Genetic diversity in *Helicobacter pylori* strains may affect the function and antigenicity of virulence factors associated with bacterial infection and, ultimately, disease outcome. In this study, DNA diversity of *H. pylori* isolates was examined by analysis of *vacA* genotypes and by restriction fragment length polymorphism (RFLP) analysis of *H. pylori*-associated genes (vacA, cagA, flaA, ureAB, and ureCD). Thirty-seven *H. pylori* isolates from 26 patients were successfully classified into distinct *vacA* allelic genotypes. The signal sequence allele s1 (31 of 37) predominated over the s2 allele (6 of 37) and was significantly associated with the occurrence (past or present) of gastric ulcers. A novel midregion allele, designated as m3, has been identified in two *H. pylori* isolates which could not be typed with midregion allele m1- or m2-specific primers. Additionally, significant nucleotide diversity yielding different amino acid sequences was demonstrated by DNA sequencing of *vacA* fragments from clinical isolates of *H. pylori*. Furthermore, RFLP analysis of 45 *H. pylori* isolates (including 15 paired isolates) obtained from antrum and corpus biopsy specimens from 30 individual patients showed remarkably high interhost diversity (one patient, one *H. pylori* strain) and intrahost identity in gene sequences coding for VacA, CagA, flagellin, and urease. Only in a single patient was a minor genotypic variation at different anatomic sites within the stomach identified. These data warrant the detailed analysis of the effect of genetic diversity on the function and antigenicity of *H. pylori*-associated virulence factors.

**Materials and Methods**

**Subjects.** Thirty patients infected with *H. pylori* were enrolled in the study (Table 1). They underwent upper gastrointestinal endoscopy in an outpatient center in Mainz, Germany. Thirteen patients (43%) were diagnosed with present or past peptic ulcers. One patient (no. 17) exhibited gastric cancer. Histologically, all of the subjects except one (no. 18) showed chronic active gastritis. The patients were from 22 to 83 years of age (mean, 54 years) and lived either within or in close proximity to Mainz.

**H. pylori strains.** Forty-five individual *H. pylori* isolates were obtained from either antrum (a) or corpus (c) specimens; they are listed in Table 1. Paired isolates from antrum and corpus specimens were obtained from 15 patients. Biopsy specimens were inoculated onto Columbia agar (Oxoid, Basingstoke, United Kingdom) to which had been added 7% human erythrocytes (kindly
provided by W. E. Hitzler of the University of Mainz blood bank) and an H. pylori-selective supplement (Oxoid). After incubation at 37°C under microaerobic conditions for up to 7 days, colonies that exhibited characteristic morphologies were identified as H. pylori by positive urease, catalase, and oxidase tests and by typical appearance on Gram’s stain. Each H. pylori isolate was further characterized by susceptibility to metronidazole, using the Epsilometer test (E test) as described in detail elsewhere (4). Individual H. pylori strains were inoculated into 5 ml of brucella broth (Oxoid) with 10% fetal calf serum (PA Laboratorien, Linz, Austria), incubated at 37°C in a microaerobic atmosphere for 2 to 3 days with continuous agitation, and pelleted for DNA preparation. For preservation, 150 µl of dimethyl sulfoxide (Merck, Darmstadt, Germany) was added to 1-ml aliquots of liquid cultures, which were then stored frozen at −70°C. Reference strains H. pylori NCTC 11638 (DSM 10524), H. pylori NCTC 11637 (DSM 4867), and Helicobacter chinensis ATCC 35683 (DSM 5359) (obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) served as controls.

**Preparation of genomic DNA.** Bacteria were pelleted, resuspended in 567 µl of TE buffer (10 mM Tris-Cl and 1 mM EDTA, pH 8.0), 30 µl of 10% sodium dodecyl sulfate, and 3 µl of a 20-mg/ml solution of proteinase K, and incubated for 1 h at 37°C. After the addition of 100 µl of 5 M NaCl solution and 80 µl of 10% hexadecyltrimethylammonium bromide (CTAB, Sigma, St. Louis, Mo.) in 0.7 M NaCl solution, the mixtures were incubated at 65°C for 10 min. DNA was extracted with an equal volume of chloroform-isopropyl alcohol (24:1) and then with phenol-chloroform-isopropyl alcohol (25:24:1), precipitated with 0.6 volume of isopropanol, washed with 70% ethanol, and redissolved in 50 µl of TE buffer, as described in detail elsewhere (26).

