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Development of Epidemiological Method for the *Helicobacter pylori* by Polymerase Chain Reaction

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The polymerase chain reaction was used to develop a method for the detection of Helicobacter pylori, a causative agent of gastritis, as well as for the elucidation of its mode of transmission. A genomic library of Helicobacter pylori DNA in Escherichia coli JM109 was constructed by cloning Hind III-digested DNA fragments into plasmid vector pUC18. The nucleotide sequences from seven recombinant clones were determined and five sets of oligonucleotide primers were synthesized on the basis of the sequences from five clones (B4, B9, B10, C15 and I22). The PCR amplifications with these primers were performed using DNA samples from five strains of Helicobacter pylori, two Campylobacter spp. and eleven species of enteric bacteria. Amplifications of the target DNA fragments in all of 5 strains of Helicobacter pylori were observed from the PCR with primers derived from clone B4, B9, C15 and I22. When the specificity was checked with the DNA samples from 13 other bacteria as template DNA for the PCR, specific amplification that produced the correct size of the target DNA of Helicobacter pylori was shown only in the PCR with primers derived from clone B9 and C15. The detection limit in the PCR amplification, determined by the heat-lysis method, was 500 cells of Helicobacter pylori.

Key Words: *Helicobacter pylori, Genomic library, Nucleotide sequence, Polymerase chain reaction, Gastritis*

INTRODUCTION

Helicobacter pylori is now recognized as a causative agent of type B chronic gastritis (Jones et al., 1984; Langenberg et al., 1984; McNulty and Watson, 1984) and it also plays a significant role in the pathogenesis of peptic ulcer disease (Lambert et al., 1985; Marshall et al., 1985; Petross et al., 1986; Price et al., 1985; Rollason et al., 1984; Steer, 1985).

Previous studies from this laboratory have shown that *Helicobacter pylori* infection in Korea begins at infancy (13% at 7-9 months of age). The infection rate reaches up to 50% at 5-6 years of age and 80-90% at 7 years of age and then maintains the percentage throughout adult life (Baik et al., 1990; Rhee et al., 1990).

The relationship between the high prevalence rate of *Helicobacter pylori* infection and the high incidence of stomach cancer in Koreans was not considered as a coincidence. Thus we proposed in 1988 that *Helicobacter pylori* should be considered as a major determinant in the hypothetical carcinogenesis of stomach cancer (Rhee, 1988).

Recently, Correa et al. (1990) and Lin et al. (1989) reported some data consistent with this hypothesis.

To reduce the rate of prevalence of gastritis and gastroduodenal disease we urgently need to develop an effective antimicrobial therapy against *Helicobacter pylori* and its definite mode of transmission has to be established to guard against reinfection.

Helicobacter pylori has not yet been detected from any organs or excreta except from gastric mucosa by

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bacteriological method. Its mode of transmission is not identified but only presumed to be the fecal-to-oral route.

In order to overcome the technical limitations of the bacteriological culture in the isolation of this organism, development of alternative methods to detect *Helicobacter pylori* is desirable.

In this study, the genomic library of *Helicobacter pylori* was constructed by recombinant DNA techniques and partial nucleotide sequences of DNA fragments were determined to perform polymerase chain reaction (PCR) as a diagnostic tool for *Helicobacter pylori*. Oligonucleotide primers were then synthesized on the basis of the sequencing data. Sensitivity of the PCR to detect heat-lysed *Helicobacter pylori* were determined.

MATERIALS AND METHODS

Bacterial Strains

The *Helicobacter pylori* strains mainly used in this study were isolated from biopsy specimens of human stomaches of patients attending Gyeongsang National University Hospital. *Campylobacter jejuni, Campylobacter coli* and eleven enteric bacteria, *Salmonella typhi, Serratia, Proteus vulgaris, Alcaligenes faecalis, Shigella dysenteriae, Morganella morganii, Escherichia coli, Enterobacter aerogenes, Citrobacter freundii, Pseudomonas aeruginosa, Klebsiella pneumoniae*, were used in this study as the control strains.

