

Construction of prokaryotic expression system of *ureB* gene from a clinical *Helicobacter pylori* strain and identification of the recombinant protein immunity

Ya-Fei Mao, Jie Yan

Ya-Fei Mao, Jie Yan, Department of Medical Microbiology and Parasitology, College of Medical Science, Zhejiang University, Hangzhou 310031, Zhejiang Province, China

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Correspondence to: Jie Yan, Department of Medical Microbiology and Parasitology, College of Medical Science, Zhejiang University, 353 Yanan Road, Hangzhou 310031, Zhejiang Province, China. yanchen@mail.hz.zj.cn

Telephone: +86-571-87217385 **Fax:** +86-571-87217044

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Abstract

AIM: To clone *ureB* gene from a clinical isolate of *Helicobacter pylori* and construct a prokaryotic expression system of the gene and identify immunity of the expressed recombinant protein.

METHODS: *ureB* gene from a clinical *H pylori* strain Y06 was amplified by the high fidelity polymerase chain reaction technique. The target DNA fragment amplified from *ureB* gene was sequenced after T-A cloning. Prokaryotic recombinant expression vector pET32a inserted with *ureB* gene (pET32a-*ureB*) was constructed. The expression of recombinant UreB protein (rUreB) in *E. coli* BL21DE3 induced by isopropylthio- β -D-galactoside (IPTG) at different concentrations was examined by SDS-PAGE. Western blot using commercial antibodies against whole cell of *H pylori* and an immunodiffusion assay using a self-prepared rabbit anti-rUreB antibody were applied to determine immunity of the target recombinant protein. ELISA was used to detect the antibody against rUreB in sera of 125 *H pylori* infected patients and to examine rUreB expression in 109 *H pylori* isolates.

RESULTS: In comparison with the reported corresponding sequences, the nucleotide sequence homology of the cloned *ureB* gene was from 96.88-97.82% while the homology of its putative amino acid sequence was as high as 99.65-99.82%. The rUreB output expressed by pET32a-*ureB*-BL21DE3 was approximate 30% of the total bacterial proteins. rUreB specifically combined with the commercial antibodies against whole cell of *H pylori* and strongly induced rabbits to produce antibody with a 1:8 immunodiffusion titer after the animals were immunized with the recombinant protein. Serum samples from all *H pylori* infected patients were positive for UreB antibody and UreB expression were detectable in all tested *H pylori* isolates.

CONCLUSION: A prokaryotic expression system with high expression efficiency of *H pylori ureB* gene was successfully established. The expressed rUreB showed qualified immunoreactivity and antigenicity. High frequencies of UreB

expression in different *H pylori* isolates and specific antibody against *UreB* in sera of *H pylori* infected patients indicate that *UreB* is an excellent antigen candidate for developing *H pylori* vaccine.

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INTRODUCTION

In China, chronic gastritis and peptic ulcer are two most common gastric diseases, and gastric cancer is one of the malignant tumors with high mortalities and morbidities^[1-34]. *Helicobacter pylori*, a microaerophilic, spiral and Gram-negative bacterium, is recognized as a human-specific gastric pathogen that colonizes the stomachs of at least half of the world's populations^[35]. Most infected individuals are asymptomatic. However, in some subjects, *H pylori* infection causes acute/chronic gastritis or peptic ulceration. Furthermore, the infection is also a high risk factor for the development of gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma and primary gastric non-Hodgkin's lymphoma^[36-43]. Recently, direct evidence that *H pylori* causes gastric carcinoma in an animal model has been reported^[44-46]. Immunization against the bacterium represents a cost-effective strategy to prevent *H pylori*-associated peptic ulcer disease and to reduce the incidence of global gastric cancer^[47]. The selection of antigenic targets is critical in the design of *H pylori* vaccine. So far, no vaccine preventing *H pylori* infection was commercially available. Almost all *H pylori* strains produce a special urease decomposing urea and forming ammonia, which is harmful to gastric mucosa. Moreover, a high pH value is benefit for organisms to colonize the stomach. Urease is composed of 4 subunits, A, B, C and D. The subunit B (UreB), a peptide with 569 amino acid residuals encoded by *ureB* gene, has been demonstrated to show the strongest antigenicity and protection among all known *H pylori* proteins^[48-50]. Furthermore, almost all *H pylori* isolates have *ureB* gene and express UreB protein, and their nucleotide and putative amino acid sequence homologies are as high as approximate 95%^[51-53]. These data strongly indicate that UreB is a potential antigen candidate for *H pylori* vaccine. In this study, a recombinant prokaryotic vector responsible for the recombinant UreB protein (rUreB) expression was constructed. Immunogenicity and immunoreactivity of rUreB were further examined. Furthermore, rUreB was used as an antigen in ELISA to detect specific antibody in sera from *H pylori* infected patients, and rabbit anti-rUreB serum was prepared to examine the UreB expression in different *H pylori* isolates. The results of this study will supply further experimental foundation for the development of *H pylori* vaccine.