**PCR.** The primers used in this study were synthesized on an automated and computer-controlled Parallel Array Synthesizer by the automated phosphoramidite coupling method and purified by standard protocols (Gibco BRL, Eggenstein, Germany). The primers F6 (5′-GCTTCTTCCCAGCAACATGC-3′) and R20 (5′-TGTCAGGGTGTCCCACTGTG-3′) were used to amplify a 1,162-bp vacA fragment (29) which is located in the midregion of vacA (bp 1657 to 2818) (20). The cap67 primers EZ3 (5′-AGTAAAAAGAGAGAAAACATAG-3′) and R009 (5′-AATAAGCCTTAGCTTTTTGGAAATC-3′) were used to amplify a product of 1,320 bp (29). The ureA primers (5′-AAGGAAAGAAGATGATG-3′ and 5′-ACCTTTTATTGCTGTTG-3′) amplified a 2,240-bp product (10), and the ureCD primers (5′-TGGAAGTAGTGAGGAGGGGAGG-3′ and 5′-ATCATGACATCGCGAGTTAAATAATGG-3′) amplified a 1,720-bp product (1). The flaA primers (5′-ATGGCTTTCAGGTCAATAC-3′ and 5′-GCTTAAGATATTTCTCTTACCACCA-3′) amplified a 1,720-bp product (20). For allelic typing, we utilized a primer set specific for amplification of m1, m2, s1, s2, s1a, and s1b as described previously (2). The m3 allele, identified in this report, was amplified with the vacA m3-specific reverse primer vraC-3 (5′-CCTTATTACGCCGCAAAAC-5′) and the forward primer VAS-3 (5′-GGATAAGTTTCCCA-3′). Two PCR mixtures contained 1× PCR buffer (10 mM Tris-Cl, [pH 8.3], 50 mM KCl, 1.5 mM MgCl2, 0.01% [wt/vol] gelatin), 200 µM each deoxynucleoside triphosphate (AppligeneOncor, Heidelberg, Germany), 25 pmol of each primer, 5 U of Taq polymerase (Eurobio, Paris, France), and 10 ng of H. pylori genomic DNA in a total volume of 50 µl. Amplifications were carried out in a OmnigenE thermal cycle carried out in an OmnigenE thermal cycle (Biozym, Teddington, Middlesex, United Kingdom). The PCR amplification included an initial denaturation step at 94°C for 2 min followed by 35 cycles with the following profiles: for vacA, 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min; for cap67, 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; for ureA, 94°C for 1 min, 50°C for 1 min, and 72°C for 2 min; and for vacC allelic typing, 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min. DNAs from H. pylori NCTC 11638 and H. pylori NCTC 11637 were assayed in each PCR run as positive controls, and DNA from H. cinaedi ATCC 35683 served as a negative control. Individual PCR products were electrophoresed on agarose gels, stained with ethidium bromide, and photographed.

**RFLP analysis.** vacA, cap67, flaA, ureAB, and ureCD gene fragments were amplified by PCR and then digested with HaeIII (for ureAB), HglI (for vacA), BglII (for vacA, flaA, and ureCD), Hinfl (for cap67), HphI (for vacA), and/or SacIII (for flaA, ureAB, and ureCD) for 3 h at 37°C in the appropriate buffer recommended by the supplier (New England Biolabs, Schwalbach/Taunus, Germany). Each, H. pylori isolate was characterized by nine individual gene-enzyme patterns. DNA digests were electrophoresed on 2% agarose gels, stained with ethidium bromide, and photographed.

**Sequencing.** PCR products to be sequenced were purified by using a QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany) and sequenced by using an Applied Biosystems model 373A DNA sequencing system and the Qiagen fluorescent dye terminator kit (5).
DyeDeoxy Terminator Cycle Sequencing Kit (Applied Biosystems, Weiterstadt, Germany). Individual PCR products were subcloned into the pCR 3.1 vector (TA Cloning Kit; Invitrogen, NV Leek, The Netherlands) for sequence analysis. The primers vaca-f2 (5'-GTGGATGCTCATACAGCT-3'; location in fragment, 268 to 285 bp) and vaca-b2 (5'-GAAATCCCCTTGAATGG-3'; location in fragment, 761 to 777 bp) were employed for vacA segment sequence analysis. The T7 and pCR 3.1 reverse sequencing primers in the TA Cloning Kit (Invitrogen) were used for sequencing of PCR products inserted in the vector.