Isolation of Chromosomal DNA and Construction of Genomic Library

The *Helicobacter pylori* 51 strain, one of the isolates, was cultured on Muller-Hinton agar plate containing bovine serum(10%, v/v) under the microareophilic condition (10% CO_2) at 37°C for 48 hours. The plates were scraped and the cells were washed by centrifugations(6000 × g for 10min at 4°C) with ice-cold phosphate-buffered saline(pH 7.2). The cells were resuspended in 5ml of 25mM Tris(pH 8.0)-50mM glucose-10mM EDTA-0.5% lysozyme (type II; Sigma) solution and incubated for 30min at 37°C and then lysed by addition of sodium dodecyl sulfate solution (final concentration, 1%). The lysate was treated with RNase A (final concentration, 100μg/ml; Sigma) for 1 hour at 37°C and treated overnight with proteinase K (final concentration, 1mg/ml; IBI). The DNA was then extracted twice with equal volume of phenol-chloroform (1:1) and once with chloroform-isoamyl acohol (24:1) and precipitated by mixing with 2 volumes of absolute ethanol for 2 hours at −20°C. It was harvested by centrifugation (12000 × g for 30 min), washed with 70% ethanol, dried and dissolved in 10mM Tris (pH 8.0)-1mM EDTA (TE).

A plasmid library of *Helicobacter pylori* DNA was constructed into pUC18 vector. The genomic DNA of *Helicobacter pylori* was completely digested with *Hind* III(IBI) for 2 hours at 37°C and ligated into *Hind*III-digested, dephosphorylated pUC 18 DNA for 6 hours at 16°C. The resulting plasmid library was transformed into *E. coli* strain JM109 by the calcium chloride method. The transformed host cells were plated and cultured on LB agar plates containing ampicillin (50μg/ml), isopropyl β-D-thiogalactopyranoside (IPTG, final concentration, 10mM; Sigma) and 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside(X-gal, final concentration, 0.2%; Sigma) for 18 hours at 37°C. The white colonies were selected as the insertcontaining recombinant clones.

Determination of Nucleotide Sequences

Sanger's dideoxy-mediated chain-termination method was used for the determination of the nucleotide sequences of *Helicobacter pylori* DNA (Sanger et al., 1977). The recombinant plasmid DNA with high purity was isolated from the small-scale culture of pUC18 clones by using a plasmid mini preparation kit (QIAGEN). 2μg(1.6pM) of double-stranded DNA was denatured with 0.2N NaOH for 5 min at 70°C, precipitated with ethanol and dissolved in distilled water. Then, this template DNA was applied to ABI Dye Primer Taq Sequencing Kit (Promega) and the reaction mixtures were loaded on 6% polyacrylamide gel which was installed in Automatic DNA Sequencer (model 370A, Applied Biosystems) and electrophoresed at 2500V, 40mA for 14 hours. The raw data from the nucleotide sequences were analyzed by Vectra software 1.30(Hewlett Packard).

Primer Synthesis and Purification

Using these sequencing data, appropriate forward and reverse primer regions were selected on the basis of the G + C base content (~ 50%) and five sets of oligonucleotide primers were synthesized with DNA synthesizer (model 380B, Applied Biosystems). The instruction of the manufacturer were followed for synthesis and completed oligomers were cleaved from the support by treatment with concentrated NH_4OH for 1.5 hours. The reaction mixture was heated for 10 hours at 55°C and 1ml of distilled water was then added to the trityl-DNA. Oligomers were purified by using an oligonucleotide purification cartridge (Applied Biosystems).

Polymerase Chain Reaction (PCR)

Purified DNA from *Helicobacter pylori* and other enteric bacteria were subjected to PCR amplification in a thermal cycler by using thermoresistant DNA polymerase from *Thermus aquaticus* (Taq polymerase, Perkin Elmer-Cetus). A typical 50-μl amplification reaction mixture contained 10μl DNA sample, 20mM $(NH_4)_2SO_4$, 70mM Tris (pH 8.8), 2mM $MgCl_2$, 1mM DTT, BSA (100μg/ml), 0.1% Triton X-100, 0.2mM of each deoxynucleotides dGTP, dATP, dTTP and dCTP (Pharmacia), and 1mM each of primers. The reaction mixture was mixed thoroughly and overlayed with 75μl of mineral oil (light, Sigma). The reaction mixture was denatured for 10 min at 98°C, cooled down to 4°C immediately and 2.5 units of Taq polymerase were then added. The microfuge tubes(0.5ml) containing the reaction mixture were placed in a programmable DNA Thermal Cycler (Ericomp) and subjected to 34 cycles of amplification. One cycle consisted of 45 sec of annealing at 55°C, 2 min of extension at 72°C and 1 min of denaturation at 90°C. After the final cycle, the tubes were incubated for an additional cycle of 1 min 40 sec at 55°C and 8 min at 72°C. To prevent unexpected contmination or carry-over of amplified DNA, negative control containing all the components of the PCR assay except for the template DNA was included with each PCR amplification.

