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Research Article

CLONING AND EXPRESSION OF A PARTIAL *UREA* ANTIGEN FOR THE PRODUCTION OF VACCINE AGAINST *HELICOBACTER PYLORI*, THE RISK FACTOR FOR GASTRIC CANCER

MOHAMMAD JAVAD MEHRAN*, SEYED HOSSEIN ZENDEHBAD, SUDHAKAR MALLA

Centre for Research & PG studies, Indian Academy Degree College, Bangalore-43. Email: sudhasanmoon2000@gmail.com

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ABSTRACT

Helicobacter pylori, a gram-negative, microaerophilic, motile, spiral-shaped bacterium, have been established as the etiologic agent of chronic gastritis and belongs to phylum Proteobacteria. Infection with *H. pylori* is the primary identified cause of gastric cancer. *H.pylori* has the capacity to invade the stomach and tolerate the habitat of the stomach. But eventually the organism dies slowly due to the low pH in the stomach. In order to protect itself from the acidic environment it produces loads of urease enzyme. This urease enzyme has drawn attention all round the world as a diagnostic agent in detecting the helicobacter infection. And moreover the antibiotic based drugs are of limited use. Novel methods are being developed for the production of antibodies to specific antigens and thus helping in the process of development of protein based vaccines.
*Ure*A (Urease) gene was isolated and ligated into pTZ57R/T cloning vector. The ligated product was then cloned into DH5α strain and allowed to propagate. The plasmids thus cloned were purified and later expressed for the gene of interest in an expression vector. The proteins specific to the gene of interest was then isolated and purified. This proteins purified can in turn be used for protein based vaccines.

Keywords: ureA gene, Helicobacter pylori, cloning, expression proteins.

INTRODUCTION

Gastric cancer is a very common malignant disease. Gastric cancer is the second, after pulmonary cancer, cause of death due to malignant cancers in the world. Symptoms of gastric cancer include weight loss, poor appetite, bloating, burping, nausea, vomiting. There is a geographic diversification in the occurrence of gastric cancer. Most cases are recorded in Japan, China, South America, and significantly less in Western Europe and in the United States (Kelley et al, 2003). It is probably the most frequent cancer in the male population with an incidence of 53.5%, 50.6% and 42.5% respectively (Gadelha et al, 1992). The mortality rate also is very high. It is the most common cause of cancer death in men and the third most common in women.

Helicobacter pylori infects mostly in childhood and stays for a long time. Majority of them infected will be potential carriers and transmit to others even though they are asymptomatic. *Helicobacter pylori* was known to cause gastric cancers and several ulcers in humans(Dunn, B. E et al, 1997). Even some non gastric *helicobacters* are also associated with human ailments, especially causing enteritis (Stanley *et al*, 1994).

These helicobacter are usually free swimming forms, protected with a thick mucous gel like substance that has wear and tear very often (Schreiber S et al, 2003). In order to colonize on the epithelial layers of stomach, the helicobacter species has to swim with its flagellar motion and at the same time it should have the capacity to control its polar movement of the flagella (McGee DJ *et al*, 2005; Ottemann KM et al, 2002). It is also observed that helicobacter species swim fastly when placed in an acidic environment (Croxen MA et al, 2006). This active swimming also stimulates other species of Helicobacter to come into close contact and start colonization on the epithelial surface. Even the colonization densities during this active swimming phase can rise to about 100 billion bacteria/ml in the mucus of the stomach layer (Croxen MA et al, 2006).

H. pylori has the capacity to invade the stomach and tolerate the habitat of the stomach. But eventually the organism dies slowly due to the low pH in the stomach. Inorder to protect itself from the acidic environment it produces loads of urease enzyme. This urease breaks down the urea content to ammonia and carbon dioxide thus neutralizing the acidic environment (Mobley, H. L et al, 1995). This ammonia liberated aids in elevating the pH levels thereby favoring the growth of helicobacter at high acidic environments (Scott, D. R et al, 1998).

This urease activity is highly conserved in helicobacter species and is thus sued as a diagnostic assay. Helicobacter cannot withstand for long in acid conditions. It can only withstand for a few minutes. Evolutionary studies also suggest that, helicobacter have evolved many strategies to avoid from the acidic environment in the stomach. It stays in very close contact with the epithelium of the stomach, and hence can escape from the acid content.