**RESULTS**

vacA and cagA genes were frequently detected in *H. pylori* isolates. Forty-five *H. pylori* isolates were obtained from 30 patients. In 40 of 45 *H. pylori* isolates, a 1,162-bp segment of vacA (8, 20, 23, 25) could be amplified with the vacA specific primers F6 and R20. However, in 5 of 45 *H. pylori* isolates obtained from four patients, PCR amplification yielded a larger vacA fragment (Fig. 1). Three of these fragments were further subjected to DNA sequence analysis. The cagA gene could be detected in 43 of 45 *H. pylori* isolates (Table 1).

**Predominance of the s1-m1 and s1-m2 vacA genotypes.** Although three individual *H. pylori* isolates (Mz10a, Mz19a, and Mz26a) could not be classified into a distinct vacA allelic genotype category, 37 *H. pylori* isolates from 26 patients were successfully classified (Table 2). Among these, five of six possible combinations of signal sequence and midregion types (s1a-m1, s1a-m2, s1b-m1, s1b-m2, and s2-m2) could be identified. The vacA signal sequence s1a allele (26 of 37) predominated over the s1b (5 of 37) and the s2 (6 of 37) alleles. Of note, both s1a- and s1b-specific fragments from one *H. pylori* isolate (Mz26a) could be reproducibly amplified. In contrast to the signal sequence alleles, the frequency of the midregion allele m1 (19 of 37) appeared to be similar to that of the m2 allele (18 of 37). However, two *H. pylori* isolates (Mz10a and Mz19a) were not typeable in the vacA midregion and were therefore subjected to DNA sequence analysis. For statistical analysis, the vacA typing data were classified into four groups: s1-m1, s1-m2, s2-m1, and s2-m2. The distribution of these groups was analyzed by using Fisher’s exact test. Among 37 *H. pylori* isolates from 26 patients, more than 80% (31 of 37 individual isolates from 21 of 26 patients) contained signal sequence type s1, approximately 50% (19 of 37 individual iso-

### TABLE 2. Distribution of vacA alleles in 37 *H. pylori* isolates from 26 patients

<table>
<thead>
<tr>
<th>Midregion type</th>
<th>No. of isolates (patients) with signal sequence type:</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>s1a</td>
<td>s1b</td>
</tr>
<tr>
<td>m1</td>
<td>17 (12)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>m2</td>
<td>9 (6)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>26 (18)</td>
<td>5 (3)</td>
</tr>
</tbody>
</table>

* Five of six possible combinations of vacA signal sequence and midregion types could be identified; the s2-m1 combination was not detected. Of note, the vacA midregion alleles and signal sequence types of three *H. pylori* isolates obtained from individual patients could not be determined (data not shown in table).

![Fig. 2](http://cvi.asm.org/...)

**FIG. 2.** Representative RFLP analyses of *H. pylori*-associated genes from four paired *H. pylori* isolates. (a) vacA fragments digested by *Hha*I; (b) cagA fragments digested by *Hin*fI; (c) ureAB fragments digested by *Hae*III; (d) ureCD fragments digested by *Sau*3AI; (e) flaA fragments digested by *Hha*I. The different RFLP patterns of one pair of *H. pylori* isolates (Mz17a and Mz17c) are shown in panel e. M, size markers; A, *H. pylori* isolate from the antrum biopsy specimen; C, *H. pylori* isolate from the corpus biopsy specimen. 1, 6, 7, 11, and 17, *H. pylori* Mz1, Mz6, Mz7, Mz11, and Mz17, respectively.
lates from 13 of 26 patients) exhibited the s1-m1 type, and less than 20% (6 of 37 individual isolates from 5 of 26 patients) were of type s2-m2; the s2-m1 genotype could not be detected ($P < 0.01$ for isolates; $P < 0.05$ for patients).

The vacA s1 allele correlates with peptic ulcers. All of the H. pylori isolates obtained from patients with a history of peptic ulcers (16 of 37) contained s1. Similarly, all of the H. pylori isolates bearing s1 (31 of 37) were found to be cagA positive (Table 3). The associations between vacA signal sequence type s1 and peptic ulcers and between s1 and cagA status were statistically significant ($P < 0.05$, Fisher's exact test [for isolates]). In contrast, no significant relationship between vacA midregion types and the presence of peptic ulcers or cagA was found ($P > 0.05$, Fisher's exact test [for isolates or patients]). Considering the small sample of cagA-negative isolates, however, the relevance of the vacA allele to cagA status must be further investigated.

