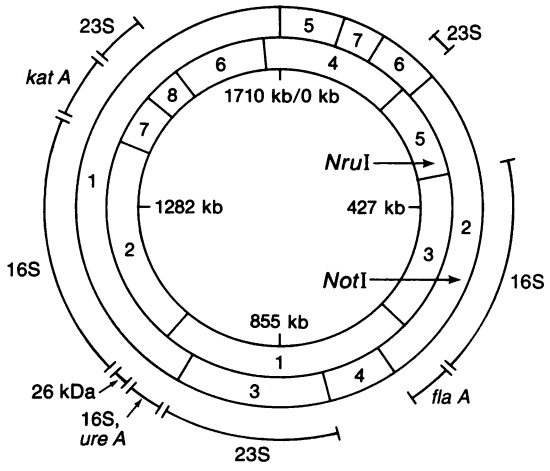

FIG. 5. Physical map of the *H. pylori* UA802 chromosome. The map was constructed from partial digestion patterns obtained with *Not*I and *Nru*I and by hybridization of ^{32}P-labelled DNA fragments after extraction from LMP agarose. DNA probes used for mapping are shown in Table 3.

The cluster of genes involved in urease production (17) was located in the same partial fragment (*Not*I-1-*Nru*I-1) as a 16S rRNA gene copy (Fig. 6); as yet, their relative positions are not known. The gene encoding the 26-kDa protein from *H. pylori* (29) overlaps the *Nru*I-1 and *Nru*I-2 fragments (Fig. 7), although no *Nru*I site was present in the published sequence of this gene (30). We have also been able to map the position of the catalase gene (*katA*) (27) within the *Nru*I-7 fragment and the flagellar gene (*flaA*) (19) which was situated in a small region at one end of the *Not*I-2 fragment (Fig. 5).

DISCUSSION

The genome sizes of *H. pylori*, which range from 1.6 to 1.73 Mb, are very close to those of *C. jejuni* and *C. coli*, at approximately 1.7 Mb (7). These sizes are small compared with those of most pathogenic bacteria (for example, genome sizes of *Staphylococcus aureus* strains range from 2.2 to 3.1 Mb [34], and *Neisseria gonorrhoeae* has a genome size of 2.3 Mb [3]) and are only about one-third the size of the *E. coli* genome (38). The small genome size of members of both the genera *Helicobacter* and *Campylobacter* is consistent with their requirement for supplemented growth medium, failure to ferment carbohydrates or degrade complex substances, and general biochemical inertness (11, 15).

C. jejuni and *C. coli* chromosomes contain three copies each of 16S and 23S rRNA genes (29, 35, 40). Similarly, the *H. pylori* chromosome also contains three copies of 16S and 23S rRNA genes. Additional mapping studies are required to determine more precisely the positions of 16S and 23S rRNA genes on the UA802 map. Only two copies were detected with the heterologous probes, whereas three were identified with homologous PCR probes. Therefore, one copy of each gene appears to have diverged more rapidly than the others. It will be of interest to determine the DNA sequences of each rRNA gene copy to ascertain their degree of relatedness. It is also notable that at least two of the three 16S and 23S

TABLE 3. DNA probes used to map gene loci on the *H. pylori* UA802 genome

Gene	Plasmid or PCR probe[a]	Species of origin[b]	Fragments hybridized[c]	Reference or source
16S rRNA	pAR140	*C. jejuni*	*Not*I-1; *Nru*I-1, -2	35
	PCR	*H. pylori*	*Not*I-1, -2; *Nru*I-1, -2, -3	33, this study
23S rRNA	pCW1	*E. coli*	*Not*I-1, -3; *Nru*I-1, -8	45
	PCR	*H. pylori*	*Not*I-1, -3, -6; *Nru*I-1, -4, -5, -8	This study
ureACDΔB	pILL594	*H. pylori*	*Not*I-1; *Nru*I-1	17
26-kDa protein	p26K	*H. pylori*	*Not*I-1; *Nru*I-1, -2	30
katA	pEX-HP2	*H. pylori*	*Not*I-1; *Nru*I-7	27
flaA	PCR	*H. pylori*	*Not*I-2, *Nru*I-1	19, this study

[a] Plasmid which carries the gene probe or PCR product obtained by using appropriate primers within the gene of interest (see text for explanation).
[b] Species from which the cloned fragment originated in the case of the DNA probes or from which the PCR product was obtained.
[c] Refer to Fig. 5 for fragment numbers. The probes were hybridized to Southern blots prepared from pulsed field gels as described in the text.

rRNA gene copies are located separately, rather than being located within an operon, as they are in *E. coli* (16, 45). We have also observed a similar separate location in at least two of three 16S and 23S rRNA genes within the *C. jejuni* UA580 and *C. coli* UA417 genomes (40).