Urease is used a clinical cofactor for diagnosing several helicobacter species like *H.pylori* and *H.mustelae*. These species attained loads of urease enzyme to safely colonise on the mucus layer on the stomach of animals (Andrutis, K. A et al, 1995; Dunn, B. E et al, 1998; Tsuda, M et al 1995).

Urease is treated as a clinical cofactor in diagnosing the helicobacter species and is found to enhance the production of cytokines and activation of phagocytes towards the gastric layers, causing inflammation of gastric layers (Harris, P. R et al, 1996). Ammonium ion released is also used as mode of diagnosis. This liberated ammonium ions are also proved to be toxic to the epithelial layers of stomach (Garner, R. M et al, 1998;Smoot, D. T et al, 1990). Urease is a metalloenzyme, with nickel as a cofactor. It is a heteromultimer and contains about 12 copies of UreA and UreB subunits, where they are encoded by urea and ureB genes respectively (Ha, N. C et al, 2001). While urease is an important virulence factor for gastric *helicobacters* which inhabit a highly acidic environment, the function of urease in the nongastric helicobacters, whose environment is not acidic, is unclear.

In this study we designed to amplify the partial ureA gene from Helicobacter pylori and clone it into a cloning vector. The vector will then be purified and cloned into a expression vector for protein production. The sequence identified from plasmid will also be sequenced to check the sequence similarity. The clones will be identified and sequenced thereafter to identify the similarity.

Materials and methods

Helicobacter pylori culture was kindly donated by Credora life sciences laboratory, Bangalore and was then subcultured on Brain

heart infusion media. The culture was revived from the glycerol stock and then incubated at 37^{0}C in an anaerobic chamber in slightly microaerophilic condition with 20% CO_2. Restriction enzymes, dNTPs, Taq polymerase were all purchased from HIMEDIA, India. Oligonucleotides were provided by the Genentech Organic Synthesis Group and were synthesized by the phosphotriester method.

Isolation of genomic DNA from bacteria

Total genomic DNA from the bacteria was isolated by N- Cetyl- N, N, N-trimethyl- ammonium bromide (CTAB) method described elsewhere (Wilson, K. 2001). In brief, the culture was centrifuged at 10000 rpm at 4°C and lysed with 675 µl extraction buffer (100 mMTrisHCl, 100 mM EDTA, 1.4 M NaCl, 1% CTAB and Proteinase K - 0.03µg/µl). The suspension was incubated at about 37^{0}C for 30 minutes. To the mixture 75µl of 20% SDS was added and incubated at 65°C for 2 hours. The suspension was then centrifuged and the supernatant was extracted with equal volumes of Chloroform and Isoamyl alcohol (24:1). The aqueous phase obtained after centrifugation was then extracted with 0.6 volumes of isopropyl alcohol. The mixture was allowed to stand undisturbed at RT for 1hour. The suspension was then centrifuged again and the DNA was pelleted with 500µl of 70% ethanol. The DNA collected was then quantified using UV spectrophotomer (Shimadzu 1800 series).

PCR amplification:

The ureA gene was amplified by PCR using purified genomic DNA as a template. Oligonucleotide primers were synthesized to amplify the intact region of ureA gene. The forward primer, 5′GAC ATT GGC GGT AAC AGA AG 3′ and the reverse primer, 5′- CTA AGG ATT TAA GGA GCA TCG 3′, were purchased from Eurofins, Bangalore. These primers correspond to the gene ureA and thus the final PCR product was 1983 Kb.

The PCR mixture consisted of 10x reaction buffer with $MgCl_2$ (1.5mM), 2µL of dNTP mix (2.5mM), 2 µL each of forward and reverse primers (10picomoles/µl each primer), 0.3µL of Taq DNA polymerase (5 U/µL), and 50ng/ µL of template DNA in a total volume of 20 µL.