Gel Electrophoresis

Ten microliters of each amplified DNA sample was electrophoresed on a 1.6% agarose gel at 120 V for 1 hour. The DNA bands were visualized by ethidium bromide staining. *Hae*III-digested bacteriophage ΦX174 DNA was used as a standard molecular weight marker. The gels were examined over a UV transilluminator and photographed with a polaroid camera (Seolin Scientifics).

Specificity and Sensitivity of PCR

To confirm whether the PCR amplification was specific to the target DNA of *Helicobacter pylori*, one microgram of DNA samples which was extracted from five strains of *Helicobacter pylori*, two species of *Campylobacter* and eleven species of enteric bacteria, were enzymatically amplified using five sets of primers derived from five recombinant clones of *Helicobacter pylori* DNA under the same conditions as above.

The detection limit in this assay system was determined by the PCR using different numbers of heat-lysed bacteria instead of purified DNA sample. Viable cells of *Helicobacter pylori* were suspended with distilled water and its concentration was adjusted to 1.0 at A_{590} (5×10^7/ml). This bacterial suspension was serially diluted 10-fold with distilled water and heated for 30 min at 95°C. The PCR amplification was performed using these heat-lysed cells as template DNA.

RESULTS

Construction of Genomic Library and DNA Sequencing

The genomic DNA of *Helicobacter pylori* was purified and completely digested with *Hin*dIII and, using the resulting fragments, a plasmid library was constructed into pUC18 vector. The inserts of recombinant clones, the size of which were in range of 350-500bp, were selected and their nucleotide sequences were determined by Sanger's dideoxy-mediated chain- termination method. The partial nucleotide sequences of *Helicobacter pylori* genomic DNA derived from seven recombinant clones (B2, B4, B9, B10, C15, I13 and I22) were shown in figure 1 and the length of their sequences were 169, 166, 242, 187, 295, 183 and 200 base pairs, respectively.

Synthesis of Oligonucleotide Primers

On the basis of the sequencing data, five sets of oigonucleotide primers were synthesized with a DNA synthesizer. The following pairs of 20-mer primers were synthesized to amplify 152, 229, 171, 226 and 181 bp fragments of clone B4, B9, B10, C15 and I22, respectively: B4(forward primer, 5'-AAGGCTAGTGAGCT AAAGGC-3' / reverse primer, 5'-TACCGAAAACACC CACGGTT-C'), B9 (forward primer, 5'-TGAACTCACT AAAGGCGGTG-3' / reverse primer, 5'-ATCCCTC ACCGTACTTGACC-3'), B10(forward primer, 5'-ATGGGAGCTTAAGGAGCATG-3' / reverse primer, 5'-TGTCGGGTGGAGACTATTTG-3'), C15(forward primer, 5'-TCCATGGCACAGGGAAATGC-3' / reverse primer, 5'-ATGG AGGGTGAGGGTTTTGG-3') and I22 (forward primer, 5'-GGCTAGTGAGCTAAAGGCCA-3' / reverse primer, 5'-TCGGATTCACCATACCGTTC-3').

