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# *IN-SILICO* DESIGN OF ANTIGENIC PEPTIDES AND MOLECULAR CLONING OF CYTOTOXIN ASSOCIATED GENE (cagA) OF *H. PYLORI* IN AN EXPRESSION VECTOR

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**ABSTRACT:** *Helicobacter pylori* (*H. pylori*) is a spiral, gram-negative pathogen which causes gastric cancer along with MALT lymphoma. It is widespread among the gut microflora and stands first among the pathogens. Cytotoxin-associated gene (cagA) is thought to be the vital protein in the pathogenesis of gastric cancer. Since there are no available vaccines at the moment, and owing to the increasing antibiotic resistance, studies towards developing a novel vaccine are very much essential. The present study was designed to clone and express the protein cagA within the bacterial systems. The protein fragment was screened for antigenic properties using *in-silico* studies. The protein of interest was confirmed using the western blots. This study further can be planned to produce a novel peptide vaccine.

**INTRODUCTION:** *Helicobacter pylori* (*H. pylori*) are considered to be the most successful of all human pathogens owing to its vast invasiveness and manifestations within the host. Since, its discovery has drawn huge attention in terms of human gastric inhabitant and was given vast importance due to its high prevalence globally (about 50%) [1, 2]. In 1994, *H. pylori* were found to be typed (I) carcinogen, and it stands high among other pathogens in terms of carcinogenicity [3]. This strain was thought to play a vital role in pathogenesis gastric cancer and intestinal ulcers along with the mucosa-associated lymphoid tissue (MALT) lymphoma [4].

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*H. pylori* colonize, manifests and subsequently induces disorders within the gut efficiently [5, 6, 7]. Usually, the stomach is said to be the sterile organ since a long time due to its strong acidity (pH<2), which kills all types of organisms like fungi, bacteria, viruses and other parasites. Among such microbes, *H. pylori* were considered to be site-specific and symbolic pathogen [8, 9].

Designing a vaccine against *H. pylori* was initiated a couple of years back owing to its invasiveness and pathogenicity [10]. Since then there is no vaccine developed which is as efficient in terms of delivery and clinical trials. It is always a challenging issue for designing a vaccine against *H. pylori* in the developing countries, where the prevalence of infection among the public is very high [11]. Although it is clear cut, of who should receive and which vaccine the humans should be given, a better approach is lacking in terms of delivery. Cytotoxin-associated gene A (CagA) is said to the best-characterized toxins of *H. pylori* and the cagA

protein product is thought to induce gastritis and eventually to gastric cancer [12]. The pathogenicity island (cag) makes *H. pylori* more virulent and pathogenic. Within cag a type IV secretion system (T4SS) made up of 20 cag proteins and its substrate, CagA is present [13]. Even though there are many proteins like vacuolating toxin A (VacA) which are thought to cause cancer, but CagA protein appears to play a critical role in cancer development.

Recent studies of expressing CagA in mice was found to lead multiple malignancies like gastric polyps and adenocarcinomas [14]. The study also reported the CagA phosphorylation in the development of *H. pylori* lead cancers [15]. Till date, CagA is the only protein substrate which was identified to be delivered and attaching epithelial cell layer of the stomach [16, 17]. CagA translocation is thought to be mediated by binding of CagL, a surface protein present on the pilus structure and also to the α5β1-integrin which is located on the basolateral surface of the gastric cells [18]. Currently, *Helicobacter pylori* infection is being treated a proton pump inhibitor along with antibiotic therapy [19]. Eradication of *H. pylori* is due to regression of gastric and MALT lymphoma, and it might aid in the prevention of gastric cancer. But these treatments slowly dropped below 80% due to the enhancement of antimicrobial resistance [20]. Since then, several modifications or combinations were used for investigating novel drugs. Hence, vaccination is the only valid approach to overcome such issues associated with increasing antimicrobial resistance [21]. Hence, designing an effective vaccine against the strains would aid in preventing gastric cancer. The present study was designed for sequencing the eluted gene of interest from the transformed clones and for cloning into an expression vector for expression of cagA proteins. The expression of proteins was confirmed by the Western blot studies.

## MATERIALS AND METHODS:

**Sample Collection:** 10 samples were collected from the Department of Gastroenterology, M. S. Ramaiah Memorial Hospital. The samples were collected from those treated for *H. pylori* infection and those with gastrointestinal bleeding in the last seven to twenty days and processed to the lab for isolation of the *H. pylori* culture. In brief, our unpublished work is about isolating the *Helicobacter pylori* species, and purification of genomic DNA. The genomic DNA obtained was used for cloning and transformation studies. pMD19-T cloning vector (Fermentas, USA) was used for the study. *E. coli* strain DH 5-α was used in the transformation studies. The DNA of interest and the clones were confirmed by double digestion with restriction enzymes.

**Sequencing:** The gene of an insert in the purified plasmid was then sequenced using ABI PRISM Big Dye Terminators v1.1 cycle sequencing kit (Applied Biosystems Foster City, CA, USA) according to the manufacturer's instruction employing T7 or 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 Expression Studies:** The purified gene of the insert was then used for expression studies.

