Isolation and Characterization of a HpyC1I Restriction-Modification System in Helicobacter pylori

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Using transposon shuttle mutagenesis, we identified six Helicobacter pylori mutants from the NTUH-C1 strain that exhibited decreased adherence and cell elongation. Inverse polymerase chain reaction and DNA sequencing revealed that the same locus was interrupted in these six mutants. Nucleotide and amino acid sequences showed no homologies with H. pylori 26695 and J99 strains. This novel open reading frame contained 1617 base pairs. The amino acid sequence shared 24% identity with a putative nicking enzyme in Bacillus halodurans and 23 and 20% identity with type IIS restriction endonuclease PseI and MlyI, respectively. The purified protein, HpyC1I, showed endonuclease activity with the recognition and cleavage site 5’-CCATC(4/5)-3’. Two open reading frames were located upstream of the gene encoding HpyC1I. Together, HpyC1I and these two putative methyltransferases (M1.HpyC1I and M2.HpyC1I) function as a restriction-modification (R-M) system. The HpyC1I R-M genes were found in 9 of the 15 H. pylori strains tested. When compared with the full genome, significantly lower G+C content of HpyC1I R-M genes implied that these genes might have been acquired by horizontal gene transfer. Plasmid DNA transformation efficiencies and chromosomal DNA digestion assays demonstrated protection from HpyC1I digestion by the R-M system. In conclusion, we have identified a novel R-M system present in H. pylori strains. Disruption of this R-M system results in cell elongation and susceptibility to HpyC1I digestion.

Helicobacter pylori, a spiral, Gram-negative bacteria, was first isolated in 1982 (1). It is an important human pathogen, responsible for type B gastritis and peptic ulcer and for increasing the risk of gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma of the stomach in humans (2–5).

Adhesion is the first step of bacterial colonization and important for persistent infection. Bacterial adhesins bind to specific host cell macromolecule receptors, resulting in tropism. Adherence to gastric mucosa may be advantageous to H. pylori by protecting against mucosal shedding into the gastric lumen. The most well-defined H. pylori adhesin-receptor interaction to date is between the Lewis b (Leb)1 blood group antigen-binding adhesin, BabA, and the H, Leb, and related ABO antigens (6, 7). Further, it has been suggested that the interaction between H. pylori adhesin, SabA, and cellular receptor, sialylated Lewis x (sLe xa), promoted persistent infection (8). However, Leb antigens are only abundant among individuals with the O blood type. Seroepidemiology studies revealed that infection rates are the same in patients with different blood types (9), and some clinical H. pylori strains did not bind either Leb or sLe xa antigens (8). Therefore, we used an H. pylori mutant library to identify genes involved in cell adhesion. After initial screening, we isolated mutants with decreased adherence. These mutants appeared elongated on Gram stain when compared with wild type controls. However, further characterization revealed that the interrupted operon was a novel restriction-modification (R-M) system in H. pylori.

R-M systems in bacteria protect against invasion of foreign DNA (10). The restriction endonuclease recognizes a specific sequence, and the cognate methyltransferase modifies the same sequence to differentiate self-DNA from foreign DNA (10). Thousands of restriction enzymes have been purified and characterized. Restriction enzymes are traditionally divided into three types according to subunit composition, cleavage site, sequence specificity, and cofactor requirements (10). Type II R-M systems, consisting of a restriction endonuclease and a paired methylase, are the most well-known and have great practical value (10).

Based on sequence similarities, there are more than 20 putative R-M systems in H. pylori 26695 strain. A previous study showed 14 Type II R-M systems with biochemical activity in H. pylori 26695 strain (11). Comparing the complete sequences of 26695 and J99 strains, the R-M systems of these two strains are diverse. The difference of R-M systems results in the barrier of interstrain plasmid DNA transfer (12) and chromosomal DNA transformation (13), but the biological significance of so many R-M systems in H. pylori is still unclear.

EXPERIMENTAL PROCEDURES

Bacteria Strains and Culture Condition—Clinical isolates were obtained at National Taiwan University Hospital (NTUH) as previously described (14). H. pylori strains were grown on Columbia blood agar plates containing 5% sheep blood and chloramphenicol (4 μg/ml) or kanamycin (10 μg/ml) and incubated for 2–3 days in microaerophilic conditions (5% O2, 10% CO2, 85% N2) at 37 °C. For bacterial growth curve examination, H. pylori were grown in the Brucella broth containing 5% fetal calf serum.