MATERIALS AND METHODS

Materials

A clinical strain of *H pylori*, provisionally named Y06, which was well-characterized by the Department of Medical Microbiology and Parasitology, College of Medical Science, Zhejiang University, was used in this study. A plasmid pET32a (Novagen, Madison, USA) and *E. coli* BL21 DE3 (Novagen, Madison, USA) were used as the expression vector and host cell, respectively. Primers for polymerase chain reaction (PCR) amplification were synthesized by BioAsia (Shanghai, China). A Taq-plus high fidelity PCR kit and restriction endonucleases were purchased from TaKaRa (Dalian, China). The T-A Cloning kit and sequencing service were provided by BBST (Shanghai, China). DAKO (Glostrup, Denmark) and Jackson ImmunoResearch (West Grove, USA) supplied rabbit antiserum against whole cell of *H pylori*, HRP-labeling sheep anti-rabbit IgG and anti-human IgG antibodies, respectively. Agents for *H pylori* isolation and identification were purchased from bioMérieux (Marcy l'Étoile, France). Overall, 126 patients (86 males and 40 females; age range: from 6-78 yr; mean age: 40.5 yr) who were referred for upper endoscopy from 4 different hospitals in Hangzhou during the period between December 2001 and June 2002 and had *H pylori* isolated from gastric mucosa were included in the study. All patients gave a written informed content. Of the 126 patients, 68 were endoscopically diagnosed as having chronic gastritis (CG, 48 superficial, 10 active and 10 atrophic gastritis), and 58 as having peptic ulcer disease (PUD, 12 gastric, 40 duodenal, and 6 gastric and duodenal ulcer). None of the patients had taken nonsteroidal anti-inflammatory drugs, antacids and antibiotics during the 2-wk before seeking medical advice. At the same time, serum specimens were also collected from these patients.

Isolation and identification of *H pylori*

Each gastric biopsy taken during endoscopy was homogenized with a tissue grinder and then inoculated on Columbia agar plates supplemented with 80 mL/L sheep blood, 5 g/L cyclodextrin, 5 mg/L trimethoprim, 10 mg/L vancomycin, 2.5 mg/L amphotericin B and 2 500 U/L cefsulodin. The plates were incubated at 37 °C under microaerobic conditions (50 mL/LO₂, 100 mL/LCO₂ and 850 mL/LN₂) for 3 to 5 d. A bacterial isolate was identified as *H pylori* according to typical Gram stain morphology, biochemical tests positive for urease and oxidase, and agglutination with the commercial rabbit antibodies against whole cell of *H pylori*. One of the isolated strains, named as Y06 that had typical characteristics of *H pylori*, was selected for further experiments.

Preparation of DNA template

Genomic DNA of *H pylori* strain Y06 was prepared by the routine phenol-chloroform method and DNase-free RNase treatment. The obtained DNA was dissolved in TE buffer, and its concentration and purity were determined by ultraviolet spectrophotometry^[54].