Specificity and Sensitivity of PCR

To check the specificity of the PCR amplification, DNA samples from five strains of *Helicobacter pylori*, which were: one standard and 4 isolated strains (strain numbers 27, 45, 51 and 81), were enzymatically amplified with primers derived from the five recombinant clones (B4, B9, B10, C15 and I22) of *Helicobacter pylori* DNA. The results were shown in figure 2.

```
< clone B2 >   01  GCCTAAAATC  CCTAGCATGA  AAATGATTGA  TCTCGCTAAA  GCCCTAGCCC
               51  CCAATACCCC  TACTAAAATC  ATAGGGATTC  GCCCGGGCGA  AAAACTCCAT
              101  GAAGTGATGA  TCCCTAAAGA  TGAAAGCCAT  TTGGCCCTAG  AATTTGAAGA
              151  CTTTTTTATC  AATCAACCC

< clone B4 >   01  ATAAAATAGA  GATTAAGGCT  AGTGAGCTAA  AGGCCACTTT  TATTGATACG
               51  GATAAAGTTT  ATGTGCTTCT  CAACATCACT  AAGAAACACA  TCGCTTTAAC
              101  GAATGAGTAA  GGATTAATAA  TGAAAAAGAT  TATTCTTGCA  TGCCTTATGG
              151  CTTTTGTGGG  TGCCAA

< clone B9 >   01  TGTTAAAACC  TGATGAACTC  ACTAAAGGCG  GTGAAAGGCG  TTACAAGTTA
               51  GAAAGTTTAG  GGCGTATCAA  TAGAAGCGCA  GTCTTTAACC  AAGATGAATT
              101  AAAAACAATA  GCTTATGCTA  GAGTAAGCTC  GCATGACCAA  CAAGATGATT
              151  TAATCAGACA  AGTTCAAGTT  TTAGAGCTTT  ATTGCGCTAG  ATGCGGCTTT
              201  AACTATGAAG  TGATACAAGA  TTTAGGGAGT  GGCATGAACT  GG

< clone B10 >  01  TCACTTATAA  TGGGAGCTTA  AGGAGCATGC  CCCCCATTTA  TAGGGCTGAA
               51  AACGGCTTGT  TGGTAATCCG  CCCTTTGATT  AAGGTTCGAG  AAGCCAGCAG
              101  CATTCATTTT  GTTACTTCTC  AAAATATCCG  CGTGGCCCCT  GATTGCAATT
              151  GCCCAGCCAA  ACAGCCCACC  TCTGATAAAG  CCCCTAT

< clone C15 >  01  TCATTCCATG  GCACAGGGAA  ATGCACAAAA  ATTTTCTCGC  ACTTGTGATG
               51  TGGCATGCTC  TCTAAAACCA  AACGGCCATC  GCCTTGTAAG  ATGTGCAGAT
              101  TTTTTAAATC  CAACAACTCA  ATTTGCTTTA  ACGCTTGCAC  GATAGATGGG
              151  GTGTGAATCT  CTATCCCTAA  GCATGTTTTA  GTGGGGTTGT  TTTTGGCTAA
              201  TTCTATCAAA  TACCTCCCAC  TCCCAAAACC  AATTTCTACT  AAAATAGGGG
              251  CTTGATTTTT  TTGAATAAAG  TCTTCAAAAG  TTTCTAAATC  AAGGG

< clone I13 >  01  CATAAATAAC  ATTACTTTTA  CACTTAAAAG  CCCTTCTATG  TGTAAAATGG
               51  GCGTGATAAT  TCCACGCAGA  AAGTCGTATC  GCTTTTGGAG  AGATTGCTCG
              101  TCCCACTTCT  CATAATCTCT  ATACAATTCT  TTAGTGATGT  CATAACAGCT
              151  AATCACTTTG  CTCGTGTCTT  TGCCGCCATA  AAT

< clone I22 >  01  GGGATAAAAT  AGAGATTAAG  GCTAGTGAGC  TAAAGGCCAC  TTTTATTGAT
               51  ACGGATAAAG  TTTATGTGCT  TCTCAACATC  ACTAAGAAAC  ACATCGCTTT
              101  AACGAATGAG  TAAGGATTAA  TAATGAAAAA  GATTATTCTT  GCATGCCTTA
              151  TGGCTTTTGT  GGGTGCCAAT  TTAAGCGCAG  AGCCTAAGTG  GTATGGCAAG
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Fig. 1. Nucleotide sequences of *Helicobacter pylori* genomic DNA derived from seven pUC18 recombinant clones and five sets of PCR primer region (shaded characters).

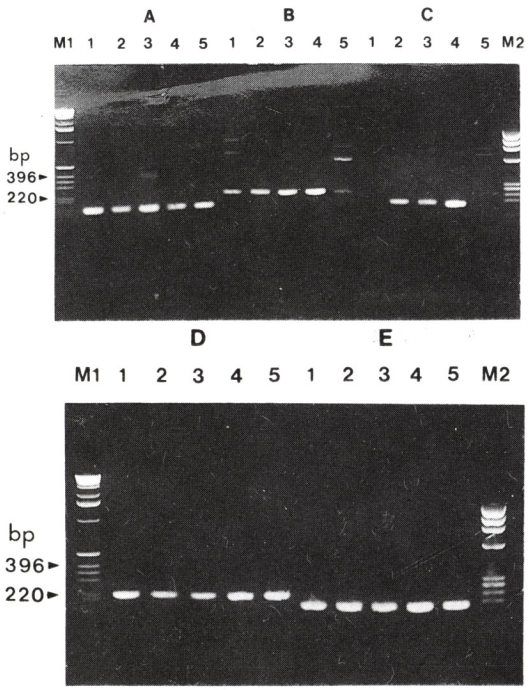

Fig. 2. PCR amplification of target DNA fragments of 5 strains of *Helicobacter pylori* using 5 sets of primers derived from 5 recombinant clones of *Helicobacter pylori*. A - E: primers of B4, B9, B10, C15 and I22, respectively. Lanes: 1, DNA from standard strain of *Helicobacter pylori*; 2 - 5, DNA from isolate strains of *Helicobacter pylori*, strain numbers 27, 45, 51 and 81, respectively; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-R marker.