Cell Adherence Assay—SC-M1, a cell line established from primary...
human gastric cancer tissue (15), was used in this study. This cell line was proved to be Leb− and aLea− positive by monoclonal antibodies against Leb (Seikagaku, Tokyo, Japan) and aLea (Chemicon, Temecula, CA), respectively. The cells were grown in a 24-well culture plate (RPMI 1640 (Invitrogen) medium, supplemented with 10% fetal calf serum and 5% CO2, 37 °C and infected with H. pylori (multiplicity of infection = 1:100). After a 30-min co-cultivation at 37 °C, nonadherent bacteria were removed by washing three times with phosphate-buffered saline buffer. SC-M1 cells with adherent H. pylori were trypsinized, serially diluted in normal saline, and spread on the Columbia blood agar plates. Recovered adherent bacterial colonies were counted. Wild type H. pylori NTUH-C1 strain served as a positive control, and each mutant strain was compared with the wild type strain.

Observation of Bacterial Morphology—The morphologies of H. pylori wild type and mutant strains were observed under a light microscope after Gram stain and captured by CoolSnap-pro software (Media Cybernetics, Silver Spring, MD). More than 10 fields were examined on each slide, and the lengths of bacteria were measured in 30 bacteria of five−10 different fields by CoolSnap-pro software (Media Cybernetics).

Inverse PCR and DNA Sequencing—To identify genetic loci interrupted by the transposon, genomic DNA of mutant strains were extracted and subjected to inverse PCR and DNA sequencing as previously described (16).

Reknockout of hpyC1IR—Intragenic sequences of hpyC1IR (nucleotides 580−895) were cloned into a TA vector, pGEM-T easy (Promega, Madison, WI). A cat gene (17) was ligated into the SalI site of pGEM-T easy/hpyC1IR (nucleotides 580–895). The plasmid was transformed into the wild type NTUH-C1 strain to generate the hpyC1IR reknockout mutant by integration.

Complementation of hpyC1IR—The upstream 198 base pair and hpyC1IR coding sequence was cloned into pGEM-T easy (Promega). A cat gene was ligated into the SalI site of pGEM-T easy/hpyC1IR (nucleotides −198 to 1617). The plasmid was transformed into a hpyC1IR mutant strain to generate the hpyC1IR complementation strain by chromosomal integration. The gene alignment of the hpyC1IR complementation strain was confirmed by PCR using different combinations of primers.

Constructions of hpyC1IM1 and hpyC1IM2 Mutants—The gene encoding M1, hpyC1IM1 was amplified from genomic DNA of wild type NTUH-C1 strain by PCR and then cloned into pGEM-T easy (Promega). MA-26R (5′-TTATGGGGCAAGACGCTGAT-3′) and MA-27F (5′-TGTAAGACCTGAAGATAC-3′) primers were used for inverse PCR with Pfu polymerase and then phosphorylated by a poly nucleotide kinase. A blunt-end cat gene was ligated into the inverse PCR product to generate the hpyC1IM1 disrupted plasmid. This plasmid was then transformed into wild type to generate the hpyC1IM1 knockout mutants such that the insertion site of the cat gene is at nucleotide 26. Transposon shuttle mutagenesis was adopted for generating hpyC1IM2 knockout mutants as previously described (16).