Each individual harbors a unique H. pylori strain. Forty-five H. pylori isolates (including 15 paired isolates) obtained from antrum and corpus biopsy specimens collected from 30 individual patients were subjected to RFLP analysis. H. pylori strains from 30 individual patients were unique; i.e., the RFLP types defined by the unique combination of nine individual gene-enzyme patterns, differed from patient to patient. In contrast, paired H. pylori isolates obtained from corpus and antrum biopsy specimens collected from individual patients yielded identical RFLP types. This striking observation was made for all paired RFLP types in 14 of 15 patients, and representative examples are shown in Fig. 2. For two H. pylori isolates obtained from an individual patient (no. 17), minor differences in just one (flaA fragment digested with HhaI) of the nine gene-enzyme patterns were observed (Fig. 2e). In summary, H. pylori strains from individual patients were unique; i.e., the RFLP types defined by the unique combination of nine individual gene-enzyme patterns differed from patient to patient. In contrast, paired H. pylori isolates obtained from corpus and antrum biopsy specimens from 14 of 15 individual patients were identical.

Direct sequencing of the flaA PCR products of H. pylori Mz17a and Mz17c revealed the existence of two flaA alleles in isolate Mz17a, which was confirmed by sequence analysis of subcloned flaA PCR products. One flaA allele in H. pylori Mz17a was identical to that in isolate Mz17c; the other flaA allele exhibited point mutations (Fig. 3). Additionally, for one H. pylori isolate (Mz26a), two types of vacA signal sequences (s1a and s1b) could be reproducibly amplified and verified by DNA sequence analysis. To this end, it is not clear whether this isolate harbors two individual functional vacA genes with different signal sequences. Since the RFLP analysis of other genes in H. pylori Mz26a showed a unique pattern, it is highly likely that isolate Mz26a indeed represents a single H. pylori strain with two different vacA signal sequences.

**Diversity of amino acid sequences existed in the vacA products.** The DNA sequences of four of the above-described variant vacA gene segments from H. pylori Mz19a, Mz26a, Mz28a, and Mz29a were analyzed, and the deduced amino acid sequences were compared with those of two reference H. pylori strains (Tx30a and NCTC 11638), as shown in Fig. 4. These four H. pylori strains were VacA positive, and all exhibited the s1-m2 vacA genotype except strain Mz19a, which contained s2 and the midregion allele designated as m3 in this report. In comparison, the H. pylori reference strain Tx30a is a Tox− strain exhibiting the s2-m2 vacA genotype (2) and NCTC 11638 is a Tox+ strain exhibiting the s1a-m1 vacA genotype (20).

Analysis of DNA and deduced amino acid sequences revealed four salient findings: (i) significant nucleotide diversity in the analyzed midregion of vacA, yielding different amino acid sequences; (ii) the existence of a novel midregion allele, designated as m3, in H. pylori Mz19a, which could not be typed with m1- or m2-specific primers; (iii) the presence of a 23-amino-

**TABLE 3. Association of vacA signal sequence type s1 with cagA status and peptic ulcers**

<table>
<thead>
<tr>
<th>vacA type</th>
<th>cagA</th>
<th>Past or present peptic ulcers</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>s1</td>
<td>31 (21)</td>
<td>0 (0)</td>
<td>16 (10)</td>
</tr>
<tr>
<td>s2</td>
<td>4 (4)</td>
<td>2 (1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>m1</td>
<td>19 (13)</td>
<td>0 (0)</td>
<td>7 (5)</td>
</tr>
<tr>
<td>m2</td>
<td>16 (12)</td>
<td>2 (1)</td>
<td>9 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>35 (25)</td>
<td>2 (1)</td>
<td>16 (10)</td>
</tr>
</tbody>
</table>

* Shown are the number of H. pylori isolates followed by the number of patients (in parentheses). Note that H. pylori isolates bearing s1 were cagA positive and that all isolates obtained from patients with peptic ulcers contained s1. No significant relationship was found between vacA midregion type and the presence of cagA or peptic ulcers ($P > 0.05$, Fisher's exact test) for isolates or patients.
acid insertion (Tx30a residues 501 to 523) encoded by vacA midregion type m2 of Tox2 strains; and (iv) amino acid divergence between a single pair of cysteine residues (11 amino acids apart in the reference strain NCTC 11638 and clinical strain Mz19a but 13 amino acids apart, with divergent sequences, in the other three clinical isolates and Tx30a).