**Ligation of the Purified Gene Product into T-vector:** The eluted PCR products were ligated separately into the pMD19-T cloning vector (Fermentas, USA). The amount of the PCR product to be used for the cloning as like in 1:1 vector to insert ratio was maintained. 30 µl of the ligation reaction was set up in 3:1 molar ratio of the insert and vector DNA. 0.52 pmol of DNA and 50 ng/µL of vector DNA were sued in the ligation mixture. The contents are mixed thoroughly and incubated at RT for about one hour.

**Transformation:** The ligated plasmids were then transformed into the bacterium DH5α and sued for screening the recombinant clones. The competent cells were prepared according to the Sambrook and Russel 1989 lab protocol. The ligated product was mixed with 200 µl of prepared, competent cells and incubated on ice for 30 min. Heat shock treatment was given to the ligation and competent cell mixture at 42 °C for about 2 min. The contents in the tube were transferred immediately onto the ice and incubated for 2-3 min. 1 ml of LB broth was added to the ligated mixture and used for plating. The ligated and transformed cells were plated onto LB agar plates containing Ampicillin (50 mg/mL), 200 µl of X- gal and 20 µl of IPTG.

White colonies containing recombinant clones were selected and streaked on a fresh LB plate containing ampicillin and incubated overnight. This serves as a master plate for each transformant. The transformed bacteria isolates were inoculated in 50 ml of LB broth containing IPTG (0.5 mM) as an 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 at 150 rpm for 24 h in an orbital incubator shaker.

**Protein Extraction:** The recombinant clones were expected to express the protein of interest along with other proteins. The protein of interest is isolated and used in subsequent research, for diagnostic and pharmaceutical production. The purity needed for the experiments should be 90-95% pure, and if used in pharmaceutical applications must be about 99.99%. Briefly to 2 ml of the culture 500 µl of lysis buffer (25mM Tris-Cl, 2mM EDTA, pH 7.6) was added along with Protease inhibitor benzamidine. The contents were mixed thoroughly, and lysozyme (100 mg/mL) was added and incubated at RT for about 20 min. The samples were sonicated and centrifuged at 18,000 rpm in a Sorvall SS-34 rotor for about 20 min at 4 ºC. The supernatant fraction or the protein of interest was collected in a fresh Eppendorf.

**Protein Estimation:** The protein concentration was estimated at 280nm using UV-VIS spectrophotometer (Shimadzu, Germany). From the stock, 1µl Protein was mixed with 99 µl sterile distilled water to get 100 times dilution. The diluted protein concentration was directly estimated by lowrys method. Bovine serum albumin was sued as standard. The phenolic group of tyrosine and tryptophan residues in a protein will produce a blue, purple color complex, with maximum absorption in the region of 660 nm wavelength, with Folin- Ciocalteau reagent. Thus the intensity of color depends on the total amount of the aromatic amino acids present. Different dilutions of BSA solutions are prepared with stock BSA solution (1 mg/mL). The contents following incubation are recorded for absorbance values at 660 nm.

**Protein Electrophoresis:** The purified protein sample was used for separation on a poly acrylamide electrophoresis gel. Proteins were separated with sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (1970) with modifications. 30% acrylamide: Bis solution (29:1), tris-HCl (1.5M pH8.8), tris-HCl (0.5 M pH 6.8), 10% ammonium per sulphate were used for SDS PAGE gel. Samples were boiled for 45 ºC in sample buffer (0.6M Tris-HCl, pH 6.8, sucrose 5.0g, mercaptoethanol 0.25 ml, bromophenol blue 0.5%). 0.1% coomassie brilliant blue R250 in 50% methanol and 10% glacial acetic acid was used as a stain. 10% methanol and 7% glacial acetic acid was used to destain. 5% stacking and 12% resolving gels were used for separation of the peptides.

**Prediction of Antigenicity of cagA Protein:** The complete amino acid sequence of cagA (GenBank: AAB58747.1) was retrieved from the protein database (http://www.ncbi.nlm.nih.gov/ protein/). The non-identical sequences were analyzed with VaxiJen v2.0 (http://www.ddg-pharmfac.net/ vaxijen/Vaxi Jen/VaxiJen.html) antigen prediction server. Since cagA is thought to be localized within the membranes, a threshold value of 0.4 was used to check the antigenicity of each full-length protein and all those that have an antigenic score >0.4 were selected as described previously [22]. Each chosen full length amino acid sequence was then used for screening the transmembrane topology analysis using TMHMM v.2.0 (http:// www.cbs. dtu.dk/services/TMHMM/) prediction server [23]. This is used to identify the exo-membrane (surface exposed) amino acid sequences of each protein.

**Prediction of Antigenic B-cell Epitopes:** The full-length sequence of cagA was used for predicting the B-cell epitopes. BCPreds (http://ailab.cs. iastate.edu/bcpreds/predict.html) was sued for the analysis where both BCpred and AAP prediction methods were used [24]. All the predicted B-cell epitopes (16-mer) having a BCPreds cutoff score >0.8 were selected and subsequently used for screening membrane topology by comparing with TMHMM results for exo-membrane amino acid sequences.