Transformation—Plasmid pHel2 (Camr) purified from Escherichia coli strain JM109 was transformed into the wild type H. pylori NTUH-C1 strain and hpyC1IM2 and hpyC1IR knockout mutants by electroporation. Plasmid pHel3 (Kanr) purified from E. coli strain ET12567 was also transformed into wild type and hpyC1IM1 knockout mutant by electroporation. (Plasmids pHel2 and pHel3 were gifts from Dr. R. Haas (Max-Planck-Institute für Biologie, Tübingen, Germany) (18).) H. pylori were washed twice with electroporation buffer (9% sucrose and 15% glycerol) prior to transformation. Electroporation was performed with 1 × 108 colony-forming unit H. pylori and 1 μg of plasmid DNA as previously described (16). Following overnight recovery, the bacteria were selected on a Columbia blood agar plate containing 10 μg/ml kanamycin and 4 μg/ml chloramphenicol, and the colony-forming units were counted. This experiment was repeated three times. On the other hand, the pHel3 and pHel2 were purified from hpyC1IM1 and hpyC1IM2 mutants, respectively, and then transformed into wild type, hpyC1IM1 mutant, and hpyC1IM2 mutant by natural transformation. Transformation was performed with 5 × 108 colony-forming unit H. pylori and 1 μg of plasmid DNA as previously described (16).

Expression and Purification of HpyC1I Protein—The gene encoding HpyC1I was amplified from DNA of wild type NTUH-C1 strain by PCR and cloned into a TA vector, pGEM-T easy (Promega). The pGEM-T easy/hpyC1I plasmid was ligated by NotI (New England Biolabs, Beverly, MA) and ligated in frame into pET28c plasmid (Novagen, Darmstadt, Germany). The resulting pET28c/hpyC1I plasmid was transformed into an E. coli strain BL21(DE3). The HpyC1I protein was expressed and purified per the manufacturer’s instruction under 1 mM isopropyl-β-D-galactopyranoside induction at 25 °C (Qiagen, Hilden, Germany).

Recognition and Cleavage Site of HpyC1I—To determine the recognition and cleavage site of HpyC1I, cloning and sequencing of the HpyC1I digestion products from bacteriophage λ DNA (New England Biolabs) were performed (19). The HpyC1I-digested fragments were blunted by T4 DNA polymerase and cloned into the EcoRV (New England Biolabs) site of pBR322 plasmid. Because the EcoRV site of pBR322 is in the tetracycline resistance gene, the Ampr, Tcr transformants were selected. Plasmid DNA was isolated from Ampr, Tcr colonies, and the restriction fragment-vector junctions were sequenced.

Diversity of HpyC1I R-M System among H. pylori Strains—Genomic DNAs of 15 randomly selected H. pylori strains, including four ATCC strains and 11 clinical isolates were extracted. To examine the modification of CCATC sequences, the DNAs were digested with purified HpyC1I protein. To determine the presence of the HpyC1I R-M system, hpyC1IM1-F (5′-TTATGGGGCAAGACGCTGAT-3′) and hpyC1IR-R (5′-TTAACGTTCTCAATGTCATTGATCTG-3′) primers corresponding to the 5′-end of hpyC1IM1 and 3′-end of hpyC1IR, respectively, were used for PCR. To characterize the location of the HpyC1I R-M system, HP1498-F (5′-TGACGCTGTATTTATGTCG-3′) and HP1501-R (5′-CTCTTACCCGTCAGATTC-3′) primers derived from the conserved region were used for PCR.

RNA Expression Profiles of Wild Type and hpyC1IR Mutant—RNAs from wild type NTUH-C1 strain and hpyC1IR mutant strain were extracted, and microarray hybridization was performed as previously described (20). The density of each microarray signal was standardized with the internal control, 23 S rRNA. The up-regulated and down-regulated genes were so defined when the variations of standardized expression levels were greater than twice the S.D.

RESULTS

Screening the Mutant Library by Adherence Assay—A total of 1500 H. pylori mutant strains were obtained from a clinical isolate NTUH-C1 by transposon shuttle mutagenesis (16). Diversity of these mutants has been confirmed by restriction pattern and random sequencing (16). To identify genes involved in adhesion, we screened each mutant strain by a 24-well culture plate in duplicate. Six mutant strains revealed a 5−10-fold decrease of the recovered adherent bacteria counts compared with wild type strain after a 30-min incubation with the SC-M1 cells (Fig. 1).
The amino acid sequences have 24% identity with a putative partial sequences with J166 strain-specific C8 sequences (23).

