Cloning and Sequence Analysis of Adhesion Gene *hpaA* of *Helicobacter pylori*

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Abstract: Objective. To clone the adhesion gene *hpaA* of *Helicobacter pylori* strain MEL-Hp27 isolated from a patient in Zhengzhou City, and analyze the *hpaA* gene nucleotide and putative amino acid sequences. **Methods.** *hpaA* gene of the *Helicobacter pylori* MEL-Hp27 was amplified by PCR. After purified, the target fragment was cloned into plasmid pBluescriptb Ⅱ and subject to nucleotide sequenced. The homologies of the nucleotide and putative amino acid sequences of *hpaA* were respectively analyzed. **Results.** *hpaA* gene of 783 bp, encoding the polypeptides of 260 amino acids, was obtained from the *Helicobacter pylori* strain MEL-HP27 genomic DNA. The homologies of the nucleotide and putative amino acid sequences compared with the published *hpaA* gene sequences were 94.76% – 97.19% and 95.38% – 98.46%, respectively. **Conclusions.** The recombined plasmid carring *hpaA* gene has been successfully constructed, and sequence analysis indicates that *hpaA* is a highly conserved prokaryotic gene and might be a potential candidate for *Helicobacter pylori* vaccine development. [Life Science Journal. 2006;3(4):42 – 48] (ISSN: 1097 – 8135).

Keywords: *Helicobacter pylori*; *hpaA* gene; cloning; sequence analysis

Abbreviations: HpaA: *Helicobacter pylori* adhesion; MALT: mucosa associated lymphoid tissue

1 Introduction

Helicobacter pylori is one of the common gram-negative bacteria causing chronic infection, which infects more than 50% of the human population. Infection of the gastric mucosa with *Helicobacter pylori* results in a number of disease outcomes including gastritis, which precedes the development of peptic ulcer disease, gastric cancer and lymphomas of the mucosa associated lymphoid tissue(MALT)[1,2]. Although significant progress has been made in treating *Helicobacter pylori* infection with current triple or quadruple therapy based on antibiotics, given in conjunction with bismuth compounds and proton pump inhibitor, the limitations of pharmacological therapy such as side effects, poor compliance, high cost, and most importantly, rapid emergence of antibiotic resistance have set the stage for the development of less costly and more efficient means to prevent and control *Helicobacter pylori* infections[3,4]. Immunization against the bacterium represents a cost-effective strategy to prevent *Helicobacter pylori* infection, the selection of antigenic targets is critical in the design of *Helicobacter pylori* vaccine[5]. *Helicobacter pylori* adhesion(HpaA) is a flagellar sheath protein with approximately 29 kDa located in the bacterial outer membrane[6]. So in this study, the recombinant plasmid inserted with *hpaA* of *Helicobacter pylori* was constructed and the homologies of the nucleotide and putative amino acid sequences were respectively analyzed, which will be helpful for determining whether the HpaA becomes one of the good candidates as an antigen in *Helicobacter pylori* vaccine.

2 Materials and Methods

2.1 Materials

The strain MEL-HP27 of *Helicobacter pylori* and cloning pBluescriptb Ⅱ were preserved by our laboratory, *E. coli* strains JM109 were purchased from New England Biolabs (Beijing) LTD (Beijing China). Pyrobest DNA polymerase, restriction endonuclease enzymes (*Bam*HI, *Hind* Ⅲ), T4 DNA

ligase, DNA gel extraction kit and 100 bp DNA marker were provided by TaKaRa Company (Dalian, China).

2.2 Bacterial culture and preparation of DNA template

Helicobacter pylori MEL-HP27 strains were grown on solid Columbia agar with 100 ml/L frozen-melting sheep blood, 50 ml/L fetal bovine serum, and antibiotic supplement (vancomycin 10 mg/L, polymyxin B 0.33 mg/L, amphotericin A 5 mg/L, trimethoprim 5 mg/L) in a microaerophilic atmosphere for 3 days to 4 days at 37 °C.