Amplifications of the target DNA fragments were observed in all of 5 strains of *Helicobacter pylori* when the PCR was performed using primers derived from clone B4, B9, C15 and I22. In the case of PCR with primer from clone B10, amplifications of the target DNA fragments were observed in only 3 strains of *Helicobacter pylori* (strain numbers 27, 45, and 51). When the DNA samples from 13 other bacteria, 2 species of *Campylobacter* and 11 species of enteric bacteria, were used in the PCR as template DNA, specific amplification that produced the correct size of the target DNA of *Helicobacter pylori* was shown only in the PCR with primers derived from clone B9 and C15 as shown in figure 4 and 6. The PCR with the other primers produced some nonspecific fragments as well as the target DNA fragment from other bacteria (Figure 3, 5 and 7).

To check the sensitivity, serially diluted suspension of heat-lysed *Helicobacter pylori* was amplified by the

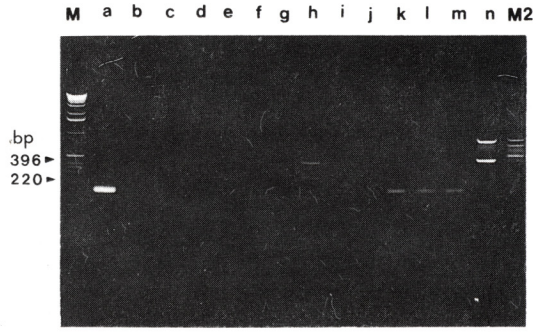

Fig. 3. PCR amplification of genomic DNA of *Helicobacter pylori* and 13 other bacteria by using primer derived from recombinant clone B4 of *Helicobacter pylori*. Lanes: a, *Helicobacter pylori*; b, *S. typhi*; c, *Serratia*; d, *P. vulgaris*; e, *A. faecalis*; f, *S. dysenteriae*; g, *M. morganii*; h, *E. coli*; i, *E. aerogenes*; j, *C. freundii*; k, *P. aeruginosa*; l, *K. pneumoniae*; m, *C. coli*; n, *C. jejuni*; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-RF marker.

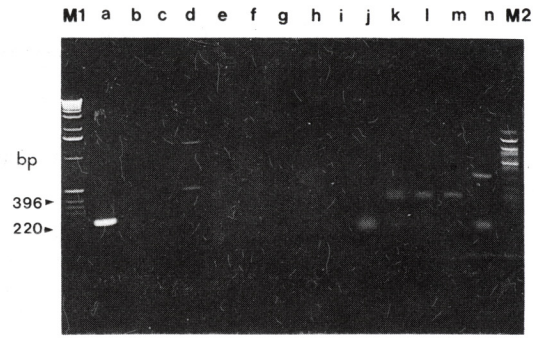

Fig. 4. PCR amplification of genomic DNA of *Helicobacter pylori* and 13 other bacteria by using primer derived fro recombinant clone B9 of *Helicobacter pylori*. Lanes: a, *Helicobacter pylori*; b, *S. typhi*; c, *Serratia*; d, *P. vulgaris*; e. *A. faecalis*; f, *S. dysenteriae*; g, *M. morganii*; h, *E. coli*; i, *E. aerogenes*; j, *C. freundii*; k, *P. aeruginosa*; l, *K. pneumoniae*; m, *C. coli*; n, *C. jejuni*; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-RF marker.

PCR with primers from clone B9 and C15. The results were shown in figure 8. The detection limits in both PCR amplifications were 500 bacterial cells.

DISCUSSION

The first instance of the microscopic detection of curved or spiral bacilli in human gastric mucosa was reported almost a century ago (Bizzozero, 1893; Salomon, 1896). Undoubtedly, these curved bacilli were,

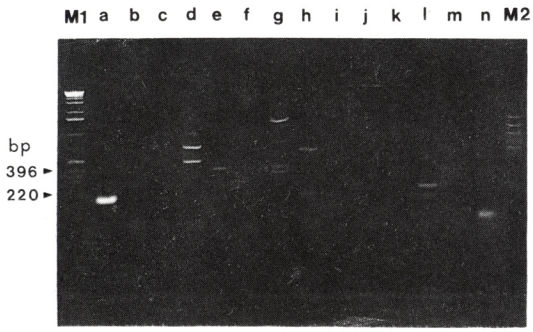

Fig. 5. PCR amplification of genomic DNA of *Helicobacter pylori* and 13 other bacteria by using primer derived from recombinant clone B10 of *Helicobacter pylori*. Lanes: a, *Helicobacter pylori*; b, *S. typhi*; c. *Serratia*; d, *P. vulgaris*; e. *A. faecalis*; f, *S. dysenteriae*; g, *M. morganii*; h, *E. coli*; i, *E. aerogenes*; j, *C. freundii*; k, *P. aeruginosa*; l, *K. pneumoniae*; m, *C. coli*; n, *C. jejuni*; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-RF marker.

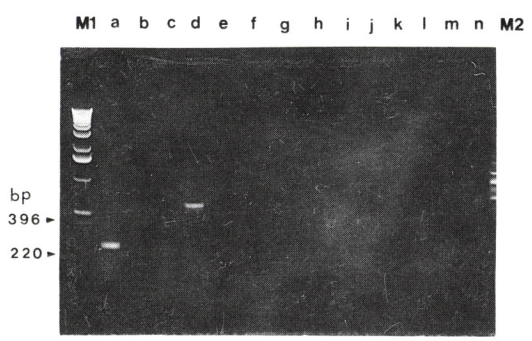