BLAST data bases. The nucleotide sequences only shared short nucleotide and protein sequences were compared with NCBI

tity with two Type IIS restriction endonucleases PleI and MlyI, respectively. There were two ORFs located upstream of this locus. Both of the upstream ORFs contained a methyltransferase domain. Based on protein function predictions and gene alignments, we proposed that these three ORFs formed an operon and functioned as a R-M system (DDBJ/EMBL/GenBank accession number AB118944). This 3.5-kb fragment was absent in both 26695 and J99 strains. This DNA fragment was located between HP1498 and HP1501 of H. pylori 26695 strain and between jhp1391 and jhp1394 of J99 strain (Fig. 3). The G + C content of the 3299-bp R-M genes in NTUH-C1 strain was 30.7%. The G + C contents of HP1499-HP1500 and jhp1392-jhp1393 (the replaced regions in 26695 and J99 strains) were 32.1 and 31.1%, respectively. In contrast, the G + C content of conserved flanking regions including HP1498 and HP1501 was 37.2%, which matches the average G + C content in 26695 and J99 strains (37.3%). The significantly lower G + C content of this R-M system implied that this R-M system might have been acquired by horizontal gene transfer.

Reknockout and Complementation of hpyC1IR—Because the transposon insertion site of these six mutants was same, we reknocked out hpyC1IR in a fresh wild type NTUH-C1 strain with a cat cassette inserted at a different site. The morphology of hpyC1IR reknockout mutant was also elongated to a same degree as mutant 6 (Fig. 2). Other phenotypic characteristics and assays of the reknockout mutant were the same as those of the original mutants. The hpyC1IR complementation strain restored the same morphology and adherence as wild type (Figs. 2 and 4).

Transformation Frequency in Mutants—R-M system differences in H. pylori are a barrier to interstrain plasmid transfer (12). We purified pHe12 and pHe13 from E. coli and transformed wild type and R-M knockout mutants. Plasmid pHe12 (Cam+), from E. coli strain JM109 was introduced into wild type NTUH-C1 strain, and hpyC1IM2 and hpyC1IR knockout mutant strains by electroporation. Plasmid pHe13 (Cam+) from E. coli strain ET12567 was introduced into wild type NTUH-C1 strain and hpyC1IM1 knockout mutant strain. By transforming plasmids from unrelated hosts, the transformation efficiencies of hpyC1IM1 (1.3 × 10–5), hpyC1IM2 (3.2 × 10–5), and hpyC1IR (2.7 × 10–6) mutants were significantly higher than those of wild type (8.4 × 10–8, 1.0 × 10–9). The transformation efficiencies of R-M knockout mutants are 100–1000-fold higher than H. pylori wild-type strain (Table 1). On the other hand, the pHe13 and pHe12 were purified from hpyC1IM1 and hpyC1IM2 mutants, respectively, and transformed wild type, and hpyC1IM1 and hpyC1IM2 mutants. The transformations into wild type (2.0 × 10–9, 3.3 × 10–7) were more difficult than back to the hpyC1IM1 (1.2 × 10–5) or the hpyC1IM2 (1.4 × 10–5) mutants. The transformation efficiency of wild type was 100–1000-fold lower than hpyC1IM1 and hpyC1IM2 mutants (Table 1, bottom). Because the HpyC1I R-M system was an operon, there was no endonuclease activity in these mutants. Therefore, plasmids isolated from either unrelated hosts or methylase mutants were easily transformed into the R-M mutants.

Expression and Purification of Novel Restriction Endonuclease—Based on earlier crystal structures, all Type II and Type IIS restriction endonucleases have a structurally similar catalytic core that spatially brings together three essential charged residues, typically two acidic residues (Asp or Glu) and one lysine residue (Lys), forming a P(D/E)X(K/R) motif (24). The amino acid sequence analysis of this novel restriction endonuclease, HpyC1I, showed such a PD-DTK motif (Pro435, Asp436, Lys442, Thr443).