The *Helicobacter pylori* strains were harvested and suspended in 1 ml sterile normal saline and pelleted by centrifugation at 10,000 g for 5 minutes. The precipitate was resuspended in lysis buffer (10 mM Tris-HCl, pH 8.0, 0.1 M EDTA, pH 8.0, 0.5 % (w/v) SDS, 20 μg/ml RNase), and then, protease K was added in to a final concentration of 100 μg/ml, the lysate was incubated in a water bath at 42 °C for 2 hours. The solution was cooled to room temperature, and mixed with an equal volume of phenol equilibrated. The two phases were separated by centrifugation at 10,000 g for 10 minutes at room temperature, and the aqueous phase was extracted with phenol twice again. Afterwards, 0.1 volume of 2.5 M ammonium acetate and 2 volume of ethanol were added to the aqueous phase. The precipitate was collected by centrifugation at 10,000 g for 2 minutes, washed twice with 70% ethanol, and dissolved in an appropriate volume of TE buffer (pH 8.0)[7,8]. The DNA concentration was measured by ultraviolet spectrophotometry.

2.3 Synthetic primers and PCR

Oligonucleotide primers were designed to amplify *hpaA* gene from *Helicobacter pylori* strain MEL-HP27 based on the published corresponding genome sequence of 26695 and J99. The sequence of sense primer with a restriction endonuclease site of *Bam* HI was: 5′-CGGGATCCATGAAAGCAA ATAATC-3′. The sequence of antisense primer with a restriction endonuclease site of *Hind* Ⅲ was: 5′-CGCAAGCTTTTATCGGTTTCT-3′. PCR was performed in a 50 μl reaction mixture in 0.6 ml tube in an automatic thermal cycler. The PCR mixture contained 5 μl of 10×PCR buffer, 2.5 μl of sample DNA, 4 μl of 2.5 mmol/L deoxynucleoside triphoshpate, 2 μl of 0.25 μmol/L oligonucleotide primers, 0.5 μl Pyrbest DNA polymerase (1.25 U), 34 μl of MilliQ H_2O. The parameters for PCR were as follows: 95 °C for 5 minutes, 1 cycle; 94 °C for 60 seconds, 45 °C for 50 seconds, 72 °C for 50 seconds, 30 cycles; 72 °C for 10 minutes, 1 cycle. The amplified products (3 μl) were observed by electrophoresis on 10 g/L agarose gel containing 0.1 μg of ethidium bromide per ml in TBE buffer. The PCR product was visualized under UV light and photographed.

2.4 Construction of recombinant plasmids

PCR products were digested by restriction endonucleases *Bam*HI and *Hind* Ⅲ, meanwhile pBluescriptb Ⅱ plasmid was digested by *Bam*HI and *Hind* Ⅲ, too. The target fragments of *hpaA* and pBluescriptb Ⅱ were recovered by DNA gel extraction kit, and then these two fragments were ligated by using T4 DNA ligase at a molar ratio of 6:1 at 16 °C for 12 hours. The recombinant plasmid was transformed into *E. coli* JM109. The *E. coli* JM109 containing the recombinant plasmid was amplified in LB solid medium containing ampicillin (100 mg/L). Clones were picked out randomly through blue/white screening and cultivated in 4 ml LB medium containing 100 mg/L of ampicillin, at 200 r/min at 37 °C overnight. Finally the recombinant plasmids were extracted by Sambrook's method and identified by PCR and restriction endomuclease enzyme digestion.

2.5 Sequence determination and homology analysis

The sequence determination of *hpaA* gene of recombinant plasmid was carried out by Shanghai DNA Biotechnologies Company (China), in the meantime, the sequence of *hpaA* gene and amino acid were analyzed by software Omiga. 2.0 and DNAmen, and compared the homology based on the GenBank (No. NC000915, strain 26695; No. NC000921, strain J99; No. X92502, strain 11637; No. AF479028, strain CH-TX1; No. U35455, strain CCUG 17874; No. X61574, strain 8826; No. DQ115385, strain K51; No. AY714223, strain Y06).

3 Results

3.1 PCR amplification of *hpaA* encoding sequence

The *hpaA* of MEL-HP27 strain was amplified by PCR from the above primers. The PCR product was electrophoresed and visualized by 10 g/L agarose gel (Figure 1). It revealed that the size of *hpaA* DNA fragment amplified by PCR was 783 bp, and was compatible with the expectant size.