The parallel DNA sequence analysis of the vacA segment from reference strain NCTC 11638 yielded identical results, as published previously (20). However, there was considerable amino acid sequence diversity (6.2%) between the three investigated clinical Tox1 strains exhibiting the s1-m2 vacA genotype and the wild Tox2 strain Tx30a, although all four of these H. pylori strains bear the midregion allele m2. Furthermore, we identified a marked amino acid sequence difference (37.7%) between the Tox1 H. pylori strain NCTC 11638, which exhibited the midregion allele m1, and the three Tox2 clinical isolates.

FIG. 4. Alignment of amino acid sequences deduced from six allelic vacA fragment nucleotide sequences. Dashes indicate amino acid identity compared with the sequence listed above. Slashes denote the absence of corresponding amino acids. The 23-amino-acid insertion is indicated by the solid rectangular box. A single pair of cysteine residues is marked by stars. The locations of m1- and m2-specific primers are indicated by arrows. The location of the m3-specific primer (5'-CTGTTAGGCCCGCAGAAAC-3') is in boldface and framed with dotted lines. Tx30a, Tox2 H. pylori strain with s2-m2 vacA genotype; its vacA sequence has been reported by Atherton et al. (2). 11638, NCTC 11638 (DSM 10242), Tox1 H. pylori strain with s1a-m1 vacA genotype; its vacA sequence has been reported by Phadnis et al. (20) and was also analyzed as a reference in this study. Mz26a, Mz28a, and Mz29a, Tox2 H. pylori strains with s1-m2 vacA genotype, isolated in this study. Mz19a, ToxH. pylori strain with vacA signal sequence type s2 and the novel vacA midregion designated as m3 in this study. This region showed 9.3% deduced amino acid diversity compared to the m1 region in strain NCTC 11638 and 42.4% deduced amino acid diversity compared to the m2 region in strain Tx30a. Cytotoxin-induced vacuolation of HeLa cells was assessed visually by light microscopy and quantified by neutral red uptake assay as described elsewhere (7).
with regard to the m2 allele. H. pylori Mz19a could not be typed with m1- or m2-specific primers. This was apparently due to a difference in the DNA sequence in this strain, corresponding to NCTC 11638 nucleotides 2374 to 2663 of vacA (20). This region showed 9.3% nucleotide or deduced amino acid diversity when compared to the m1 region in strain NCTC 11638, specifically within the DNA sequence of the 20-nucleotide reverse primer employed for the m1 typing (6 of 20 nucleotides different), and 29% nucleotide or 42.4% deduced amino acid diversity when compared to the m2 region in strain Tx30a (Fig. 4). This region exhibited a higher degree of DNA sequence similarity to the m1 region than to the m2 region. This vacA midregion allele was designated as m3 and could be successfully amplified with the m3-specific primers, as described in Material and Methods. Thus, two H. pylori strains (Mz10a and Mz19a) considered to be untypeable by the m1-m2 scheme described by Atherton and coworkers (2) contained the m3 allele identified in this report, in combination with vacA signal sequence type s1a and type s2.

**DISCUSSION**

We employed vacA genotyping to characterize 37 individual H. pylori isolates derived from 26 patients. Thirty-one isolates contained the signal sequence type s1, predominantly s1a, and 19 isolates exhibited the s1-m1 genotype. The s2-m1 genotype was not found. These data support the pioneering study of Atherton and coworkers, who also failed to detect the s2-m1 genotype (2). However, signal sequence type s1, particularly s1a, and the genotype s1-m1 appeared to occur more frequently in our study, suggesting that the distribution of H. pylori vacA genotypes obtained was considerably different from those previously reported (2). This is further supported by the detection of a high frequency of the cagA gene (43 of 45 isolates [Table 1]) in our study, whereas Atherton and coworkers detected cagA gene only in 60% (35 of 59) of H. pylori strains. Despite these differences, the proportion of the human subjects in this study with a history of peptic ulcers (43%) was similar to that (41%) reported by Atherton et al. (2), indicating that the clinical H. pylori isolates in both studies were obtained from patients with similar severity of clinical disease.