The *H. pylori* genome displays considerably more genomic variability than that of either *C. jejuni* or *C. coli* (7, 46) or those of other species for which PFGE fingerprinting has been done (2, 13, 25). Of 30 strains examined, only 2 had identical genomic fingerprints. Conventional gel electrophoresis of *H. pylori* genome DNAs with enzymes with more frequent cut sites has also demonstrated significant genome variability (18, 21, 31). Diversity among *H. pylori* strains has also been observed by using arbitrary primer PCR (1). In addition, Ferrero and coworkers (10) reported a high degree of DNA polymorphism in different isolates of *H. pylori* examined during the construction of urease-negative mutants. However, although genomic patterns of individual *H. pylori* isolates from unrelated patients show a large degree of variability, they remained constant during subculture in the laboratory, and isolates of *H. pylori* from the antrum, fundus, and body of the stomach of one patient had identical genome patterns. Protein profiles (41) and enzyme studies (11, 24) have suggested little phenotypic variation among *H. pylori* isolates.

The unusual genomic diversity of *H. pylori* isolates observed by us and by others (1, 10, 18, 21, 31) requires further study. It is possible that *H. pylori* strains undergo genomic rearrangements after they infect a new human host, perhaps in response to stresses associated with colonization and adaptation to a new environment. This could explain the wide variation in genomic patterns observed among strains isolated from different individuals.

Several hypotheses can be advanced to account for the observed genomic diversity in *H. pylori*. (i) Variability could be explained by movement of short repetitive DNA sequences (20) which have been noted in a number of different species. (ii) Another mechanism, which has been observed in *Streptomyces* spp., could involve the amplification of particular chromosomal DNA sequences, possibly accompanied by the deletion of adjacent DNA (4). (iii) *H. pylori* DNA may undergo changes in nucleotide sequence which are not associated with phenotypic changes (silent mutations). The presence of an *Nru*I site within the 26-kDa protein gene in *H. pylori* UA802 but not in the original DNA sequence (30) would support this suggestion, as would the apparent sequence diversity observed in one of each of the three 16S and 23S rRNA gene copies. (iv) Genomic rearrangements may be associated with uptake of DNA by natural transformation (18, 26, 43). However, *C. jejuni* and *C. coli* also undergo natural transformation (42) but show considerably less genomic diversity than *H. pylori*. Preliminary experi-

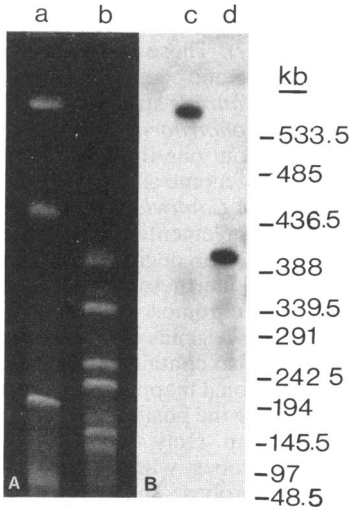

FIG. 6. (A) PFGE of *H. pylori* UA802 digested with *Not*I (lane a) and *Nru*I (lane b) as described for Fig. 1. Southern blot of the gel shown in panel A hybridized to a ^{32}P-labelled urease gene (*ureACDΔB*) probe (pILL594) (17) (lanes c and d).

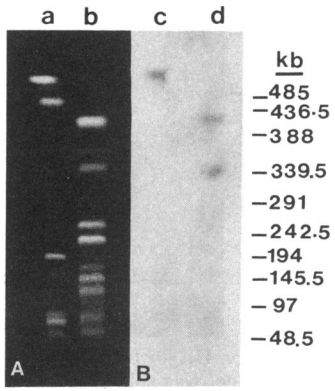

FIG. 7. PFGE of *H. pylori* UA802 digested with *Not*I (lane a) and *Nru*I (lane b). Southern blot of gel shown in panel A hybridized with the ^{32}P-labelled 26-kDa gene (p26K) (30) (lanes c and d).

ments suggest that natural transformation under laboratory conditions (43) does not appear to be associated with genomic diversity in *H. pylori*. (v) DNA in some strains of *H. pylori* may be protected from restriction endonuclease digestion by the production of an endogenous methylase(s) (22) which is able to methylate nucleotides within the recognition sequences for *Not*I, *Nru*I, or other endonucleases. Any or all of these factors may play a role in the genomic diversity observed in *H. pylori*.

ACKNOWLEDGMENTS

We thank R. W. Sherbaniuk for biopsy material; A. Labigne, P. O'Toole, and P. Nuijten for DNA probes; and H. Lior for *H. pylori* strains. We acknowledge the contributions of E. Stockdale in the design and hybridization of PCR probes and D. E. Berg for helpful discussion.

A portion of this work was supported by the Canadian Bacterial Diseases Network (Centres for Excellence Program). D.E.T. is a Medical Scientist with the Alberta Heritage Foundation for Medical Research.

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