3.2 Construction and identification of recombinant plasmids

Recombinant plasmid pBluescriptb Ⅱ-hpaA was digested with *Bam*HI and *Hind* Ⅲ and confirmed by PCR, then digestive product and PCR product were visualized on 10 g/L agarose gel (Figure 2). It demonstrated that recombinant plasmid was digested to 3,000 bp and 783 bp DNA fragment, which contained the objective gene, and

hpaA gene was amplified from the recombinant plasmid by PCR.

3.3 Sequence analysis

Sequencing results showed that the *hpaA* gene consists of 783 base pairs and encodes the polypeptides of 260 amino acids. The sequencing results of *hpaA* from strain MEB-HP27 are published in the

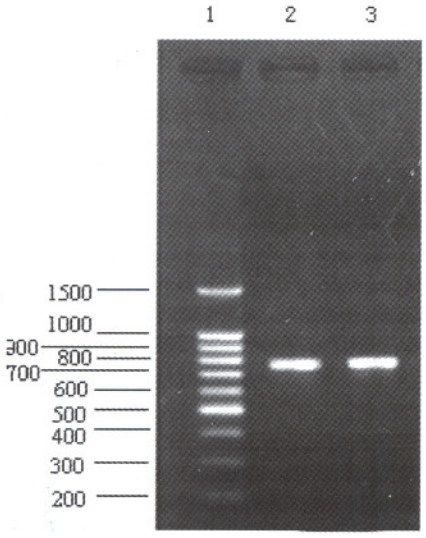

Figure 1. The result of *hpaA* gene amplification using PCR Lane 1:100 bp DNA ladder; Lane 2 and Lane 3: PCR product of *hpaA* gene.

GenBank, the accession number is DQ353891. The homologies of the nucleotide and putative amino acid sequences compared with eight published *hpaA* gene sequences were 94.76% – 97.19% and 95.38% – 98.46%, respectively (Figures 3, 4). The strain MEL-HP27 was quite identical to NCTC11637 than the others with nucleotide homologies of 97.19%, and the amino acid identity was 97.31% against NCTC11637. There are only 22 base pairs different between MEL-HP27 and NCTC11637, at 62nd site codon AAG/N→AGG/R, at 100th site codon AAT/N→AGC/S, at 112th site codon GCG/A→TCG/S, at 124th site codon AGT/S→AAT/N at 137th site codon ACA/T→ATA/I, at 164th site codon ATC/I→GCT/V, at 256th site codon AAC/N→GGC/G (codon/amnio acid). These analysis indicated that the *hpaA* gene sequence and the putative amino acid sequence were quite conservative and might be a potential antigen candidate for *Helicobacter pylori* vaccine development.

4 Discussion

Helicobacter pylori adhesion is a flagellar sheath potein located in the bacterial outer membrane. The outer membrane is a continuous structure on the surface of gram-negative bacteria, which have bilateral particular significance as a potential target for protective immunity and bacterial pathogens[9,10]. In other studies, outer membrane vaccines have been used with considerable success to induce protection against a number of organisms[4,11]. The *hpaA* gene is located in genome DNA of *Helicobacter pylori* and considerably conservative for its nucleotide and amino acid sequences. HpaA is one of the major structural outer membrane proteins of *Helicobacter pylori* and plays an important role in adhesion of the microbe[12,13]. Furthermore, antibody against HpaA almost could be found in all *Helicobacter pylori* infected patients sera, which will be an ideal antigen candidate for *Helicobacter pylori* vaccine. In this study, the *hpaA* gene was cloned from strain MEL-HP27, which consists of 783 base pairs and encodes the polypeptides of 260 amino acids. The homologies of the nucleotide and putative amino acid sequences of *hpaA* gene from *Helicobacter pylori* strain MEL-HP27 compared with the 8 published *hpaA* gene sequences were as high as 94.25% – 97.32% and 95.38% – 98.46%, respectively. These data indicate that the mutation level of the *hpaA* gene of *Helicobacter pylori* strain MEL-HP27 is within the range reported by GenBank, and suggest that HpaA is an excellent and ideal antigen for developing *Helicobacter pylori* vaccine.