Polymerase Chain Reaction

Primers were designed to amplify the whole sequence of *ureB* gene from strain Y06 based on the published sequences and reading frames^[51-53]. The sequences of *ureB* sense primer with an endonuclease site of EcoRV and antisense primer with an endonuclease site of *XhoI* were 5'-GGAGATATCATGATTAGCAGAAAAG AATATGTTTC-3' and 5'-GGACTCGAGCTAGAAAATGCTAAAGATTTGTGC-3', respectively. Total volume per PCR was 100 µL containing 2.5 mol/L each dNTP, 250 nmol/L each of the 2 primers, 15 mol/L MgCl₂, 3.0 U Taq-plus polymerase, 100 ng DNA template and

1×PCR buffer (pH8.3). The parameters for PCR were 94 °C for 5 min, ×1; 94 °C for 30 s, 56 °C for 30 s, 72 °C for 120 s, ×10; 94 °C for 30 s, 56 °C for 30 s, 72 °C for 130 s (10 s addition for each of the following cycles), ×20; and then 72 °C for 12 min, ×1. The results of PCR were observed under UV light after electrophoresis in 10 g/L agarose, pre-stained with ethidium bromide. The expected size of target amplification fragment was 1 704 bp.

Cloning and sequencing

The target amplification DNA fragment from *ureB* gene was cloned into pUCm-T vector (pUCm-T-*ureB*) by using the T-A Cloning kit according to the manufacturer's instruction. The recombinant plasmid was amplified in *E. coli* strain DH5α and then extracted by Sambrook's method^[54]. A professional company (BBST) was responsible for nucleotide sequence analysis of the inserted fragment. Two *E. coli* DH5α strains containing pUCm-T-*ureB* and expression vector pET32a, respectively, were subcultured in LB medium. Then the plasmids were extracted^[54] and digested with *EcoRV* and *XhoI*, respectively. The *ureB* target fragments and pET32a were recovered for ligation. The recombinant expression vector pET32a-*ureB* was transformed into *E. coli* BL21DE3, which was named as pET32a-*ureB*-BL21DE3. The target *ureB* gene fragment inserted in pET32a plasmid was sequenced again.

Expression and identification of the fusion protein

pET32a-*ureB*-BL21DE3 was rotatively cultured in LB medium at 37 °C and induced by isopropylthio-β-D-galactoside (IPTG) at different concentrations of 1.0, 0.5 and 0.1 mmol/L. The supernatant and precipitate were separated by centrifugation after the bacterial pallet was ultrasonically broken (300 V, 5 s×3). The molecular mass and output of rUreB were measured by SDS-PAGE. The expressed rUreB was collected by Ni-NTA affinity chromatography. The commercial rabbit antiserum against whole cell of *H pylori* and HRP-labeling sheep anti-rabbit IgG were used as the first and second antibodies, respectively, to determine the immunoreactivity of rUreB by Western blot. Rabbits were immunized with rUreB to prepare the antiserum, and an immunodiffusion assay was applied to determine the antigenicity of rUreB.

ELISA

By using rUreB as antigen at the coated concentration of 20 µg/mL, a patient serum sample (1:400 dilution) as the first antibody and HRP-labeling sheep anti-human IgG (1:4 000 dilution) as the second antibody, the specific antibody against UreB in sera from the 126 patients infected with *H pylori* were detected by ELISA. The result of ELISA for a patient's serum sample was considered as positive if the *A* value at 490 nm (*A*₄₉₀) was over the mean plus 3 SD of 6 negative serum samples^[55]. UreB expression in *H pylori* isolates was examined by ELISA using the ultrasonic supernatant of each isolate (50 µg/mL) as a coated protein antigen, the self-prepared rabbit anti-rUreB serum (1:2 000 dilution) as the first antibody and HRP-labeling sheep anti-rabbit IgG (1:3 000 dilution) as the second antibody. The result of ELISA for a *H pylori* ultrasonic supernatant sample was considered as positive if its *A*₄₉₀ value was over the mean plus 3 SD of 6 ultrasonic supernatant samples at the same protein concentration of *E. coli* ATCC 25922^[55].