Fig. 6. PCR amplification of genomic DNA of *Helicobacter pylori* and 13 other bacteria by using primer derived from recombinant clone C15 of *Helicobacter pylori*. Lanes: a, *Helicobacter pylori*; b, *S. typhi*; c. *Serratia*; d, *P. vulgaris*; e. *A. faecalis*; f, *S. dysenteriae*; g, *M. morganii*; h, *E. coli*; i, *E. aerogenes*; j, *C. freundii*; k, *P. aeruginosa*; l, *K. pneumoniae*; m, *C. coli*; n, *C. jejuni*; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-RF marker.

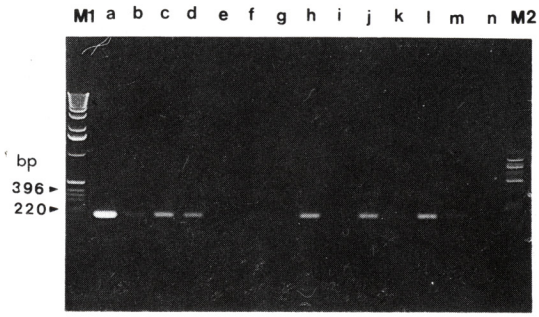

Fig. 7. PCR amplification of genomic DNA of *Helicobacter pylori* and 13 other bacteria by using primer derived from recombinant clone I22 of *Helicobacter pylori*. Lanes: a, *Helicobacter pylori*; b, *S. typhi*; c. *Serratia*; d, *P. vulgaris*; e. *A. faecalis*; f, *S. dysenteriae*; g, *M. morganii*; h, *E. coli*; i, *E. aerogenes*; j, *C. freundii*; k, *P. aeruginosa*; l, *K. pneumoniae*; m, *C. coli*; n, *C. jejuni*; M1, 1Kb ladder marker (Bethesda Research Lab.); M2, *Hae*III-digested ΦX 174-RF marker.

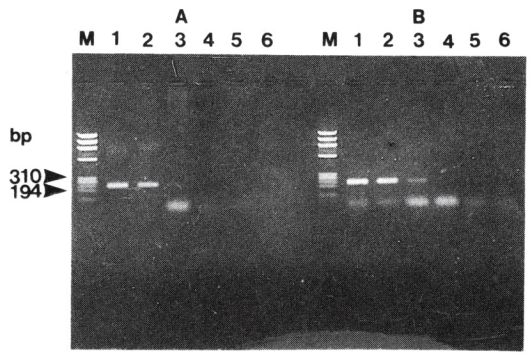

Fig. 8. Detection limits in the PCR amplification of heat-lysed *Helicobacter pylori* with primers from clone B9 and C15. A: primer derived from recombinant clone B9 of *Helicobacter pylori* DNA, B: primer derived from recombinant clone C15 of *Helicobacter pylori* DNA. Lanes: 1, 50000 bacteria; 2, 5000 bacteria; 3, 500 bacteria; 4, 50 bacteria; 5, 5 bacteria; 6, 0 bacteria; M, *Hae*III-digested ΦX 174-RF marker. The specific target DNA fragment in both PCR were visualized to lane 3 (500 bacterial cells) by naked eyes.

in most instances, the organisms which are now identified as *Helicobacter pylori*. In the early twentieth century, several investigators demonstrated the curved bacilli in postmortem human stomaches by silver staining technique (Doenges, 1938; Freedberg, 1940).

By 1940, on the basis of autopsy studies, the general opinion was that the organisms were unimportant commensal flora.

In 1954, Palmer reported the results of a study of 1180 biopsy specimens obtained by gastric suction and stained with hematoxylin and eosin (H & E). The curved bacilli observed by earlier investigators could not be found because these organisms were very faintly visible in H & E staining. Palmer concluded that the organisms represented contaminants related only to the agonal stage of illness e.g. hypochlorohydria or achlorohydria.

In the early 1980s, Warren and Marshall(1983) returned to the use of silver staining (Warthin-Starry staining) for microscopic examination of endoscopically

obtained biopsy specimens of gastric mucosa. Curved bacilli were again noted, particularly in association with antral gastritis.

Helicobacter pylori was initially called *Campylobacter pyloridis*, the name was soon changed to *Campylobacter pylori* (Marshall and Goodwin, 1987). Although the spiral, microaerophilic, non-spore-forming, gram-negative rods befit the genus *Campylobacter*, a number of significant morphologic, structural, biochemical and genomic features indicated that these organisms should be placed in a new genus, now named *Helicobacter* (Goodwin, 1989).