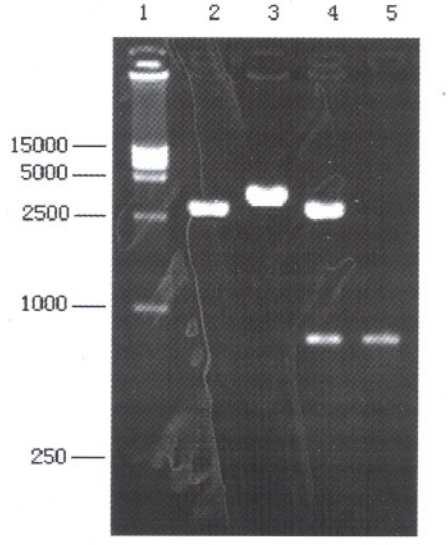

Figure 2. Identification of recombinant plasmid pBluescript-hpaA by restriction enzyme digestion
Lane 1: 15000bp DNA ladder; Lane 2: pBluescriptb Ⅱ digested by *Bam*HI and *Hind* Ⅲ; Lane 3: pBluescript-hpaA digested by *Hind* Ⅲ; Lane 4: pBluescript-hpaA digested by *Bam*HI and *Hind* Ⅲ; Lane 5: *hpaA* gene amplified by PCR from recombinant plasmid pBluescript-hpaA.

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MEL-HP27    ATGAAAGCAAATAATCATTTTAAAGATTTTGCATGGAAAAAATGCCTTTT   50
26695       --------------------------------------------------   50
J99         ------a-----gg---------g--------------------t----   50
11637       ----g---------------------------------------------   50
CH-CTX1     ------a-----gg---------g--------------------------   50
CCUG17874   ------a-----gg---------g--------------------------   50
8826        ------a-----gg---------g--------------------------   50
K51         --------------------------------------------------   50
Y06         ----g---------------------------------------------   50

MEL-HP27    AGGCGCGAGCGTGGTGGCTTTGTTAGTGGGATGCAGTCCGCATATTATTG   100
26695       ---t----------------------------------c----------   100
J99         --------------------a--------g--t--c--------------   100
11637       --------------------a-------------c--------------   100
CH-CTX1     ---------t----------a-------------c--------------   100
CCUG17874   ------------gc-----a-------------c--------------   100
8826        ----a---------------a--------g-----c--------------   100
K51         ---t----------------------------c--------------   100
Y06         ----------------------g----------c--------------   100

MEL-HP27    AAACCAATGAAGTCGCTTTGAAATTGAATTACCATCCAGCTAGCGAGAAA   150
26695       --------------------------------------------------   150
J99         ------------t-------------------------------------   150
11637       --------------------------------------------------   150
CH-CTX1     --------------------------------------------------   150
CCUG17874   ---------------------g----------------------------   150
8826        ---------g-----------------------------------------g   150
K51         --------------------------------------------------   150
Y06         --------------------------------------------------   150

MEL-HP27    GTTCAAGCGTTAGATGAAAAGATTTTACTTTTAAAGCCAGCTTTTCAATA   200
26695       ----------------------------g-------g---------------   200
J99         ----------------------------------g---------c-----   200
11637       ----------------------------g-------g---------c-----   200
CH-CTX1     ----------------------------g-------g---------c-----   200
CCUG17874   ----------------------------g-------g---------------   200
8826        ----------------------------g-----------------c-----   200
K51         ----------------------------g-------g---------------   200
Y06         ------------------------c--g-------g---------------   200

MEL-HP27    CAGCGATAATATTGCTAAAGAGTATGAAAACAAATTCAAGAATCAAACCG   250
26695       --------------------------------------------------   250
J99         ----------------------------------------------------a   250
11637       t----------c-------------------------------------   250
CH-CTX1     --------------------------------------------------   250
CCUG17874   --------------------------------------------------   250
8826        ---------c--------------------t------------------a   250
K51         --------------------------------------------------   250
Y06         ----------------------------------------------------a   250