Data analysis

The nucleotide sequences of the cloned *ureB* gene inserted in the two recombinant plasmid vectors were compared for homology with the 3 published *ureB* gene sequences (NC000915, M60398, X57132, AB032429)^[51-53] by using a molecular biological analysis soft ware.

RESULTS

PCR

The target fragment of *ureB* gene with the expected size amplified from DNA template of *H pylori* strain Y06 is shown in Figure 1.

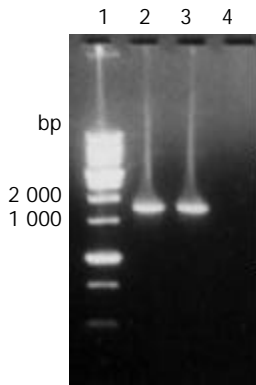


Figure 1 The target fragment of *ureB* gene amplified from *H pylori* strain Y06 DNA. Lane 1, 1 kb DNA marker (BBST); lanes 2 and 3, the target amplification fragment of *ureB* gene from genomic DNA of *H pylori* strain Y06; lane 4: blank control.

Nucleotide sequence analysis

The nucleotide sequences of *ureB* gene in *pUCm-T-ureB* and *pET32a-ureB* were completely same. The homologies of the nucleotide and putative amino acid sequences of the cloned *ureB* gene compared with the published *ureB* gene sequences^[51-53] were from 96.88% to 97.82% and from 99.65% to 99.82%, respectively (Figures 2 and 3).

Expression of the target fusion protein

IPTG at concentrations of 1.0, 0.5 and 0.1 mmol/L efficiently induced the expression of rUreB in the pET32a-ureB-BL21DE3 system. The product of rUreB was mainly presented in ultrasonic precipitates and the output was approximate 30% of the total bacterial proteins (Figure 4).

Immunoreactivity and antigenicity of rUreB

The commercial rabbit antibodies against the whole cell of *H pylori* combined with rUreB as confirmed by Western blot (Figure 5), and the titer demonstrated by immunodiffusion assay between rUreB and self-prepared rabbit anti-rUeB serum was 1:8.

ELISA

Since the mean±SD of A₄₉₀ values of the 6 negative serum

```
(1)1   ATGAAAAAGATTAGCAGAAAAGAATATGTTTCTATGTATGGCCCTACTACAGGCGATAAA
(2)1   .....
(3)1   .....
(4)1   ATG.....T.....

(1)61  GTGAGATTGGGCGATACAGACTTGATCGCTGAAGTAGAACATGACTACACCATTATGGC
(2)61  .....
(3)61  .....
(4)61  .....

(1)121 GAAGAGCTTAAATTCGGTGGCGGTAACCCCTGAGAGAAGGCATGAGCCAATCCAACAAC
(2)121 .....
(3)121 .....CC.....T.....T.....T.....
(4)121 .....G..TT...G.....

(1)181 CCTAGCAAAGAAGAATTGGATCTAATCATCACTAACGCTTTAATCGTGGATTACACCGGT
(2)181 .....G.....T...T.....
(3)181 .....C.....T.....G.....
(4)181 .....C.....T.....

(1)241 ATTTATAAAGCGGATATTGGTATTAAAGATGGCAAAATCGCTGGCATTGGTAAAGGCGGT
(2)241 .....
(3)241 .....
(4)241 .....C.....

(1)301 AACAAAGACATGCAAGATGGCGTTAAAAACAATCTTAGCGTAGGTCCTGCTACTGAAGCC
(2)301 .....
(3)301 .....G.....
(4)301 .....G.....

(1)361 TTAGCCGGTGAAGGTTTGATCGTAACTGCTGGTGGTATTGACACACACATCCACTTCATT
(2)361 .....G.....
(3)361 .....C.....
(4)361 .....T.....C.....C.....

(1)421 TCACCCCAACAAATCCCTACAGCTTTTGCAAGCGGTGTAACAACCATGATTGGTGGCGGA
(2)421 .....T...
(3)421 ..C.....G.....
(4)421 ..C.....

(1)481 ACTGGTCCTGCTGATGGCACTAATGCGACTACTATCACTCCAGGCAGAAGAAATTTAAAA
(2)481 ..C.....
(3)481 .....C.....C..A..C.....
(4)481 .....C..A..C.....C.....

(1)541 TGGATGCTCAGAGCGGCTGAAGAATATTCTATGAACTTAGGTTTCTTGGCTAAAGGTAAC
(2)541 .....T.....
(3)541 .....
(4)541 .....C.....A.....
```