Helicobacter pylori are motile, gram-negative, curved rods with a size of 3.0×0.5um that are oxidase-, catalase- and urease-positive. These organisms are typically located within or beneath the mucus layer adjacent to the gastric epithelium within the antrum, body or fundus. Also these organisms are found adjacent to the surface epithelium, in the gastric fits and in the necks of gastric glands. They are seldom found deep within the gastric glands and they tend to congregate near intercellular junctions (Marshall and Warren, 1984; Goodwin et al., 1986; Hazell et al., 1986; Rhee et al., 1988).

Studies have shown a high correlation between histologically proven antral gastritis and the recovery of *Helicobacter pylori* from biopsy specimens (Jones et al., 1984; Kilbridge et al., 1988; Rauws et al., 1988) as well as between duodenal ulcer and the presence of this organism in the stomach (Buck et al., 1986; Price et al., 1985). Further evidence of the causal relation between *Helicobacter pylori* and human disease is provided by Marshall et al.(1985) and Morris and Nicholson (1987) who satisfied Koch's postulates by demonstrating that ingestion of the viable organisms induced histologically confirmed gastritis. Moreover, experimental infection leading to acute gastritis was successful in germ-free piglets(Eaton et al., 1989; Krakowka et al., 1987; Lambert et al., 1987) as well as germ-free beagle dog (Radin et al., 1990). Alleviation of symptoms in patients with gastritis or peptic ulcer disease and absence of relapse after antimicrobial therapy to *Helicobacter pylori* (Lambert et al., 1986; Langenberg et al., 1985) as well as infectious nature of gastritis (Ramsey et al., 1957; Gledhill et al., 1985; Peterson et al., 1987) are additional supportive evidences for the causative role of this organism in the B type gastritis.

There are also a lot of data on the prevalence of *Helicobacter pylori* infection in asymptomatic persons (Barthel et al., 1988; Graham et al., 1988; Havey et al., 1986; Kaldor et al., 1986; Langenberg et al., 1984; Rauws et al., 1988; Marshall et al., 1985; Megraud et al., 1989; Petross et al., 1988). Thus, it is well Known that the prevalence rates of *Helicobacter pylori* infection are different in ethnic origins, geographic location as well as socioeconomic status.

Helicobacter pylori infection is infrequent in young children in developed countries and becomes progressively a little more frequent during adulthood but, the prevalence of infection is less than 50% even in old age. However, in developing countries, infection is more common, and begins earlier and, the infection, once acquired, appears to persist possibly for life.

Previously, we studied the prevalence of *Helicobacter pylori* infection in normal population in Korea (Baik et al., 1990; Rhee et al., 1990). This high rate of prevalence is unparalleled anywhere else in the world, and implies that there are many sources of infection, stemming from the life style or customs native to Korea.

Therefore, eliminating these organisms from our stomaches requires immediate attention to discovering a means of tracing the sources of infection in our environment and, the design of an appropriate therapeutic modality.

Although fecal-to-oral route of infection is highly suspicious in the case of *Helicobacter pylori*, this organism has not yet been isolated from fecal specimens. As an alternative method of bacteriological culture to detect the major source of *Helicobacter pylori* infection, we tried to apply polymerase chain reaction.