MEL-HP27    CGCTCAAGGTTGAACAGATTTTGCAAAATCAGGGCTATAAGGTTATTAAT   300
26695       ----------------------------------------------gc   300
J99         ----t--a------g----c--------------------------------   300
11637       ------------------------------a-----------------gc   300
CH-CTX1     ------------------------------------c--------c---   300
CCUG17874   t-------------------------------------------------   300
8826        ----t--a------g----c-----------------c--------c---   300
K51         ----------------------------------------------gc   300
Y06         ----t---------g----c-----------a----------------gc   300

MEL-HP27    GTAGATAGCAGCGATAAAGACGATCTTTCTTTTGCGCAAAAAAAAGAAGG   350
26695       ------------------------------t-----------------   350
J99         --g---------------------t--------------------------   350
11637       ------------------------t----------a---------------   350
CH-CTX1     --g-----t---------------t--------------------------   350
CCUG17874   --g---------------------t--------------------------   350
8826        --g---------------------t--------------------------   350
K51         ------------------------------t-----------------   350
Y06         ------------------------------t-----------------   350

MEL-HP27    GTATTTGGCCGTTGCTATGAGTGGCGAAATTGTTTTACGCCCCGATCCTA   400
26695       ---------t----------a--------------------------   400
J99         -------*--t--c-------a--------------------------   400
11637       ---------g----------a--------------------------   400
CH-CTX1     ---------g----------a--------------------------   400
CCUG17874   ---------t--c-------a---t----------------------   400
8826        ---------g----------t--------------------------   400
K51         ---------t----------a--------------------------   400
Y06         ------------c-------a--------------------------   400
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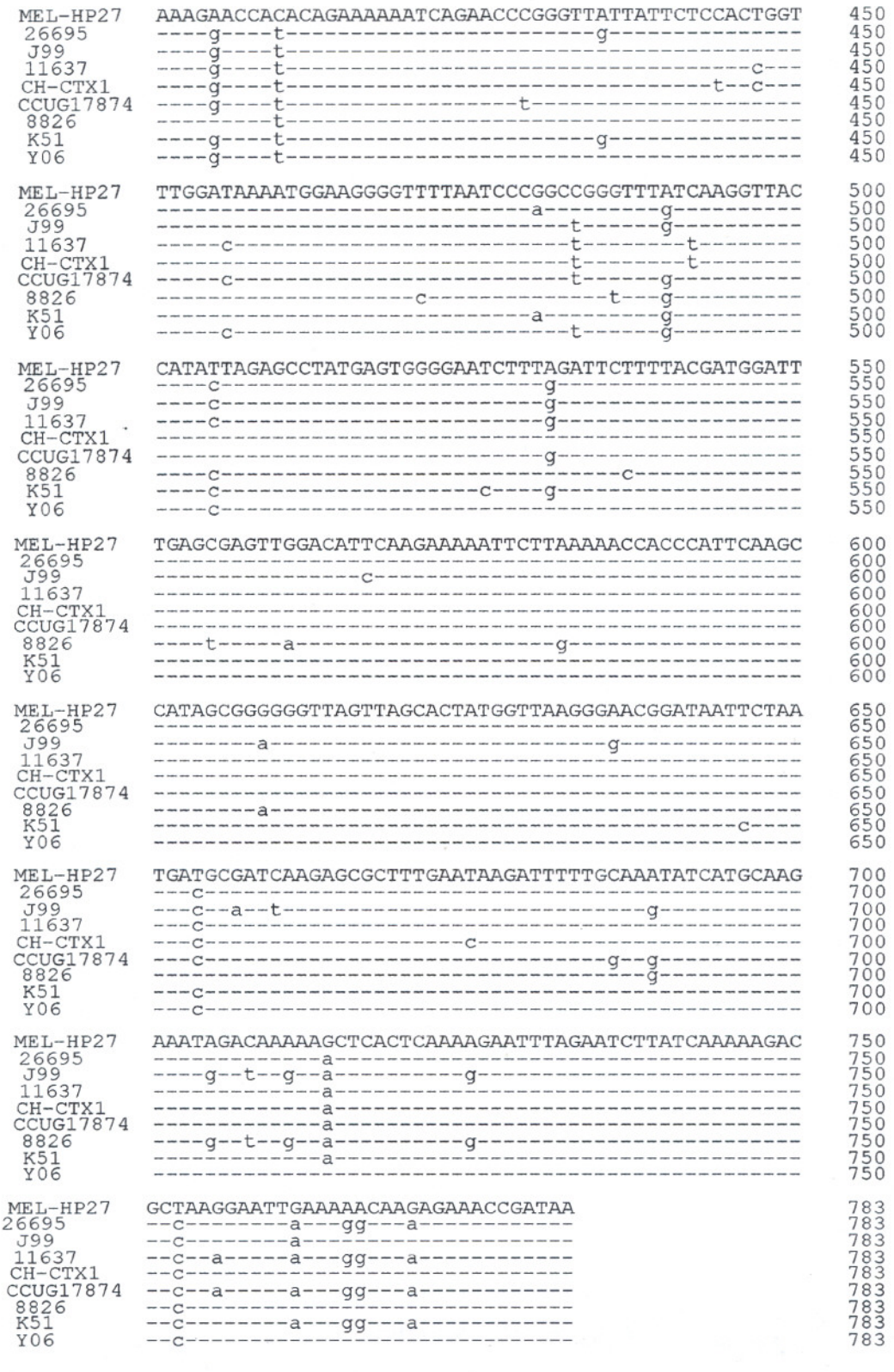