Fig. 2. Cell morphologies of wild type, mutant strains, hpyC1IR reknockout mutant, and complementation strain observed under a light microscope. Observations were done under 1000 magnitudes of light microscope after Gram stain and were examined with more than 10 fields in each slide. A, wild type strain. B, mutant strain with decreased adherence. C, hpyC1IR reknockout mutant. D, hpyC1IR complementation strain.
B. halodurans was not yet characterized in detail (25), PleI and MlyI were chosen to digest the chromosomal DNA of wild type NTUH-C1 strain. DNA from wild type NTUH-C1 strain was digested by both PleI and MlyI. These results indicated that the PleI and MlyI R-M system were both absent in H. pylori NTUH-C1 strain. To assay its activity, HpyC1I was expressed in E. coli. His tag fusion protein was generated by using pET28c plasmid and was purified by Ni²⁺-NTA agarose. The endonuclease activity of purified protein was detected by cleavage of DNA. The optimal reaction conditions were under 1× NEB buffer 1 (10 mM Bis-Tris propane-HCl, 10 mM MgCl₂, 1 mM dithiothreitol, pH 7.0) supplemented with 100 μg/ml bovine serum albumin and incubated at 37 °C. About 60 ng of purified protein (0.1 μl) could digest 1 μg of λ DNA in 1 h at 37 °C.

The Recognition and Cleavage Site of HpyC1I—To determine the recognition and cleavage site of HpyC1I, λ phage DNA was digested with HpyC1I and then blunted by T4 DNA polymerase (19). The HpyC1I restriction fragments were cloned into a pBR322 plasmid and then sequenced. Comparisons of the 10 junction sequences indicated that HpyC1I was a Type IIS re-
Striction endonuclease, because a putative nonpalindromic recognition sequence was identified in the cloned inserts at a constant distance from the junction (Table II). The enzyme recognized a 5-bp asymmetric sequence CCATC and cleaved DNA downstream, after nucleotides 4 and 5 in the top and the bottom strand, respectively. The double strand cleavage of HpyC1I produces a one-base 5'-protruding end. REBASE searches (available on the World Wide Web at rebase.neb.com) revealed that the recognition and cleavage site of HpyC1I was upstream from the restriction endonuclease, because a putative nonpalindromic recognition sequence was identified in the cloned inserts at a constant distance from the junction (Table II). The enzyme recognized a 5-bp asymmetric sequence CCATC and cleaved DNA downstream, after nucleotides 4 and 5 in the top and the bottom strand, respectively. The double strand cleavage of HpyC1I produces a one-base 5'-protruding end. REBASE searches (available on the World Wide Web at rebase.neb.com) revealed that the recognition and cleavage site of HpyC1I was identical with restriction endonuclease BciI (25). Therefore, HpyC1I was an isoschizomer of BciI. The reaction condition, R-M gene alignment, and digestion pattern of λ, pBR322, and phiX174 DNA by HpyC1I were identical with BciI (Fig. 5). DNA of wild type H. pylori NTUH-C1 strain was resistant to BciI digestion (data not shown). Therefore, the CCATC sequences of NTUH-C1 strain were modified, and these modifications were resistant to HpyC1I and BciI digestion.

The Diversity of HpyC1I R-M Systems among H. pylori Strains—Chromosomal DNAs from 15 H. pylori strains, including four ATCC strains and 11 clinical isolates, were examined for the prevalence of HpyC1I R-M systems. The chromosomal DNAs of the nine strains with HpyC1I R-M system were resistant to HpyC1I digestion (Fig. 6A), whereas those from the other six strains without the HpyC1I R-M system were digested by HpyC1I. These results of DNA digestion were consistent with PCR results of HpyC1I R-M genes. HpyC1IM1-F and hpyC1IR-R primers (corresponding to the 5'-end of the gene encoding M1.HpyC1I and 3'-end of the gene encoding HpyC1I, respectively) were used to amplify HpyC1I R-M genes (Fig. 6B). PCR products with the predicted size of 3.3 kb were only observed in the nine strains that were resistant to HpyC1I digestion and not in the other six strains. To examine the chromosomal locations of the HpyC1I R-M genes among these strains, HP1498-F and HP1501-R primers corresponding to the conserved HP1498 and HP1501 genes were used for PCR (Fig. 6C). DNA from the nine strains (yielding wild type NTUH-C1 strain) yielded PCR products with a predicted size of ~4.5 kb. These results indicated that the size and location of the integrated region were the same in the nine strains harboring the HpyC1I R-M genes. DNAs from the other six strains without the HpyC1I R-M genes yielded a predicted size of about 2.1 kb as did 26695 and J99 strains.

