Antigenic properties of *Finegoldia magna* protein L and Type IV Pilin (PilA) for in-silico multi epitope peptide vaccine designing

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Abstract

**Background & Objectives:** *Finegoldia magna* is a potential opportunistic pathogen for humans. *F. magna* as the most frequent pathogenic species of Gram positive anaerobic cocci accounts for up to 5-12% of all anaerobic infections. *F. magna* possess Protein L super antigen and Type IV Pilin (PilA) that due to trigger intense immune responses are invaluable for designing multi epitope peptide vaccines in current study.

**Materials & Methods:** In this study, immunoinformatics tools were used to predict B and T cell epitopes of Protein L and Type IV Pilin (PilA). The epitopes were evaluated for antigenicity, allergenicity and binding energy to appropriate DRB3*01:01, DRB1*03:01 and DRB1*15:01 HLA alleles and then were fused together by GPGPG and EAAAK spacers. *Vibrio cholera* Toxin B Subunit was introduced at N-terminus of the constructed vaccine as adjuvant, and with an eye on further identification and purification, a 6×HisTag was introduced at C-terminus. Codon optimization performed for further expression in *Escherichia coli* host. The amino acid sequence of the multi epitope peptide vaccine used for 3D structure prediction and refinement. Then structural evaluation via ramachandran plot analysis performed. Physicochemical properties and solubility of the constructed vaccine was also studied.

**Results:** Results showed the selected epitopes with high antigenicity and no allergenicity. These epitopes manifest high affinity toward recommended HLA alleles. The predicted 3D model of constructed vaccine showed high stability, solubility and half-life for expression in *E. coli* host.

**Conclusion:** In this study, Protein L and Type IV Pilin (PilA) used for in-silico designing an effective vaccine against *F. magna*.

**Keywords:** *Finegoldia magna*, protein L PilA, multi epitope peptide vaccine, HLA.

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ویژگی‌های آنتی‌ژنی پروتئین L و پیلین تایپ ۴ (PilA) باکتری فاینگولدیا ماگنا به منظور طراحی درون رایانه‌ای واکسن پیش‌بندی چند اپی توپی (Finegoldia magna)

حسین جاوید

چکیده

سالنده و هدف: فاینگولدیا ماگنا یک پاتوژن فرصت طلبی است و شایع‌ترین گونه بیماری کوکسی‌های بی‌هوایی مثبت می‌باشد. این باکتری عامل ایجاد ۵ تا ۲۱ درصد تمام عفونت‌های بی‌هوایی است. در این پژوهش سوئیر آنتی-ژن پروتئین L و پیلین نوع ۴ (PilA) آن به دلیل ایجاد پاسخ‌های ایمنی قوی و اهمیت در طراحی واکسن‌های پیش‌بندی چند اپی توپی مورد استفاده قرار گرفتند.

مواد و روش‌ها: ابزارهای ایمونولوژی محاسباتی برای پیش‌بینی اپی توپ‌ها به کار برده شد. اپی توپ‌ها از نظر ویژگی آنتی‌ژنی و آلرژی‌زا بوده و اپی توپ‌های دارای قدرت اتصال مناسب به آلت‌های HLA DRB1*01:01، DRB1*03:01 و DRB1*15:01 بررسی شدند. سپس این اپی توپ‌ها در خصوص ویژگی‌های خاص صورت‌گیری کردند. همچنین به بهبود کدون استاندارد واکسن نقش بسزایی داشتند. نتایج نشان داد که حسین جاوید در سال سیزدهم شماره اول (پیاپی ۲۴) بهار ۹۹ صفحات ۱۹-۹۸

یافته‌ها: اپی توپ‌های انتخابی دارای ویژگی آنتی‌ژنیتی و بدون آلرژی‌زا بوده و قدرت اتصال مناسب به آلت‌های HLA DRB1*01:01، DRB1*03:01 و DRB1*15:01 داشتند. ساختار سه‌بعدی واکسن با استفاده از سی‌اس‌آکوئی، اصلاحات ساختاری، و سپس تحلیل نمودار راماجدی را انجام داد. نتایج نشان داد که حسین جاوید در سال سیزدهم شماره اول (پیاپی ۲۴) بهار ۹۹ صفحات ۱۹-۹۸

نتیجه‌گیری: در این مطالعه، پروتئین L باکتری فاینگولدیا ماگنا به منظور طراحی درون رایانه‌ای واکسن‌های استفاده قرار گرفت.