The PCR was performed with the following cycling profile: initial denaturation at 94°C for 2 min, followed by 30 cycles of 50s denaturation at 94°C, annealing at 51°C for 30s, and extension at 72°C for 1min. The time for the final extension step was increased to 6 min. The PCR products amplified were then qualitatively analysed on 1% agarose gel. The PCR product was recovered using the QIA quick gel extraction kit, and the amplified product was then purified and used for cloning purpose.

Primer Details

Table 1: Table showing the details of the forward and reverse primers designed towards amplification of the urea gene of *Helicobacter pylori.*

Primer	Sequences (5'-3')	GC %	Tm Value	Length	Product Size
FW	GAC ATT GGC GGT AAC AGA AG	50	51.8^{0}C	20	1710bp
RV	CTA AGG ATT TAA GGA GCA TCG	43	50.5^{0}C	21	

Cloning of the *ureA* gene

The purified PCR product was ligated into the pTZ57R/T cloning vector (Fermentas, USA) and the resulting plasmid was transformed into the competent *E. coli* JM109. A 30 µl ligation reaction was setup in 3:1 molar ratio of insert and vector DNA as follows. 6µl of PCR product (0.52pmol) was ligated with T4 DNA ligase (5 weiss units) and vector of concentration 50ng/µl was used. The total volume of the reaction mixture was made up to 30µl with nuclease free water (Sigma Aldrich). Ligation mixture was incubated at room temperature (25°C) for one hour after a short spin. The ligated product was later kept on ice until the transformation experiment started.

Competent cell preparation and transformation

The competent cells were prepared using the protocol as described in Molecular cloning (Sambrook and Russel, 1989). *E. coli* DH-5-α was revived from the glycerol stock and inoculated into 5ml of Luria Bertani (LB) broth. The culture was incubated overnight at 37°C in an orbital shaker at 200rpm. The culture was allowed to grow until the optical density reached about 0.375(A600). Then the cultures were transferred to pre-chilled centrifuged tubes and incubated for 10 minutes on ice and later subjected to calcium chloride treatment (0.1M $CaCl_2$).

From the final suspension, aliquots of 200 µl were stored for further use at -80°C in 40% glycerol. The aliquots are used for transformation.

The ligated product was mixed with 200µl of prepared competent cells and incubated on ice for 30 minutes without disturbing followed by heat shock treatment at 42°C for 2 minutes. The tubes were then incubated on ice for 2minutes. To the treated cells 1ml of LB broth was added and the tubes were incubated in an orbital shaker at 37°C for 1 hour with an agitation of ~200rpm.

During the incubation period, 50ml of LB agar was melted and allowed to cool to 40°C. To the 50ml of molten LB agar, 50 µl of Ampicillin (50mg/ml) to a final concentration of 50µg/ml, 200µl of X-Gal to a final concentration of 80µg/ml and 20µl of IPTG to a final concentration of 80µg/ml was added. The incubated culture after 1 hour was centrifuged at 1000rpm for 10 minutes at room temperature and the pellet was resuspended in 100 µl of fresh LB broth. From the suspension, 100 µl was spread on LB agar plate. The plates were then incubated at 37°C overnight.

White colonies containing recombinant plasmids due to the insertional inactivation of the *lacZ* gene were selected and streaked on a fresh LB plate containing ampicillin and incubated overnight and served as a master plate for each tranformant. All colonies from the master plate were subjected to plasmid DNA isolation and restriction analysis to identify the positive recombinants.

Plasmid isolation by alkaline lysis method

The selected colonies were inoculated separately into 2ml of LB medium containing Ampicillin (50µg/ml) in a 15ml sterile tube. The cultures were incubated overnight at 37°C with vigorous shaking. 1.5ml of overnight culture was centrifuged at 12000 rpm for 30 seconds at 4°C in a microcentrifuge. The bacterial pellet was resuspended in 100 µl of ice cold solution I (50mM glucose, 25mM Tris-Cl and 10mM EDTA; pH 8.0) by vigorous vortexing. To the suspension 200µl of freshly prepared solution II (0.2N NaOH, 1% w/v SDS) was added and mixed properly. The contents of the tube were mixed by inverting the tube rapidly for five times and 150µl of ice cold solution III (5M Potassium acetate, 12.5 ml glacial acetic acid and the resultant solution made upto 100 ml) was added. The tubes were then vortexed gently and the tubes were incubated on ice for 3-5 minutes and later centrifuged at 12000rpm for 5 minutes at 4°C. The supernatant collected was extracted with an equal volume of chloroform and isoamyl alchohol (24:1). The Plasmid DNA was precipitated with 0.6 volumes of ice cold isopropanol and the DNA was pelleted at 12000rpm and the pellet was washed with 70% ethanol.