RNA Expression Profiles of Wild Type and hpyC1IR Mutant Strain—The RNA expression profiles of wild type and hpyC1IR mutant were analyzed by microarray hybridization. Compared with the expression level of wild type, 10 genes were up-regulated and seven genes were down-regulated in hpyC1IR mutant. Five of the 17 genes had no data base match and no predicted function (Table III).

**DISCUSSION**

The initial aim of our study was to identify adhesins in H. pylori. However, the mutants with decreased adherence revealed elongated cell structure; the interrupted locus was predicted to be a restriction endonuclease. Therefore, this locus was unlikely to be an adhesin, and the decrease of cell adherence might be caused by changes in cell structure or other indirect changes caused by the knockout of R-M system.

The transposon insertion of these six mutants was at the same site in hpyC1IR. Therefore, these six mutants might have descended from one insertion mutation event. However, reknocked hpyC1IR in a fresh wild type strain caused the same phenotypic changes, and complementation restored the morphology of wild type; both findings indicated that disruption of hpyC1IR rather than other chromosomal mutations caused these phenotypic alterations.

**H. pylori** is a genetically diverse species (26). Strain-specific genes may be of great interest biologically, and some may be associated with drug resistance, bacterial surface structure, or restriction-modification. Comparison of 26695 and J99 genome sequences revealed that more than 20 putative R-M systems

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**Table II**

| Position in λ DNA | DNA sequences flanking HpyC1I cleavage sites in λ DNA |
|------------------|-------------------------------------------------------|
| 1325–1364        | 5'-CTGGCCAAAGTCATCCATGGT∥GTCACGCCAATAAGTGAGA-3'     |
| 1596–1635        | 5'-GAAAAGACGGCGATCGGAGAC∥GGCAAGTCGGGCTGCGGCGG-3'   |
| 4797–4836        | 5'-CGGACAGCGTCATCCGCG∥CTAGCTGGCTCACAGTTATT-3'      |
| 9581–9620        | 5'-CATGTTATGCACATCCGG∥TGAACGCGCTTGCAGAGCC-3'       |
| 9855–9894        | 5'-TCTGGAAGACGGCAATGAC∥CCGGCGCTTCTGAGGCC-3'         |
| 11833–11872      | 5'-TCCGGCAGCGGAGATTAC∥AGCCTGATGGCGCGCGCA-3'        |
| 12404–12445      | 5'-TCAAGACCGTTCCGCGG∥ACTGGAAGCGCAGCTGAGA-3'        |
| 39312–39531      | 5'-AGACATACGACCATGAG∥AGAAAAACGGATTTTGTCCG-3'       |
| 39588–39627      | 5'-ATCTATGAGAAAATCATGCCC∥CAGAACGTGTTAAGCTTGAC-3'   |

* Boldface type, the cloned λ DNA sequence digested with HpyC1I; italic type, λ DNA sequence adjacent to the cloned HpyC1I restriction fragment; underline, HpyC1I recognition sequence; vertical arrow, HpyC1I cleavage position.
could be identified in each strain (11). Unique genes of the J166 strain that were identified by PCR-based subtraction hybridization were predominantly (7 of 18) R-M genes (23). The HpyC1I R-M genes were present in NTUH-C1 strain but absent in both 26695 and J99 strains. We also proved that the HpyC1I R-M system were present in >60% H. pylori strains.

**FIG. 6.** Prevalence of HpyC1I R-M systems among H. pylori strains. The products of DNA digestion or PCR were analyzed on a 1% agarose gel. A, Chromosomal DNAs of 15 H. pylori strains digested by purified HpyC1I. B, HpyC1I R-M genes amplified by PCR with hpyC1IM1-F and hpyC1IR-R primers, corresponding to the 5′-end of hpyC1IM1 and 3′-end of hpyC1IR, respectively. C, DNA fragments amplified by HP1498-F and HP1501-R primers, derived from the conserved regions of HP1498 and HP1501 genes.