Surface-exposed B-cell epitope sequences having the cutoff value >0.8 for BCPreds were then analyzed using VaxiJen at threshold 0.5 to check the antigenicity. The selected B-cell epitope sequences were then analyzed using VaxiJen at

threshold 0.4 to check the antigenicity. And finally, four epitopes with the top VaxiJen scores were used to screen for T-cell epitopes [25].

**Prediction of T-cell Epitopes from Selected B-cell Epitopes:** T-cell epitopes were predicted from the selected B-cell epitopes using the following parameters. Firstly, the T-cell epitope sequence should bind to both the MHC I and MHC II molecules, and the least number of total interacting MHC molecules should be >15. The T-cell epitope sequence must also interact with HLADRB1*0101 of MHC-II and should be antigenic based on VaxiJen score. Propred-1 (http://www. imtech.res. in/raghava/propred1/) and Propred (http://www. imtech.res.in/raghava/propred/) servers were used to identify common epitopes that bind to both MHC I and MHC II molecules [26].

**Western Blotting:** Protein bands of interest are excised from the gel and homogenized in the extraction buffer. The protein purified was stored in the PBS buffer and used for the synthesis of primary antibodies and secondary antibodies. Primary and secondary antibodies were ordered from Thermofisher Inc, Bangalore. The western blotting procedure was followed according to the instructions given in the kit (Credora Biotech. Pvt. Ltd). The excised bands were electroblotted onto a nitrocellulose membrane for 2½ h. The membrane was blocked with blocking reagent incubated with primary and secondary antibodies. Following which 1X antibody HRP was added incubated for 30 min with TMB $H_2O_2$ as substrate.

## RESULTS:

**Sequencing:** The sequence obtained from the sequence report was analyzed on the BLAST program. The BLAST program results were shown above in figure. From the sequence obtained it was clear that the query sequence was 92% matching with the *Helicobacter pylori* strains. The sequences when analyzed for multiple sequence alignment on clustal omega. The phylogenetic tree obtained predicts of the possible sequence similarity and the sequence family. The sample sequence obtained was closely matching with FR666848.1 strain. They both are in close similarity and are matching 92% with each other. They might belong to the same family tree.

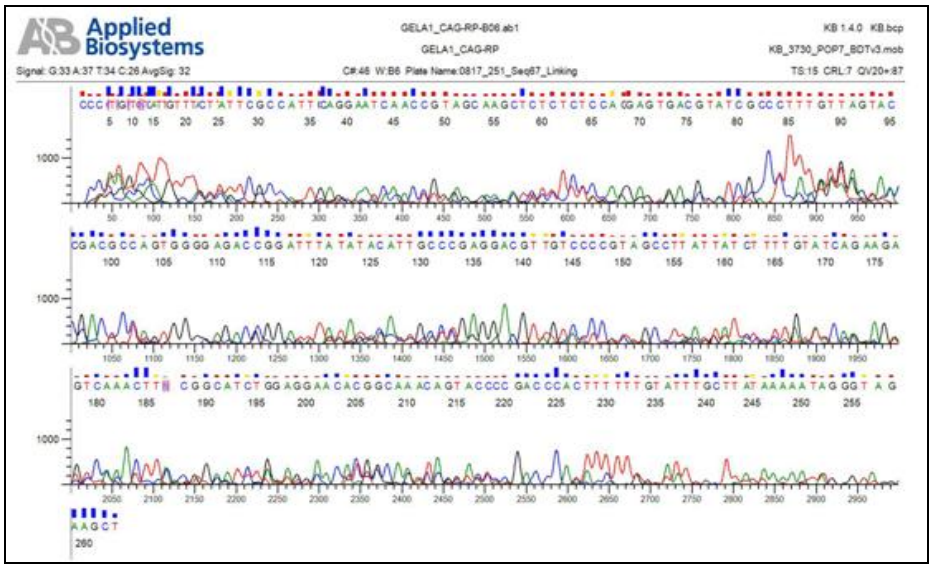



**FIG. 1: CHROMATOGRAM REPORT OF THE SEQUENCED DATA OF THE GENE OF INTEREST**

**Gene Expression Studies:** Eluted gene insert was quantified and ligated with the expression vector pMD19-T. Following incubation, at 27 ºC for 3-4 h, the ligated mixture was stored until further use.

**Protein Extraction:** The sample 6, 7, 8, 9, and 10 all showed good content of protein. The concentration of the protein was calculated using the BSA standard graph (y=0.0011x; $R^2$=0.9903). Sample 10 was found to contain more protein content followed by S6 and then S9. S7 and S8 also showed good amount of protein content, but when compared to the former three, they are found to be less. The three samples S10, S6 and S7 were used for the protein electrophoresis and western blotting.