Confirmation of clones by restriction digestion

The purified plasmid was subjected to restriction digestion using restriction endonucleases (Merck, India). Restriction digestion was performed in 20 µl reaction volumes with recommended units of enzyme and appropriate buffers at 37°C for 2hours. The plasmid DNA (0.2µg/µl) was double digested with restriction enzyme *Bam*HI (10U/µl) and *Eco* RI (10U/µl). The contents of the mixture were mixed properly by pipetting and then the tubes were briefly spin down. The tubes were then incubated at 37°C for 2 hours. The digested samples were resolved on 1% low melting agarose gel for confirming the release of the insert by the restriction endonucleases.

The released gene of insert was eluted from the agarose gel using gel extraction kit (Bioline USA).

Sequencing

The insert in the purified plasmid was sequenced using ABI PRISM Big Dye Terminators v1.1 cycle sequencing kit (Applied Biosystems Foster city, CA, USA) according to the manufacturer's instructions using T7/M13 primers. The comparison of the nucleotide sequences of the unique fragment with the sequences available in the GenBank database was carried out using the NCBI BLAST program (http//www.ncbi.nlm.nih.gov/blast)

Gene cloning into expression vector:

The gene of insert was eluted from the agarose gel using gel extraction kit (Bioline USA). Eluted gene of insert was quantified and ligated with the expression vector pET20b. The reaction mixture was incubated at 27 ^{0}C for 3-4 hours. Vector pET20b (50ng/µl) together with eluted gene of insert(0.52pmole) was added to the ligation mixture and then incubated.

Ligated plasmid was transformed in to the bacterium DH5α as described earlier in this paper and plated on LB medium containing ampicillin (50mg/ml) to a final concentration of 50µg/ml. The white colonies from the LB- Ampicillin agar plates were inoculated in LB-Ampicillin broth and incubated at 37°C for overnight and plasmid DNA was isolated by Alkaline lysis method as described earlier in this paper. The transformed bacteria were inoculated in 50ml of LB broth containing IPTG (0.5mM) as inducer for the expression of the gene. *E.coli* strain without plasmid was used as control. The inoculated culture flask was incubated at 37°C in an orbital shaker at 150 rpm for 24 hours.

Protein extraction

The bacterial cells were harvested by centrifugation (7,000 x g) at 4°C and washed with sterile distilled water. The cells were then suspended in 10 ml of ice-cold acetone (analytical grade), and allowed to stand on ice for 5 min. the suspension was then centrifuged (7,000 x g) at 4°C. Residual acetone was removed by air drying, and the proteins were then extracted by incubating with 1.0 ml of 1% sodium dodecyl sulfate (SDS) for 2 min.

The protein concentration was estimated at 280nm using UVspectrophotomer (Shimadzu 1800 series). From the stock 1µl Protein was mixed with 99-µl sterile distilled water to get 100 times dilution.

SDS PAGE analysis of protein sample:

The collected protein fractions in sample buffer (10% SDS, 10mM Dithiothreitol, 20% Glycerol, 0.2M Tris-HCl and 0.05% Bromophenol blue) were then separated on SDS PAGE electrophoresis containing 5% stacking gel and 12% resolving gel. The glass plates were removed from the electrophoresis apparatus and placed on a paper towel. The plates were carefully separated apart using a spatula. The gel was stained using Coomassie Brilliant Blue dye, by agitating slowly on a rocker, overnight and subsequently destained for a few times until protein bands were visualized. The molecular weight of protein bands were determined by comparing them with the molecular weight markers.

RESULTS AND DISCUSSION

Genomic DNA isolation and quantification

The Gram negative bacteria were cultured in the Brain heart infusion broth and genomic DNA was isolated by modified CTAB method. The isolated DNA was electrophorized in 1% Agarose gel. The quantity and quality of DNA was analyzed by UV spectrophotometer.