(1)601 GCTTCTAACGACGCGAGCTTAGCCGATCAAATTGAAGCTGGTGCATTGGCTTTAAAATC
(2)601T.....C.....T
(3)601T..T.....C.....
(4)601 A.....T.....C.....T.....

(1)661 CACGAAGACTGGGGCACCCTCTTCTGCAATCAATCATGCGTTAGATGTTGCAGACAAA
(2)661G.....
(3)661T.....A..A.....
(4)661A..A.....G.....

(1)721 TACGATGTGCAAGTCGCTATCCACACAGACACTTTGAATGAAGCCGGTTGCGTGGGAAGAC
(2)721T..A.....
(3)721T..A.....
(4)721T..A.....

(1)781 ACTATGGCAGCTATTGCCGGACGCACTATGCACACTTTCCACACTGAAGGTGCTGGCGGC
(2)781T.....T.....C.....
(3)781C.....T.....
(4)781C.....C.....T.....

(1)841 GGACACGCTCCTGATATTATTAAAGTAGCTGGTGAACACAACATTCTTCCCCTTCCACT
(2)841C.....
(3)841C.....G.....
(4)841G..C..C.....G.....

(1)901 AACCCCACTATCCCTTTCCTGCTGAATACAGAAGCAGAACACATGGACATGCTTATGGTG
(2)901C.....C.....G.....
(3)901
(4)901
(5)901

(1)961 TGCCACCCTTGATAAAAAGCATTAAAGAAGATGTTTCAGTTCGCTGATTCAAGGATCCGC
(2)961
(3)961
(4)961

(1)1021 CCTCAAACCATGCGGCTGAAGACACTTTGCATGACATGGGGATTTTCTCAATCACCAGC
(2)1021T.....
(3)1021T..T.....
(4)1021T.....C.....T..T.....

(1)1081 TCTGACTCTCAAGCTATGGGTCGTGGGTGAAGTTATCACTAGAACTGGCAAACAGCT
(2)1081G.....C.....
(3)1081C.....
(4)1081

(1)1141 GACAAAAACAAAAAGAATTGGCCGCTTGAAAGAAGAAAAAGGCGATAACGACAACCTTC
(2)1141G.....
(3)1141
(4)1141

(1)1201 AGGATCAAACGCTACTTGTCTAAATACACCATTAACCCAGCGATCGCTCATGGGATTAGC
(2)1201
(3)1201
(4)1201

(1)1261 GAGTATGTAGTTCTGTAGAAGTGGGCAAAGTGGCTGACTTGGTATTGTGGAGTCCCAGCA
(2)1261A.....A...
(3)1261A.....A...
(4)1261A.....

(1)1321 TTCTTTGGCGTAAAACCAACATGATCATCAAAGCGGGTTCATTGCGTTGAGTCAAATG
(2)1321G.....A.....A..C.....
(3)1321G.....T.....A.....
(4)1321G.....A.....

(1)1381 GGTGACGCGAACGCTTCTATCCCTACCCACAACCAGTTTATTACAGAGAAATGTTTCGCT
(2)1381 ..C..T.....G.....
(3)1381 ..C..T.....
(4)1381 ..C..T.....