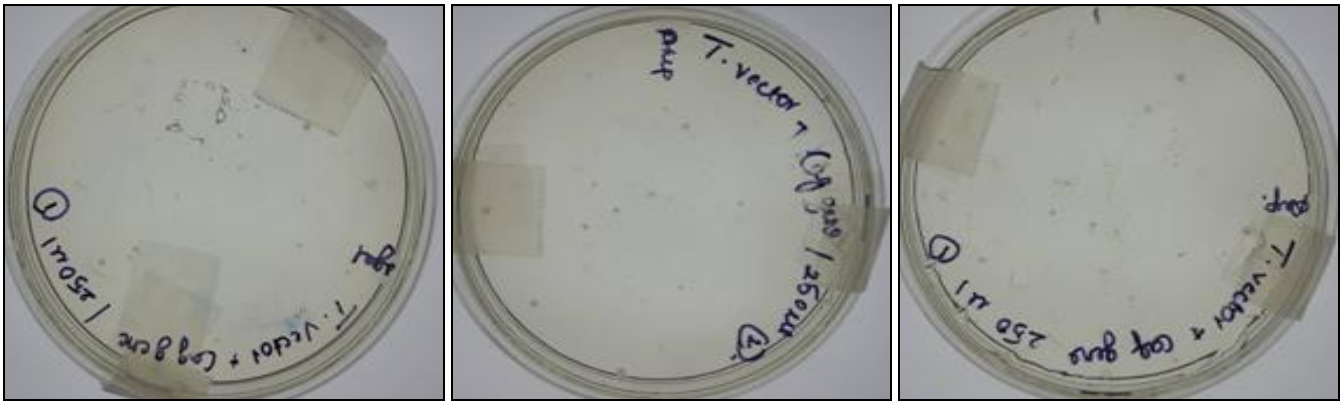


**FIG. 2: LB AGAR PLATES SHOWING THE TRANSFORMATION CULTURES.** Plate 1, Plate 2, Plate 3: Transformed cultures containing the recombinant DNA.

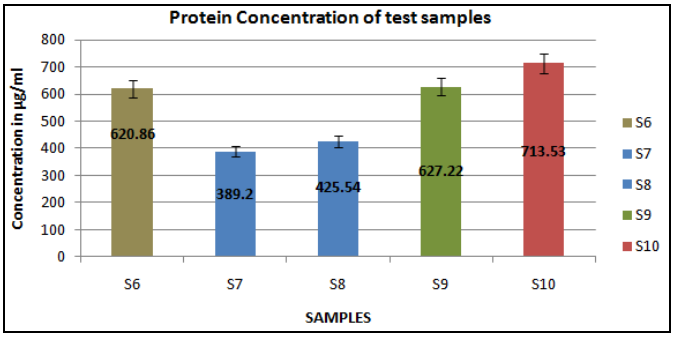



**FIG. 3: GRAPH SHOWING THE CONCENTRATION OF PROTEIN ESTIMATED USING THE STANDARD CURVE OF BSA.** All the values were average of triplicates. The Values are expressed as µg/mL ± S.D

**Protein Electrophoresis:** The crude protein samples were further purified on SDS-PAGE gel using 12% resolving gel. The protein of interest was found to be approximately 14Kda.

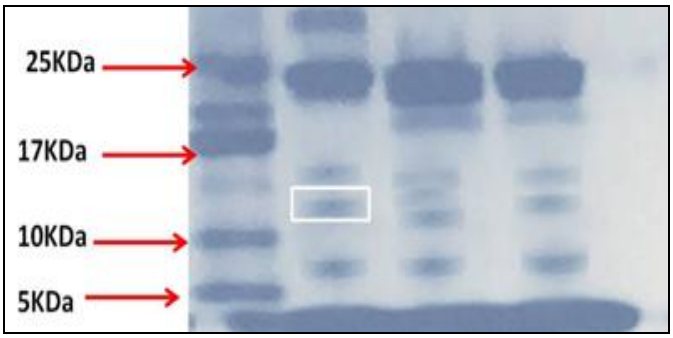



**FIG. 4: SDS PAGE GEL SHOWING THE PROTEIN BAND OF INTEREST. MOLECULAR MARKER WAS RUN ON THE GEL.** Lane 1: Protein sample 10; Lane 2: protein sample 9; Lane 3: protein sample 6. A protein band of interest shown in white rectangle was found to be approximately 12KDa.

The protein samples run on the SDS PAGE gel showed distinct bands. The bands after staining with CBB was then destained with distilled water. The three samples showed uniform banding pattern confirming the strains and the protein samples. A faint but distinct band was seen at about 11-13 kDa. The protein of interest was calculated to be approximately 12786.01Da (˜12KDa). This confirms the presence of the desired protein, **Fig. 4**. Further confirmation is needed by western blotting.

The Vaxigen antigen prediction software revealed a score of 0.4327, which predicts the protein of interest to be a good antigen. The TMHMM results predict that the protein to be localized but secretory. Basing on the topology prediction, it was found to be outside.