PCR amplification of the *ure*A gene

Species specific primers were designed for the *Helicobacter pylori* using the sequences of *Ure* A gene available in NCBI GenBank using Primer 3 Software. The predicted primers were validated initially *insilico* and subsequently on the thermocycler. The primers could yield an amplicon of the expected size specific to u*re*A gene. The primers were found to produce ~1060 bp amplicon which shown in the figure.

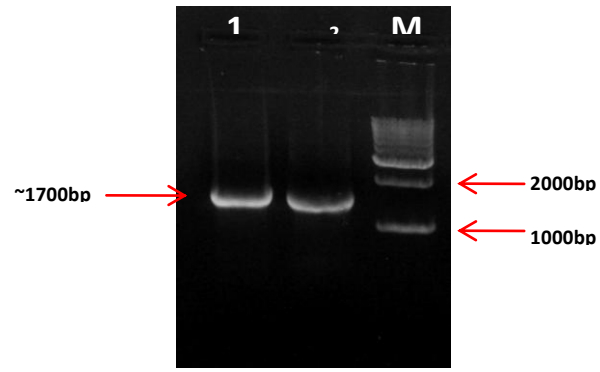

Fig.: 1 Gel showing the PCR amplification of *Ure*A partial gene by specific primers. (Lane M: 1kb ladder, Lane 1, 2: *Ure* A gene product.

16S rDNA PCR

16S rDNA sequence was done to identify the bacteria. The obtained sequence was then BLAST to identify the bacteria. The sequencing is 98% matching with *Helicobacter pylori* strains in BLAST analysis. The 16S rDNA sequence obtained was mentioned bellow.

5'ACTTGATCCTGGCTCAGAAGTGAACGGCTGGCGGCGTGCCTAATACA
TGCAAGTCGGAACAATGAAGCTTCTAGCTTGCTAGAAGTGGATTAGTG
GCGCACGGGTGAGTAACGCATAGGTCATGTGCCTCTTAGTTTGGGAAA
GCCATTGGAAACGATGATTAATACCAGACACTCCCGACGGGGGAAAGA
TTTATCGCTAAAAGATCAGCCTATGTCCTGTCAGCTTGTTGGTAAGGTA
ATGGCTTACCAAGGCTATGACGGGTATCCGGCCTGGGAGGGTGAACGG
ACACACTGGAACTGAGACACGGGTCCAGACTCCTACGGGAGGCAGCAGT
AGGGAATATTGCTCAATGGGGGAAACCCTGAAGCAGCAACGCCGCGTG
GAGGATGAAGGTTTTAGGATTGTAAACTCCTTTTGTTAGAGAAGATAA
TGACGGTATCTAACGAATAAGCACCGGCTAACTCCGTGACAGCAGCCGC
GGTAATACGGAGGGTGCAAGCGTTACTCGGAATCACTGGGCGTAAAGA
GCGCGTAGGCGGGATAGTCAGTCAGGTGTGAAAGCCTATGGCTTAACC
ATAGAACTGCATTTGAAACTACTATTCTAGAGTGTGGGAGAGGTAGGT
GGAATTTCTTGGTGTAGGGGTAAAATCCGTAGAGATCAGAGGAATACT
CATTGCGAAGGCGACCTGCTGGAACATTACTGACGCTGATTGCGCGAAA
GCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTGAACGCCCTGAA
CGATGGATGCTAGTTGTTGGAGGGCTTAGTCTCTCCAGTAATGCAGCTA
ACGCATTAAGCATCCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCA
AAGGAATAGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAATT
CGAAGATACACGAAGAACCTTACCTAGGCTTGACATTGAGAGAATCCG
CTAGAAATAGTGGAGTGTCTAGCTTCCTAGACCTTGAAAACAGGTGCT
GCACGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCGGCGC
AACGAGCGCAACACCCTTTCTTAGTTGCTAACAGGTCATGCTGAGAACT
CTAAGGATACTGCCTCCGTAAGGAGGAGAATGGTGGGGACGACGTCAA
GTCATCATGGCCCTTACGCCTAGGGCTACACACGTGCTACAATGGGGTG
CACAAAGAGAAGCAATACTGCGAAGTGGAGCCAATCTTCAAAACGCCT
CTCAGTTCGGATTGTAGGCTGCAACTCGCCTGCAAGAAGCTGGAATCGC
TAGTAACTCGCAAATCAGCCATTGTTAGAGGCCAATACGTTCCCGGGTC
TTGTACTCACCGCCCGTCACACCATGGGAGTTGTGTTTGCCTTAAGTCA
GGATGCTAAACGAGCTACTGCCCACGGCACAGCACAGGGACTGGGGTGA
AGTCGTAACAAGGTAACCGTA3'