Figure 3. Homology comparison of *hpaA* gene nucleotide sequences

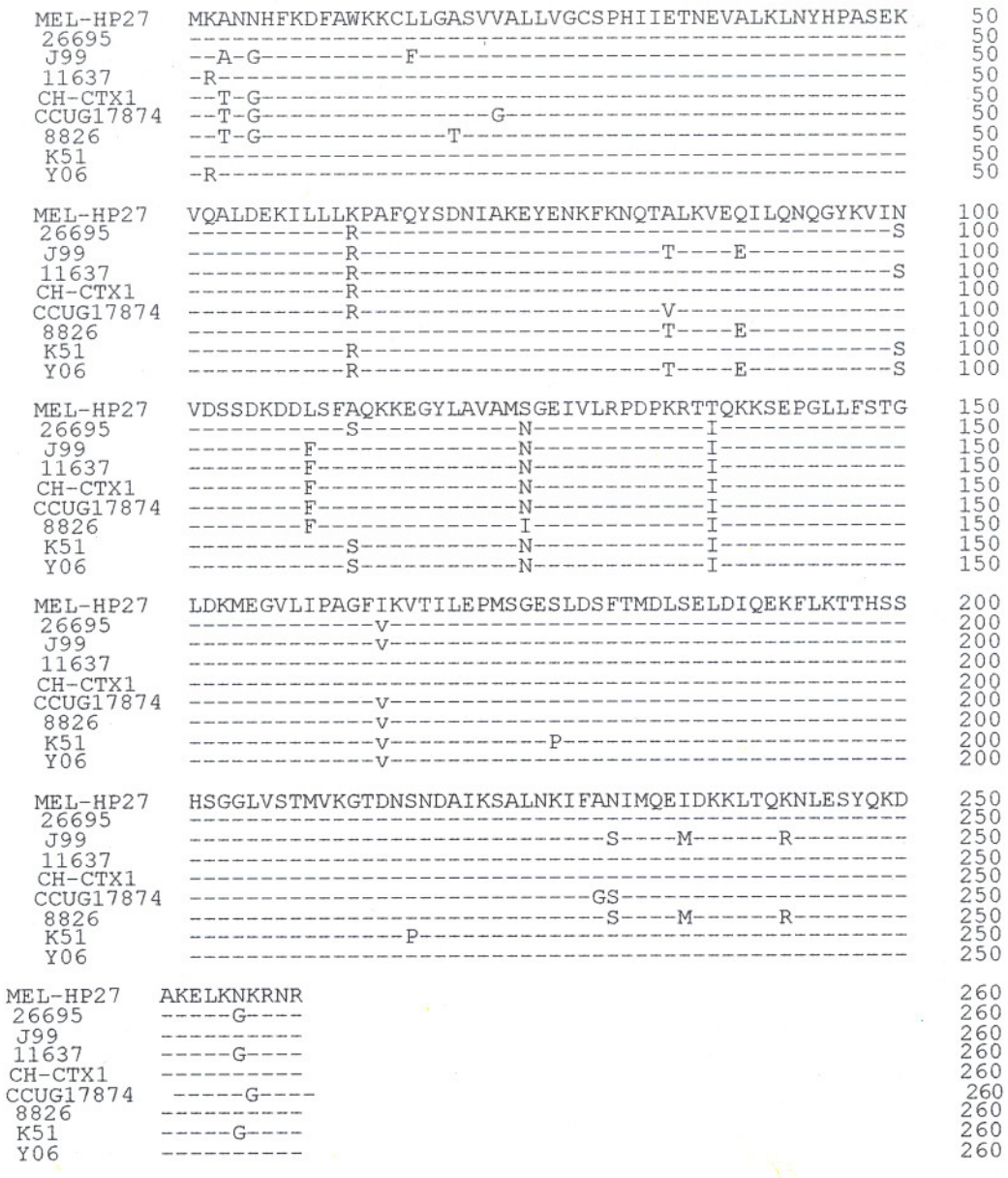

Figure 4. Homology comparison of the putative amino acid sequences of *hpaA* gene

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References

1. Ohata H, Kitauchi S, Yoshimura N, *et al*. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. Int J Cancer 2004; 109(1):138－43.
2. Kauser F, Hussain MA, Ahmed I, *et al*. Comparing genomes of *Helicobacter pylori* strains from the high-altitude desert of Ladakh, India. J Clin Microbiol 2005; 43(4):1538－45.
3. Graham DY. Therapy of *Helicobacter pylori*: current status and issues. Gasteroenterology 2000; 118: S2－S8.
4. Liu XF, Hu JL, Zhang X, *et al*. Oral immunization of mice with attenuated *Salmonella typhimurium* expressing *Helicobacter pylori* urease B subunit. Chinese Medical Journal 2002;115(10):1513－6.
5. Hatzifoti C, Wren BW, Morrow JW. *Helicobacter pylori* vaccine strategies-triggering a gut reaction. Immuno Today 2000; 21: 615－9.

6. Valkonen KH, Wadstrom T, Moran AP. Identification of the N-acetylneuraminyllactose-specific laminin-binding protein of *Helicobacter pylori*. Infect Immun 1997; 65 (3):916 – 23.
7. Yuan JP, Li T, Shi XD, *et al*. Deletion of *Helicobacter pylori* vacuolating cytotoxin gene by introduction of directed mutagenesis. World J Gastroenterol 2003;9(10): 2251 – 7.