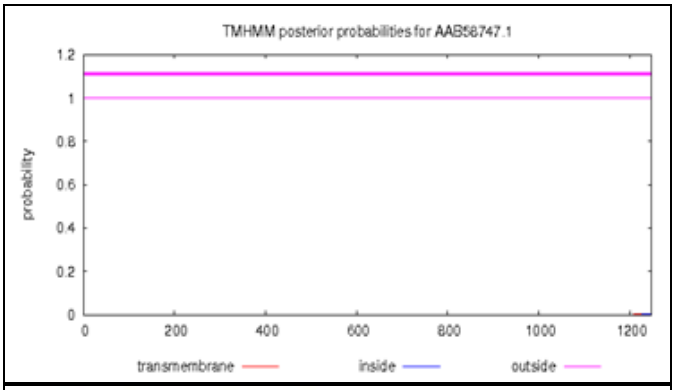
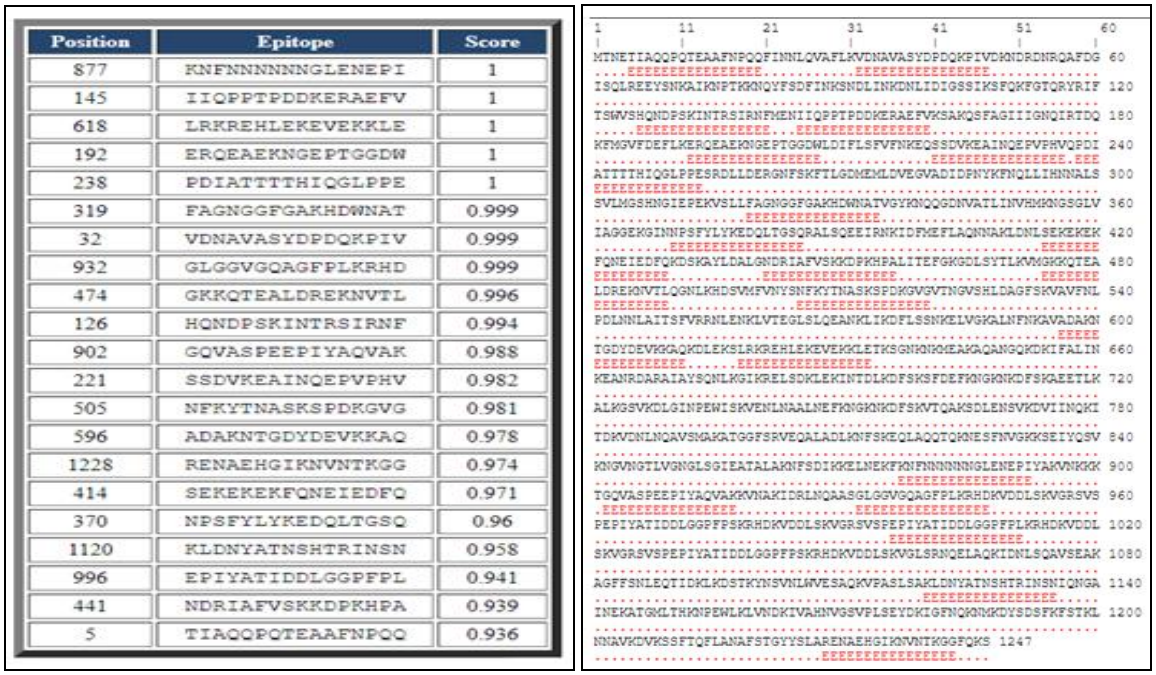



**FIG. 5: IMAGE SHOWING THE PREDICTED TOPOLOGY VALUES FROM THE TMHMM SOFTWARE**

All the sequences with a cut off score more than 0.9 were selected. The first four peptide sequences with cut off score 1 were found to be best epitopes for the study. KNFNNNNNNGLENEPI, IIQPPTPDD KERAEFV, LRKREHLEKEVEKKLE, ERQEAE KNGEPTGGDW, and PDIATTTTHIQGLPPE were selected.



| Position | Epitope | Score |
|---|---|---|
| 877 | KNFNNNNNNNGLENEPI | 1 |
| 145 | IIQPPTPDDKERAEFV | 1 |
| 618 | LRKREHLEKEVEKKLE | 1 |
| 192 | ERQEAEKNGEPTGGDW | 1 |
| 238 | PDIATTTTHIQGLPPE | 1 |
| 319 | FAGNGGFGAKHDWNAT | 0.999 |
| 32 | VDNAVASYDPDQKPIV | 0.999 |
| 932 | GLGGVGQAGFPLKRHD | 0.999 |
| 474 | GKKQTEALDREKNVTL | 0.996 |
| 126 | HQNDPSKINTRSIRNF | 0.994 |
| 902 | GQVASPEEPIYAQVAK | 0.988 |
| 221 | SSDVKEAINQEPVPHV | 0.982 |
| 505 | NFKYTNASKSPDKGVG | 0.981 |
| 596 | ADAKNTGDYDEVKKAQ | 0.978 |
| 1228 | RENAEHGIKNVNTKGG | 0.974 |
| 414 | SEKEKEKFQNEIEDFQ | 0.971 |
| 370 | NPSFYLYKEDQLTGSQ | 0.96 |
| 1120 | KLDNYATNSHTRINSN | 0.958 |
| 996 | EPIYATIDDLGGPFPL | 0.941 |
| 441 | NDRIAFVSKKDPKHPA | 0.939 |
| 5 | TIAQQPQTEAAFNPQQ | 0.936 |