Cloning of PCR product in to T vector

PCR yielded a specific amplicon of 1060-bp in *Helicobacter pylori* strain. The fragment was separated on 1% agarose gel and purified using gel extraction kit. The purified PCR product was quantified and then ligated with cloning vector using T4 DNA ligase enzyme. The ligated plasmid was transformed in to *E.coli* bacterial strain DH5α. The plates were screened for blue white colonies.

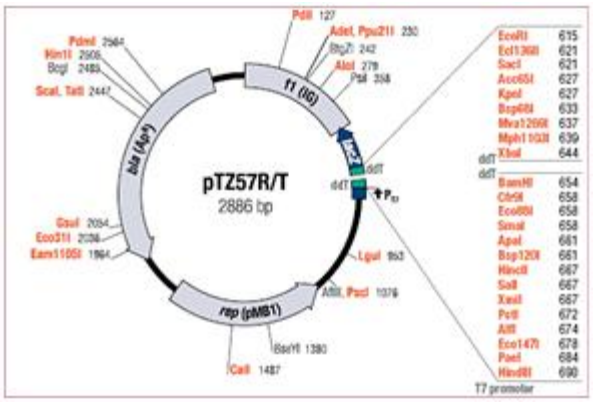

Fig 2: Map of T vector pTZ57R/T (Fermentas, Germany

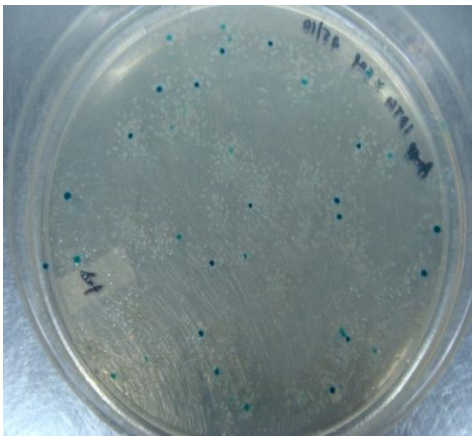

Fig 3: LB agar plate showing blue white colonies for the transformed bacterial cells. The white colonies correspond to the recombinant clones

Plasmid Isolation

Plasmids were isolated from the transformed cells by using alkaline lysis method. The isolated plasmid was then electrophoresed on 1% Agarose gel. The purified plasmid were subjected to double digested using *Bam*HI and *Eco*RI (Merck, India). After incubation at 37°C for 2 hours the restricted product was electrophoresed on 1% Agarose gel. The release of the gene product was visualized on the gel.

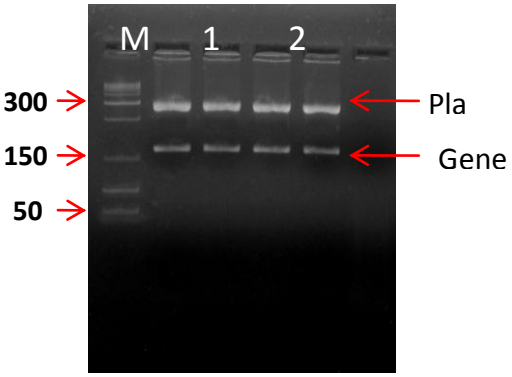

Fig 4: Restriction digestion of ligated plasmid using *Bam*HI and *Eco*RI on 1% agarose gel. Lane M: Molecular marker, Lane 1, 2, 3 &4: Digested sample.