**TABLE III**

Result of RNA expression profile of NTUH-C1 and hpyC1IR mutant

| Expression level (20) | Gene number | Up-regulated gene description |
|-----------------------|-------------|-------------------------------|
| NTUH-C1 wild type     | hpyC1IR mutant | HP0443 Hypothetical protein |
| 0                     | 53.8        | HP0991 Hypothetical protein |
| 5.6                   | 19.3        | HP0895 Hypothetical protein |
| 20.4                  | 75.4        | HP0613 ABC transporter, ATP-binding protein |
| 3.7                   | 47.1        | HP0624 Solute-binding signature and mitochondrial signature protein (aspB) |
| 3.4                   | 26.1        | HP0816 Flagellar motor rotation protein (motB) |
| 3.2                   | 47.1        | HP1315 Ribosomal protein S3 (rps3) |
| 42.5                  | 60.8        | HP1403 Type I restriction enzyme M protein (hsdM) |
| 7.6                   | 16.0        | HP1416 Lipopolysaccharide 1,2-glucosyltransferase (rfaJ) |
| 0                     | 46.2        | HP1040 Ribosomal protein S15 (rps15) |

| Expression level (20) | Gene number | Down-regulated gene description |
|-----------------------|-------------|---------------------------------|
| NTUH-C1 wild type     | hpyC1IR mutant | HP0689 Hypothetical protein |
| 36.0                  | 30.6        | HP1129 Biopolymer transport protein (exbD) |
| 43.0                  | 8.5         | HP0062 Hypothetical protein |
| 33.0                  | 27.9        | HP0372 Deoxycytidine triphosphate deaminase (dcd) |
| 28.6                  | 9.1         | HP1316 Ribosomal protein L2 (rpl2) |
| 30.3                  | 9.8         | HP1366 Type II restriction enzyme R protein (MboIIIR) |
| 53.2                  | 18.9        | HP0301 Dipeptide ABC transporter, ATP-binding protein (dppD) |
| 53.4                  | 18.9        |
Compared with the G + C content of the conserved flanking regions (−37.2%) and whole genome (−37.3%) of 26695 and J99 strains, the integrated R-M system has significantly lower G + C content (30.7%). This R-M system might be acquired by horizontal transfer during evolution.

R-M systems in bacteria were responsible for cleavage of unmodified foreign DNAs to protect their own chromosomal DNA. *H. pylori* strains have diverse R-M systems resulting in barriers of interstrain DNA transformation (12). Transformation efficiency results also demonstrated CCATC-unmodified plasmids from *E. coli*, and *hpyC1IM1* and *hpyC1IM2* mutants were easily digested by the wild type NTUH-C1 strain. However, small amounts of unmodified plasmid DNA could still be transformed. This indicated that either some plasmids might be protected by the methylase in wild type, or a DNA recombination event might have taken place prior to digestion by the restriction endonuclease.

The Type I R-M system consists of DNA methyltransferase and restriction endonuclease. An endonuclease always contains a N-terminal domain for DNA binding and a C-terminal domain for DNA cleavage. The conserved catalytic residues were identified in HpyC1I. Amino acid sequence comparison revealed 23 and 20% similarity with Type IIS restriction endonuclease. An endonuclease always contains an N-terminal domain for DNA binding and a C-terminal domain for DNA cleavage. The conserved catalytic residues were identified in HpyC1I. Amino acid sequence comparison revealed 23 and 20% similarity with Type IIS restriction endonuclease.

Knockout mutants of *hpyC1IR* showed cell elongation and decreased adherence to the gastric cancer cell line (SC-M1). The mechanism for the morphological change remained unclarified. However, R-M systems could affect many cellular genes as shown by microarray study. There were 10 genes with increased expression and seven genes with decreased RNA expression levels in the *hpyC1IR* mutant. These results may shed light on the regulatory role of HpyC1I R-M system. Interestingly, a methylase gene of the Type I R-M system, *hsaIM*, was up-regulated in the *hpyC1IR* mutant. On the other hand, a restriction endonuclease of the Type IIS R-M system, *mboIR*, had a decreased expression level in *hpyC1IR* mutant.

In conclusion, we have identified a novel HpyC1I R-M system in *H. pylori* and have documented its R-M function and recognition site. This R-M system is present in ~60% of the *H. pylori* isolates. Inactivation of HpyC1I R-M resulted in cell elongation.
Isolation and Characterization of a HpyC11 Restriction-Modification System in Helicobacter pylori

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