8. Chen XJ, Yan J, Shen YF. Dominant cagA/vacA genotypes and coinfection frequency of *H. pylori* in peptic ulcer or chronic gastritis patients in Zhejiang Province and correlations among different genotypes, coinfection and severity of the diseases. Chinese Medical Journal 2005; 118(6):460 – 7.
9. Richard AA, James B, Beth M, *et al*. Comparative genomics of *Helicobacter pylori*: analysis of the outer membrane protein families. Infection and Immunity 2000; 68 (7):4155 – 68.
10. Keenan IJ, Allardyce AR, Bagshaw FP. Lack of protection following immunization with *Helicobacter pylori* membrane vesicles highlight santigenic differences between *H. felis* and *Helicobacter pylori*. FEMS Microbiology Letters 1998;161:21 – 7.
11. Jiang Z, Tao XH, Huang AL, *et al*. A study of recombinant protective *H. pylori* antigens. World J Gastroenterol 2002; 8: 308 – 11.
12. Jones AC, Logan RP, Foynes S, *et al*. A flagellar sheath protein of *Helicobacter pylori* is identical to HpaA, a putative N-acetylneuraminyllactose-binding hemagglutinin, but is not an adhesin for AGS cells. J Bacteriol 1997;179: 5643 – 7.
13. Alm RA, Ling SL, Moir DT, *et al*. Genomic-sequence comparison of two unrelated isolates of the human gastric pathogen *Helicobacter pylori*. Nature 1999;397: 176 – 80.

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