(1)1441 CATCATGGTAAAGCCAAATACGATGCAAACATCACTTTTGTGTCTCAAGCGGCTTATGAC
(2)1441T.....
(3)1441A..C.....A.....
(4)1441

```

(1)1501 AAAGGCATTAAAGAAGAATTAGGGCTTGAAAGACAAGTGTGCGGTAAAAAATTGCAGA
(2)1501 .....A.....
(3)1501 .....
(4)1501 .....

(1)1561 AACATCACTAAAAAGACATGCAATTCAACGACACTACCGCTCACATTGAAGTCAATCCT
(2)1561 ..T.....T.....
(3)1561 .....
(4)1561 .....

(1)1621 GAAACTTACCATGTGTTTCGTGGATGGCAAAGAAGTAACTTCTAAACCAGCCAATAAAGTG
(2)1621 .....
(3)1621 .....C.....
(4)1621 .....C.....

(1)1681 AGCTTGGCGCAACTCTTTAGCATTCTTAG
(2)1681 .....
(3)1681 .....
(4)1681 .....A.....

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Figure 2 Homology comparison of the cloned and reported *H pylori ureB* nucleotide sequences.(1)-(3): the reported sequences from GenBank (No. NC000915, strain 26695; No. M60398, X57132; No. AB032429); (4): the sequencing result of *H pylori* strain Y06 *ureB* gene. Underlined areas indicate the positions of primer sequences.

```

[1]1  MKKISRKEYVSMYGPTTGDKVRLGDTDLIAEVEHDYTIYGEELKFGGGKTLREGMSQSNN
[2]1  .....
[3]1  .....
[4]1  //M.....

[1]61  PSKEELDLIITNALIVDYTGIIYKADIGIKDGKIAGIGKGGNKMDQGVKNNLSVGPATEA
[2]61  .....
[3]61  .....
[4]61  .....

[1]121 LAGEGLIVTAGGIDTHIHFISPOQIPTAFASGVTTMIGGGTGPADGTNATTITPGRRLK
[2]121 .....
[3]121 .....
[4]121 .....

[1]181 WMLRAAEEYSMNLGFLAKGNASNDASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADK
[2]181 .....
[3]181 .....
[4]181 .....

[1]241 YDVQVAIHDTLNEAGCVEDTMAAIAGRTMHTFHTEGAGGGHAPDIKIVAGEHNILPAST
[2]241 .....
[3]241 .....
[4]241 .....

[1]301 NPTIPFTVNTAEHMDMLMVCHHLKSIKEDVQFADSRIRPQTIAAEDTLHDMGIFSITS
[2]301 .....
[3]301 .....
[4]301 .....

[1]361 SDSQAMGRVGEVITRTWQTADKNKKEFGRLKEEKGDNDNFRIKRYLSKYTINPAIAHGIS
[2]361 .....
[3]361 .....
[4]361 .....

[1]421 EYVGSVEVGKVADLVLWSPAFFGVKPNMIKGGFIALSQMGDANASIPTPQPVVYREMFA
[2]421 .....
[3]421 .....
[4]421 .....

[1]481 HHGKAKYDANITFVSQAAYDKGIKEELGLERQVLPVKNCRNITKKDMQFNDTTAHIEVNP
[2]481 .....
[3]481 .....
[4]481 .....