**FIG. 6: IMAGE SHOWING THE PREDICTED SCORES FOR EPITOPE ANALYSIS USING BCPreds**

**TABLE 1: TABLE SHOWING THE PROBABLE ANTIGEN SCORE FROM THE BCPreds**

| Epitope Sequence | Probable antigen score |
|---|---|
| KNFNNNNNNGLENEPI | 0.8682 |
| IIQPPTPDDKERAEFV | 0.2833 |
| LRKREHLEKEVEKKLE | 0.9916 |
| ERQEAEKNGEPTGGDW | 1.4724 |
| PDIATTTTHIQGLPPE | 0.8827 |

All the epitope sequences were found to be more than 0.4 except second sequence (0.2833). Neglecting the second sequence, the remaining four sequences were used for the prediction of T-cell epitopes **Table 1**. All the predicted binders with real scores were represented in the table. The predicted best models were shown in the highlighted region. The predicted binder was marked red. Only five sequences with a real score more than 1 were selected **Table 2**. All these five sequences were used for prediction of antigen using Vaxigen. Basing on the Vaxigen antigenic score the peptide HLEKEVEKK (Vscore 1.4) and NGEPTGGDW (Vscore 1.0128) were selected as the best antigens. And NGEPTGGDW was selected since it is having real high score and antigenic score **Table 3**.

**TABLE 2: TABLE SHOWING THE PREDICTED REAL SCORES AND POSITION OF THE PREDICTED BINDER**

| Rank | Sequence | At Position | Real Score | *Log* Score |
|---|---|---|---|---|
| ALLELE: HLA-A2 | TTTTHIQGL | 5 | 0.0125 | -3.6889 |
| ALLELE: HLA-A*0201 | TTTTHIQGL | 5 | 2.16391456 | 0.7719 |
| ALLELE: HLA-A*0205 | TTTTHIQGL | 5 | 1.4 | 0.3365 |
| ALLELE: HLA-A2 | NGEPTGGDW | 8 | 2.25 | 0.8109 |
| ALLELE: HLA-A*0201 | EKNGEPTGG | 6 | 0.000938838 | -4.4073 |
| ALLELE: HLA-A*0205 | EAEKNGEPT | 4 | 0.000181494 | -8.3207 |
| ALLELE: HLA-A2 | HLEKE**V**EKK | 6 | 1.8 | 0.5878 |
| ALLELE: HLA-A*0201 | KREHLEKEV | 3 | 0.047482321 | -2.0021 |
| ALLELE: HLA-A*0205 | LEKEVEKKL | 7 | 0.0024 | -0.6935 |
| ALLELE: HLA-A2 | NNNNNGLEN | 4 | 0.0025 | -4.382 |
| ALLELE: HLA-A*0201 | FNNNNNNGL | 2 | 0.002656591 | -1.6456 |
| ALLELE: HLA-A*0205 | F**N**NNNNNGL | 2 | 1.05 | 0.0488 |

**TABLE 3: TABLE SHOWING THE PREDICTED ANTIGENIC EPITOPIC SEQUENCES ALONG WITH REAL SCORE FROM BCPreds AND ANTIGEN SCORE FROM THE Vaxigen v.2.0**

| Rank | Sequence | Real Score | Antigen score |
|---|---|---|---|
| ALLELE: HLA-A*0201 | TTTTHIQGL | 2.16391456 | 0.9041 |
| ALLELE: HLA-A*0205 | TTTTHIQGL | 1.4 | 0.9041 |
| ALLELE: HLA-A2 | NGEPTGGDW | 2.25 | 1.0128 |
| ALLELE: HLA-A2 | HLEKEVEKK | 1.8 | 1.4 |
| ALLELE: HLA-A*0205 | FNNNNNNGL | 1.05 | 0.8434 |

The amino acid sequence of the protein of interest was given below.

>AAB58747.1 CagA [Helicobacter pylori]
MTNETIAQQPQTEAAFNPQQFINNLQVAFLKVDNAVASYDPDQKPIVDKNDRDNRQAFDGIS
QLREEYSNKAIKNPTKKNQYFSDFINKSNDLINKDNLIDIGSSIKSFQKFGTQRYRIFTSWV
SHQNDPSKINTRSIRNFMENIIQPPTPDDKERAEFVKSAKQSFAGIIIGNQIRTDQKFMGVF
DEFLKERQEAEKNGEPTGGDWLDIFLSFVFNKEQSSDVKEAINQEPVPHVQPDIATTTTHIQ
GLPPESRDLLDERGNFSKFTLGDMEMLDVEGVADIDPNYKFNQLLIHNNALSSVLMGSHNGI
EPEKVSLLFAGNGGFGAKHDWNATVGYKNQQGDNVATLINVHMKNGSGLVIAGGEKGINNPS
FYLYKEDQLTGSQRALSQEEIRNKIDFMEFLAQNNAKLDNLSEKEKEKFQNEIEDFQKDSKA
YLDALGNDRIAFVSKKDPKHPALITEFGKGDLSYTLKVMGKKQTEALDREKNVTLQGNLKHD
SVMFVNYSNFKYTNASKSPDKGVGVTNGVSHLDAGFSKVAVFNLPDLNNLAITSFVRRNLEN
KLVTEGLSLQEANKLIKDFLSSNKELVGKALNFNKAVADAKNTGDYDEVKKAQKDLEKSLRK
REHLEKEVEKKLETKSGNKNKMEAKAQANGQKDKIFALINKEANRDARAIAYSQNLKGIKRE
LSDKLEKINTDLKDFSKSFDEFKNGKNKDFSKAEETLKALKGSVKDLGINPEWISKVENLNA
ALNEFKNGKNKDFSKVTQAKSDLENSVKDVIINQKITDKVDNLNQAVSMAKATGGFSRVEQA
LADLKNFSKEQLAQQTQKNESFNVGKKSEIYQSVKNGVNGTLVGNGLSGIEATALAKNFSDI
KKELNEKFKNFNNNNNNGLENEPIYAKVNKKKTGQVASPEEPIYAQVAKKVNAKIDRLNQAA
SGLGGVGQAGFPLKRHDKVDDLSKVGRSVSPEPIYATIDDLGGPFPSKRHDKVDDLSKVGRS
VSPEPIYATIDDLGGPFPLKRHDKVDDLSKVGRSVSPEPIYATIDDLGGPFPSKRHDKVDDL
SKVGLSRNQELAQKIDNLSQAVSEAKAGFFSNLEQTIDKLKDSTKYNSVNLWVESAQKVPAS
LSAKLDNYATNSHTRINSNIQNGAINEKATGMLTHKNPEWLKLVNDKIVAHNVGSVPLSEYD
KIGFNQKNMKDYSDSFKFSTKLNNAVKDVKSSFTQFLANAFSTGYYSLARENAEHGIKNVNT
KGGFQKS