Sequence data

The gene was identified by sequencing of plasmid. An approximately 1060-bp region of the *ure*A gene was sequenced at Eurofins, Bangalore. The sequence data was shown below. Nucleotide sequence analysis of gene was used to investigate the identity of bacterial *ure*A gene of *Helicobacter pylori*. To demonstrate the quality and accuracy of results provided from a public database, we compared sequences to their corresponding GenBank sequences. The sequence had "perfect" match (similarity, 99%) with sequences of their corresponding gene (*ure*A) from GenBank as determined by using BLAST (version 2.7).

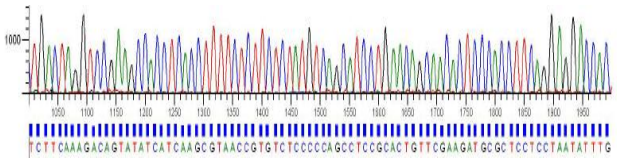

Fig 6: Showing the sequencing dendrogram

>*ure*A gene sequence

5'AGCACGTATGAAAAAGATTAGCAGAAAAGAATATGCTTCTATGTATG
GCCCTACTACAGGCGATAAAGTGAGATTGGGCGATACAGACTTATCGCT
GAAGTAGAACATGACTACACCATTTATGGCGAAGAGCTTAAATTCGGT
GGCGGTAAAACTTTAAGAGAAGGCATGAGCCAATCCAACAACCCTAGC
AAAGAAGAACTGGATTTAATCATCACTAACGCTTTAATCGTGGATTAT
ACCGGTATTTATAAAGCGGATATTGGTATTAAAGATGGCAAAATCGCT
GGCATTGGCAAAGGCGGTAACAAAGACATGCAAGATGGCGTTAAAAAC
AATCTTAGCGTGGGTCCTGCTACTGAAGCCTTAGCTGGTGAAGGTTTGA
TCGTAACTGCTGGTGGTATTGACACACACATCCACTTCATCTCCCCCCA
ACAAATCCCTACAGCTTTTGCAAGCGGTGTAACAACGATGATTGGTGGC
GGAACTGGCCCTGCTGATGGCACTAACGCAACCACTATCACTCCAGGCA
GAAGAAATTTAAAATGGATGCTCAGAGCGGCTGAAGAATATTCCATGA
ACTTAGGTTTCTTAGCTAAAGGTAACGCTTCTAACGATGCGAGCTTAGC
CGATCAAATTGAAGCCGGTGCGATCGGTCTTAAAATCCACGAAGACTG
GGGAACAACTCCTTCTGCAATCAATCATGCATTAGATGTTGCGGACAA
ATACGATGTGCAAGTCGCTATCCACACAGACACTTTGAATGAAGCCGGT
TGTGTAGAAGACACTATGGCAGCCATTGCCGGACGCACTATGCACACTT
TCCACACTGAAGGCGCTGGCGGCGGACACGCTCCTGATATTATTAAAGT
GGCCGGTGAACACAATATTCTACCCGCTTCCACTAACCCCACTATCCCT
TTCACTGTGAATACAGAAGCAGAACACATGGACGTGCTTATGGTGTGC
CACCACTTGGATAAAAGCATTAAAGAAGATGTTCAGTTCGCTGATTCA
AGGATTCGCCCTCAAACCATTGCGGCTGAAGACACTTTGCATGACATGG
GGATTTTCTCAATCACCAGTTCTGACTCTCAAGCTATGGGTCGTGTGGG
TGAAGTTATCACCAGAACTTGGCAAACAGCTGACAAAAACAAAAAAGA
ATTTGGCCGCTTGAAAGAAGAAAAAGGCGATAACGACAACTTCAGGAT
CAAACGCTACTTGTCTAGATACACCATTAACCCTGCGATCGCTCATGGG
ATTAGCGAGTATGTAGGTGCTGTAGAAGTGGGCAAAGTGGCTGACTTG
GTATTGTGGAGTCCCGCATTCTTTGGCGTAAAACCCAACATGATCATCA
AAGGCGGGTTCATCGCATTAAGTCAAATGGGTGATGCGAACGCTTCTA
TCACTACCCCACAACCGGTTTATTACAGAGAAATGTTCGCTCATCATGG
TAAAGCCAAATACGATGCAAACATCACTTTTGTGTCTCAAGCGGCTTAT
GACAAAGGCATTAAAGAAGAATTAGGGCTTGAAAGACAAGTGTTGCCG
GTAAAAAATTGCAGGAACATCACTAAAAAAGACATGCAATTCAACGAC
ACTACCGCTCACATTGAAGTCAATCCTGAAACTTACCATGTGTTCGTGG
ATGGCAAGGAAGTAACTTCTAAACCAGCCAATAAAGTGAGCTTGGCGC
AACTCTTTAGCATTTTCTAGACTGTGCA3'