[1]541 ETYHVFVDGKEVTSKPANKVSLAQLFSIF
[2]541 .....
[3]541 .....T.....
[4]541 .....

```

Figure 3 Homology comparison of the putative amino acid sequences of the cloned and reported *H pylori ureB* gene. [1]-[3]: the reported sequences from GenBank (No. NC000915, strain 26695; No. M60398, X57132; No. AB032429); [4]: the putative amino acid sequence of the cloned *H pylori* strain Y06 *ureB* gene.

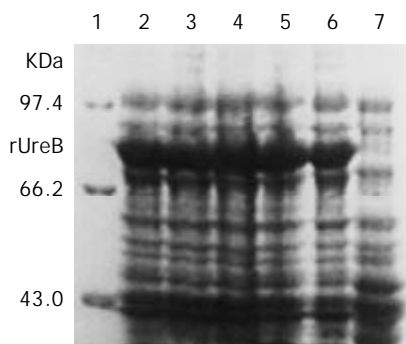


Figure 4 Expression of *rUreB* induced by IPTG at different concentrations. Lane 1, molecular mass marker; lanes 2-4, IPTG at 1.0, 0.5, and 0.1 mmol/L, respectively; lanes 5 and 6, bacterial precipitate and supernatant with IPTG at 1 mmol/L, respectively; lane 7, control.

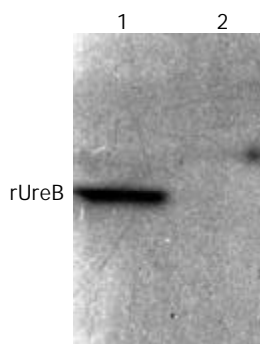


Figure 5 Western blot result of commercial rabbit antibodies against the whole cell of *H pylori* and *rUreB*. Lane 1, *rUreB*; lane 2, blank.

samples was 0.173 ± 0.025 , the positive reference value for the specific antibody detection in patients' sera was 0.248. According to the reference value, 100% (125/125, one serum sample was contaminated) of the tested patients' serum samples were positive for antibody against *rUreB* with an A_{490} value range of 0.27-1.97. Since the mean \pm SD of A_{490} of the 6 negative bacterial controls was 0.170 ± 0.026 , the positive reference value was 0.248 for *UreB* detection in *H pylori* isolates. According to the reference value, 100% (109/109, the other 17 isolates could not be revived from -70°C) of the tested *H pylori* isolates were positive for *rUreB* epitopes with an A_{490} value range of 0.47-1.93.

DISCUSSION

Amoxicillin and metronidazole, used as routinely therapeutic antibiotics in a triple therapy, effectively eradicate *H pylori* infection *in vivo*^[56-58]. However, there are problems such as side effects, emergence of drug resistance and re-infection after withdrawal of antibiotics, etc. It is generally considered that inoculation with *H pylori* vaccine is of value in prevention and control of *H pylori* infection. Previous studies have demonstrated that inoculation with sonic-broken *H pylori* resist the challenge of live *H pylori* and even eliminate existing infection of *H pylori* in animal models. But high nutrition requirements, poor growth after several passages, easy contamination during cultivation and difficulty in bacterial conservation make whole cell vaccine of *H pylori* impracticable. Genetic engineering vaccine seems to be a possible pathway for developing *H pylori* vaccine. *UreB*, used as a candidate antigen for *H pylori* genetic engineering vaccine, has advantages of high sequence conservation, a high frequency of distribution and large expression in different isolates, strong

antigenicity due to its big molecular mass, granular structure and exposure on the surface of bacteria^[59,60].

In the present study, a prokaryotic expression system of *rUreB* was constructed. In comparison with the three published *ureB* gene sequences^[51-53], high homologies of 96.88-97.82% and 99.65-99.82% of the nucleotide and putative amino acid sequences of the cloned *ureB* gene from the *H pylori* strain Y06, respectively, were found, whereas homologies were 96.78-97.83% and 99.82-100%, respectively, among the *ureB* genes from the 3 *H pylori* strains. These data indicate that the mutation level of *ureB* gene from different *H pylori* strains is considerably low.

The results of SDS-PAGE performed in this study demonstrate that the constructed prokaryotic expression system pET32a-*ureB*-BL21DE3 efficiently produces *rUreB* even at the concentration of IPTG as low as 0.1 mmol/L. *rUreB* mainly is presented in the form of dissoluble inclusion body and appears in the supernatant of culture only in a small amount. The output of *rUreB* is relatively high (approximately 30% of the total bacterial proteins), which is beneficial to industrial production.

Western blot assay performed in this study confirms that the commercial rabbit antiserum against whole cell of *H pylori* recognizes and combines to *rUreB*, indicating a high immunoreactivity of the recombinant protein. The immunodiffusion assay also demonstrates that *rUreB* efficiently induces rabbit to produce specific antibody with a higher titer, exhibiting a favorable antigenicity of the recombinant protein.

The findings by ELISA that all the tested *H pylori* isolates expressed *UreB* and all the tested sera from *H pylori* infected patients were positive for *UreB*-specific antibody indicate the universal existence of *UreB* in *H pylori* strains and a high frequency of *UreB*-specific antibody in human, which supports application of *rUreB* as an antigen for the development of *H pylori* vaccine.

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