**Western Blotting:** The sequence highlighted in yellow was used for the synthesizing of the antibodies. Monoclonal antibodies were procured for the highlighted sequence. The procured antibodies were used for the western blotting analysis. Protein bands of interest obtained on the gel were excised from the gel and homogenized in the extraction buffer. The protein purified was stored in the PBS buffer and stored for further experiments. The bands were found after hybridization and were found to be approximately about 12KDa. The bands confirm the expression of the protein of interest within the clones **Fig. 7**. Such a successful expression might lead to the development of a novel vaccine.

**FIG. 7: IMAGE OF WESTERN BLOT SHOWING THE BANDS OF DESIRED PROTEIN AFTER HYBRIDIZATION WITH THE ANTIBODIES**

**DISCUSSION:** *Helicobacter pylori* strains were isolated, and the DNA content was purified from the culture. The DNA was used for cloning (unpublished) and expression studies. The sequence obtained from the sequence report was analyzed on the BLAST program. From the sequence obtained, it was clear that the query sequence was 92% matching with the Helicobacter pylori strains. The ligated plasmids were then transformed into the bacterium DH5α and plated on LB agar- Ampicillin plates. The blue-white colonies were observed, and the white colonies were picked up and grown overnight in LB broth. The total protein content was purified and separated on the SDS PAGE gel. The three samples showed uniform banding pattern confirming the strains and the protein samples.

A faint but distinct band was seen at about 11-13 kDa. The protein of interest was calculated to be approximately 12786.01Da (˷12KDa). This confirms the presence of the desired protein. Further confirmation on western blot also confirmed of the protein of interest.

All the present studies done confirm of the possible hypothesis that cagA can be used as a peptide vaccine. Peptide vaccines have been proved very effective in terms of potentiality and decreased immune rejection. Such a development would surely aid in the development of new vaccines. Hence the study in isolating and purifying a pathogenic peptide from the protein cagA was successful. Further experiments need to be designed to isolate and test for the efficacy levels on the patients.

**CONCLUSION:** Owing to its invasiveness and pathogenicity, numerous treatment and control methods were employed to target the Helicobacter species. Hence, the last available option is to develop a vaccine against the pathogen. But there are currently some issues in developing the vaccine like each host responds differently to the pathogen and moreover, there is a high genetic diversity seen in *H. pylori*. And only with a vaccine, physicians can claim complete protection and decreases the risk of infection. Hence, it would be more appropriate to recommend vaccines in developed countries. Hence we should search for a better vaccine and adjuvants for eradicating the pathogen.

Hence, our study on the cagA protein cloning and expression was successful and might aid in the development of potential candidate for the vaccine. The gene of interest was isolated and successfully cloned into the bacterial strain and then cloned to express the protein of interest. The protein isolated

was sequenced and designed antibodies for a small peptide. Such antibodies were used for the western blot confirmation. Further, studies need to be planned to design proteins of several other peptides and use them for vaccination. Further these peptide fragments need to be tested among the animal models. Once, if found the protein of interest was expressed, then it can be used for the clinical study.

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**CONFLICT OF INTEREST:** Authors declare no conflict of interest.