SDS PAGE analysis of *ure*A gene expression analysis

Inserted *ure*A gene was expressed significantly in the prokaryotic expression system, and specific strip at ~ 70KDa was demonstrated in SDS-PAGE. The collected protein fractions in sample buffer (10% SDS, 10mM Dithiothreitol, 20% Glycerol, 0.2M Tris-HCl and 0.05% Bromophenol blue) were then separated on SDS PAGE electrophoresis containing 5% stacking gel and 12% resolving gel. The gel was stained using Coomassie Brilliant Blue dye, by agitating slowly on a rocker, overnight and subsequently destained for a few times until protein bands were visualized. The molecular weight of protein bands were determined by comparing them with the molecular weight markers.

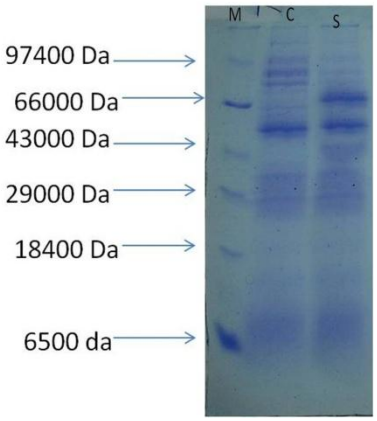

Fig 7: SDS PAGE analysis of *ure*A gene expression [Lane M: Protein ladder (Phosphorylase B-97400, Bovine Serum Albumin-66000, Ovalbumin-43000, Carbonic Anhydrase-29000, Lactoglobulin-18400, Aprotinin-6500), Lane C:Control, Lane S: *ureA* gene expression]

Summary and conclusion

In the present study we cloned and characterized the *ure*A gene from *Helicobacter pylori* to develop an antigen for the protective immunity against gastric helicobacters. To overcome the limits of antibiotic-based therapies, the vaccine approach has been undertaken since the last decade, leading us to identify some relevant bacterial antigens as candidates for vaccines. The *ure*A gene from *Helicobacter pylori* was amplified using specific primers and cloned in pTZ57R/T and transformed into DH5α cells. The plasmid DNA obtained was then confirmed by restriction digestion and sequence analysis. The sequence was found to be 99% similar to that obtained in GenBank. The sequence of *ure*A gene amplified by the specific primer is closely matching (99%) with a *Helicobacter pylori* strain.

The gene restricted from the cloning vector was ligated to expression vector. Transformation was confirmed with plasmid extraction and followed by restriction digestion. IPTG was used as an inducer for the expression of *ure*A protein and the protein was successfully isolated and quantified. The quantified protein was subjected to SDS PAGE to evaluate the expression of that protein. After staining with commassie staining a specific band was observed at an approximate molecular weight of 70 KDa. Further study is required to go for a conclusion that the expressed protein will act as an antigen for the humoral immunity against the *Helicobacter pylori.* Based on the rapid emergence of antibiotic resistance among *Helicobacter pylori* strains isolated from patients all over the world, alternative strategies for treatment and prevention, e.g. both therapeutic and prophylactic *Helicobacter pylori* vaccines, are urgently needed.

The experience from recent studies in our and other laboratories suggest that such vaccines should be given orally, ideally together with an effective mucosal adjuvant and provide protection against key pathogenic mechanisms of the bacteria. The discovery of a protective antigen of *Helicobacter pylori* offers further hope that an effective vaccine can be produced for human usage.

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