In this study, three H. pylori isolates could not be classified into distinct vacA genotypes. DNA sequence analysis of the vacA midregion from one midregion-untypable H. pylori isolate revealed significant nucleotide diversity when compared to the m1 or m2 region, especially a mutation of 6 nucleotides within the region of the 20-nucleotide primer employed for m1 typing. Furthermore, this midregion could be combined with both vacA signal sequence types s1 and s2. Thus, we propose that m1-m2 untypeable H. pylori strains harbor a different midregion allele, designated as m3 in this report, which can be amplified with the m3-specific primer vacm3-b and the primer VA3-F. In another H. pylori isolate, both the s1a and s1b fragments, confirmed by DNA sequence analysis, could be amplified with primer sets specific for typing s1a and s1b. These results suggest that a supplement to the present primer sets may be helpful for classification of individual H. pylori strains.

The H. pylori-associated genes vacA, cagA, flaA, ureAB, and ureCD, all encoding virulence factors, showed significant interhost DNA sequence diversity as defined by RFLP analysis of PCR products (Fig. 2). Remarkably, H. pylori strains from individual patients appeared to be unique (one patient, one strain). Strict clonality in each host was underlined by the genotypic identity of paired isolates obtained from corpus and antrum biopsy specimens of individual patients. Only in a single patient was a minor genotypic variant identified at different anatomic sites within the stomach.

In general, significant DNA diversity may reflect a high mutation frequency and/or recombination of individual gene alleles, as well as host immune selection against particular genotypes (2, 9). Additionally, this PCR-based RFLP analysis is useful for clinical applications and for epidemiological studies of H. pylori infections. We are currently implementing the evaluation of genetic diversity in H. pylori-associated genes to characterize the clonality and epidemiology of H. pylori in defined populations (i.e., family members).

Five of 45 H. pylori isolates exhibited variant vacA gene fragments as determined by PCR analysis (Fig. 1). DNA sequence analysis of the variant vacA segments from three Tox+ H. pylori strains (vacA genotype s1-m2) indicated the presence of a deduced 23-amino-acid insertion, encoded by the midregion of vacA, which was absent in the Tox+ reference strain NCTC 11638 (vacA genotype s1a-m1) and in strain Mz19a (s2-m3). The insertion contained a potential ATP or GTP binding site motif (GNYLGKS), corresponding to a Walker A consensus sequence or a phosphate-binding loop (13, 22). A similar insertion was previously detected in Tox+ H. pylori strains (Tx30a, 87-203, and 86-313) exhibiting the m2 vacA allele (2). It was reported to be absent in Tox+ H. pylori strains with type m1 vacA alleles (2, 8, 20, 23, 25). Taken together, these data indicate that this insert occurs in Tox+ as well as Tox+ H. pylori strains which exhibit the m2 allele of vacA.

Two cysteine residues were identified in the amino acid sequences deduced from all of the published vacA gene sequences (2, 8, 20, 23, 25). These amino acid residues are located at the C-terminal end of the mature proteins (23), 11 or 13 amino acids apart. In the four Tox+ strain amino acid sequences published, the cysteines flank 11 amino acids of identical sequence (8, 20, 23, 25). In contrast, the cysteine residues flank 13 amino acids with divergent sequences in two Tox− strains (Tx30a and 87-203) exhibiting the s2-m2 vacA genotype (2, 8). In our s1-m2 vacA H. pylori strains (Fig. 4), two cysteine residues are also separated by 13 amino acids with divergent sequences. Moreover, these strains are all Tox−. Thus, there is apparently no direct correlation between VacA function and the cysteine-to-cysteine sequences.

Recent data indicate that VacA represents an oligomer (>600 kDa) composed of at least six or seven 95-kDa monomers, which are processed to yield a 37-kDa N-terminal subunit and a 58-kDa C-terminal subunit (16, 25). VacA binding can be inhibited by antibodies reacting exclusively with the 58-kDa fragment (11). The sequenced vacA segments in this study contained a part of the region encoding the 58-kDa fragment. The amino acid sequence diversity, the presence or absence of a potential ATP or GTP binding site, and the sequence divergence between a single pair of cysteine residues within portions of the vacA product may impact on the structure, antigenicity, and function of the 58-kDa fragment.

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