With this method, detection of low numbers of microorganisms is possible by selectively amplifying specific microbial genes or fragments of DNA *in vitro* (Mullis and Faloona, 1987).

PCR requires two primers: a forward primer, which anneals to one strand of the DNA and a reverse primer, which anneals to the opposite strand. One cycle involves denaturation of double-stranded DNA, annealing of the two primers and extension of the primers in opposite directions, thus doubling the amount of target DNA. Repetition of this three-step reaction cycle at different temperatures results in an exponential amplification of fragment. By repeating the cycle n times, the amount of DNA rises to 2^n and the concentration of the specific DNA sequence generally increases to $\geq 10^6$-fold.

The length of the newly synthesized fragment of DNA can be determined either directly by agarose gel electrophoresis or indirectly by hybridization with a labeled complementary DNA probe.

This ability to amplify a target sequence to a specific length allows for a very specific, sensitive and rapid diagnostic test.

To apply this PCR technique, sequence of a genome specific to the organism should be known.

The genomic library of *Helicobacter pylori* DNA in *E. coli* JM109 was constructed by cloning *Hin*dIII-digested DNA fragments into plasmid vector pUC18 and one hundred of white colonies containing insert DNA were selected. Plasmid DNA isolated from these clones were digested with *Hin*dIII and electrophoresed on agarose gel. The seven clones (B2, B4, B9, B10, C15, I13 and I22) that contained insert DNA with the size of 350-500bp were selected and their nucleotide sequences were partially determined from 5'-terminal. The length of their sequence were in the range of 166-295bp. Among the nucleotide sequences from seven clones, appropriate sequences suitable for the synthesis of oligonucleotide primer, of which G+C rations were 50%, were found in both 3'- and 5'-terminal region of the sequences from clone B4, B9, B10, C15 and I13. Then five sets of oligonucleotide primers of recombinant clones of *Helicobacter pylori* DNA were synthesized and PCR amplification with these primers was performed.

The specificity of PCR amplification was checked by using each of five primers and DNA samples from 5 strains of *Helicobacter pylori* and 13 species of other enteric bacteria. Among the 5 sets of primers, PCR amplification with primers only from clone B9 or C15 produced the target DNA fragments specific to *Helicobacter pylori*.

The detection limits with primers from clone B9 and C15 were determined by using different numbers of heat-lysed *Helicobacter pylori*. As noted in the results, the detection limits in both PCRs were 500 bacterial cells.

The PCR amplification with primers from clone B9 and C15 could be used to detect small numbers of *Helicobacter pylori* in feces or other human excreta and to identify the route of infection.

On-going study using this PCR will detect *Helicobacter pylori* from clincal specimens, e.g. gastric biopsy materials or fecal specimens.

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