## REFERENCES:

1. Marshall BJ and Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 1: 1311-15.
2. Mégraud F: A humble bacterium sweeps this year's Nobel Prize. Cell 2005; 123: 975-976.
3. Malla S, Mehran MJ and Zendehbad SH: Cloning and expression of a partial urea antigen for the production of vaccine against helicobacter pylori, the risk factor for gastric cancer. Asian Journal of Pharmaceutical and Clinical Research 2014; 7(1): 111-17.
4. Hagymási K and Tulassay Z: *Helicobacter pylori* infection: new pathogenetic and clinical aspects. World J Gastroenterol 2014; 20: 6386-99.
5. Basso D, Plebani M and Kusters JG: Pathogenesis of *H. pylori* infection. Helicobacter 2010; 15(S-1): 14-20.
6. Delahay RM and Rugge M: Pathogenesis of *Helicobacter pylori* infection. Helicobacter 2012; 17(S-1): 9-15.
7. Shiota S and Yamaoka Y: Strategy for the treatment of *H. pylori* infection. Curr Pharm Des 2014; 20: 4489-00.
8. Yamaoka Y: Pathogenesis of *Helicobacter pylori*-Related gastroduodenal diseases from molecular epidemiological studies. Gastroenterol Res Pract 2012; 371503.
9. Kusters JG, van Vliet AH and, Kuipers EJ: Pathogenesis of *Helicobacter pylori* infection. Clin Microbiol Rev 2006; 19: 449-90.
10. Guo L, Liu K, Zhao W, Li X, Li T, Tang F, Zhang R, Wu W and Xi T: Immunological features and efficacy of the reconstructed epitope vaccine CtUBE against Helicobacter pylori infection in BALB/c mice model. Appl Microbiol Biotechnol 2013; 97: 2367-78.
11. Chen J, Lin L, Li N and She F: Enhancement of *Helicobacter pylori* outer inflammatory protein DNA vaccine efficacy by co-delivery of interleukin-2 and B subunit heat-labile toxin gene encoded plasmids. Microbiol Immunol 2012; 56: 85-92.
12. Crabtree JE, Taylor JD, Wyatt JI, Heatley RV, Shallcross TM and Tompkins DS: Mucosal IgA recognition of *Helicobacter pylori* 120 kDa protein, peptic ulceration, and gastric pathology. Lancet 1991; 338: 332-10. 1016/0140-6736(91)90477-7.
13. Covacci A, Falkow S, Berg DE and Rappuoli R: Did the inheritance of a pathogenicity island modify the virulence of *Helicobacter pylori*? Trends Microbiol 1997; 205-10. 1016/S0966-842X(97)01035-4
14. Cover TL, Glupczynski Y, Lage AP, Burette A, Tummuru MK and Perez-Perez GI: Serologic detection of infection with cagA+ *Helicobacter pylori* strains. J Clin Microbiol 1995; 33: 1496-00.
15. Ohnishi N, Yuasa H, Tanaka S, Sawa H, Miura M and Matsui A: Transgenic expression of *Helicobacter pylori* CagA induces gastrointestinal and hematopoietic neoplasms in mouse. Proc Natl Acad Sci USA 2008; 105: 1003-8.10.1073/pnas.0711183105.
16. Stein M, Rappuoli R and Covacci A: Tyrosine phosphorylation of the *Helicobacter pylori* CagA antigen after cag-driven host cell translocation. Proc Natl Acad Sci USA 2000; 97: 1263-8. 10.1073/pnas.97.3.1263.
17. Segal ED, Cha J, Lo J, Falkow S and Tompkins LS: Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. Proc Natl Acad Sci, USA 1996: 14559-64. 10. 1073/pnas.96.25.14559.
18. Ong SP and Duggan A: Eradication of Helicobacter pylori in clinical situations. Clin Exp Med 2004: 30-8. doi: 10. 1007/s10238-004-0035-2.
19. Talley NJ, Fock KM and Moayyedi P: Gastric cancer consensus conference recommends *Helicobacter pylori* screening and treatment in asymptomatic persons from high-risk populations to prevent gastric cancer. Am J Gastroenterol 2008; 103: 510-4. doi: 10.1111/j.1572-0241. 2008.01819.x
20. Kosunen TU, Pukkala E, Sarna S, Seppala K, Aromaa A and Knekt P: Gastric cancers in Finnish patients after cure of Helicobacter pylori infection: a cohort study. Int J Cancer 2011; 128: 433-9. doi: 10.1002/ijc.25337.
21. Irayyif SM, Kumar RS and Malla S: Identification of methicillin-resistant *Staphylococcus aureus* by a rapid Polymerase chain reaction technique. Asian J Pharm Clin Res 2014; 7(5): 16-19.
22. Yamaoka Y: Mechanisms of disease: Helicobacter pylori virulence factors. Nat Rev Gastroenterol Hepatol 2010; 7: 629-41. 10.1038/nrgastro.2010.154.
23. Singh H and Raghava GPS: ProPred: prediction of HLA-DR binding sites. Bioinformatics 2001; 17(12): 1236-37. https://doi.org/10.1093/bioinformatics/17.12.1236.
24. Doytchinova IA and Flower DR: Bioinformatic approach for identifying parasite and fungal candidate subunit vaccines. Open Vaccines Journal 2008; 1: 22-26.
25. Chen J, Liu H, Yang J and Chou K: Prediction of linear B-cell epitopes using amino acid pair antigenicity scale. Amino Acids 2007; 3: 423-428.
26. EL-Manzalawy Y, Dobbs D and Honavar V: Predicting linear B-cell epitopes using string kernels. J Mol Recognit 2008; 21: 243-55.

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