واژگان کلیدی: Finegoldia magna، پروتئین L، واکسن پیش‌بندی چند اپی توپی، HLA دریافت مقاله: اسفند ۹۸ پذیرش برای چاپ: فروردین ۹۹
Introduction
Gram positive coccus, *Finegoldia magna*, formerly *Peptostreptococcus magnus*, is a member of strict anaerobic commensal microbiome in skin, oral cavity, gastrointestinal and genourinary tracts and is considered as a potential opportunistic pathogen for humans and animals. *F. magna* is the most frequent and pathogenic species of Gram Positive Anaerobic Cocci (20-40%) and accounts for up to 5-12% of all anaerobic infections (1). Vast clinical infections and various symptoms are thought to be somehow connected to *F. magna* (2), including wound infections, cardiac and pulmonary infections, pericarditis, mediastinitis, necrosing pneumonia, empyema, soft tissue and musculoskeletal e.g., necrotizing fasciitis, septic arthritis, native and prosthetic joint infections (3) and polymicrobial vaginosis (2), breast abscesses (4) and diabetic feet (5). Fetal monomicrobial bacteremia and Toxic Shock Syndrome (TSS) cases have been reported ethologically connected to *F. magna* (6). *F. magna* has been found in seminal fluid of idiopathic infertile men analyzed by the Next Generation Sequencing (NGS) techniques (7). *F. magna* possesses five virulence factors (Protein L, Type IV Pilin (PilA), PAB, Suf A and FAF) as major parts of its pathogenic arsenal. Among these factors, Protein L (PL) and Type IV Pilin (PilA) (T4P) are studied vastly. PL is approximately 108 kDa and contains 992 amino acid residues with the ability to bind different classes of immunoglobulins through the light chains (8) with the outcome of protecting *F. magna* from being killed by immune system. T4P which helps adherence of *F. magna* to the host tissues via kerinoocytes and extracellular matrix enables *F. magna* colonization, biofilm formation and interaction with the immune system (9). T4P is approximately 14.8 kDa and contains 134 amino acid residues. Immunization against infections is considered as most triumphant medical treatment ever achieved in which, peptide based vaccines are prospective of immunization to trigger intense long lasting protection via recruiting minimal microbial elements and compounds (10). In the current study by using different immunoinformatics platforms, we predict T cell and B cell epitopes of PL super antigen and T4P of *F. magna* ATCC 29328 as model organism. In addition, in-silico, by fusing the predicted epitope peptides via spacers and adjuvant, we designed an ultimate multi epitope peptide vaccine (MEPV) with possibility to prevent infections related to *F. magna*.

Materials and methods
Retrieval of PL and P4T sequences and assessment of antigenic properties
FASTA format files of amino acid sequences of PL (Q51918) and T4P (B0RZY1) of *F. magna* ATCC 29328 and *Vibrio cholera* Toxin B Subunit (CTB; Q7X2D2) were obtained from the Uniprot database at [http://www.uniprot.org](http://www.uniprot.org). VaxiJen server at [http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html](http://www.ddg-pharmfac.net/vaxijen/VaxiJen/VaxiJen.html) (11) was used to predict antigenic-immunogenic attributes of these proteins where the threshold value was set at ≥ 0.4 to increase prediction precision and decreasing unfavorable positively false results.

Prediction and selection of linear B cell epitopes
Prediction of PL and T4P linear B cell epitopes was performed by the immune epitope database and analysis resource (IEDB) server at [http://tools.immuneepitope.org/bcell/](http://tools.immuneepitope.org/bcell/) (12) via
Antigenic properties of *Finegoldia magna* protein L and Type IV Pilin (PilA) for in-silico multi-presses.

**Construction and engineering of MEPV, secondary and tertiary structure prediction**

The repeated sequences of B cell and T cell epitopes were omitted and then the selected epitopes were fused together via a G and P rich spacer (GPGPG). CTB was used as adjuvant and fused to the amino terminus of the construct via EAAAK linkers. In addition, for further convenient purification and identification, a 6×HisTag was introduced at the carboxyl terminus of the final vaccine construct. The secondary structure of the constructed MEPV was predicted based on primary amino acid sequences using PSIPRED web server at http://bioinf.cs.ucl.ac.uk/psipred/ (17). The amino acid sequences of MEPV, then was uploaded on the I-TASSER server at http://zhanglab.ccmb.med.umich.edu/I-TASSER/ for 3D structure prediction (18). For high resolution protein structure refinement, the best produced models (based on C and TM scores and RMSD) were uploaded into Modrefiner server at https://zhanglab.ccmb.med.umich.edu/ModRefiner/ (19). After protein structural refinement, the quality and validation assessment were performed by using Ramachandran plot analysis in RAMPAGE server at http://mordred.bioc.cam.ac.uk/~raptor/rampage.php (20).

**Assessment of physicochemical parameters of the constructed MEPV**

Assessment of various physicochemical parameters and properties of the constructed MEPV was performed using the ProtParam server at https://web.expasy.org/protparam/ (21). The major assessed parameters include theoretical isoelectric point (pI), grand average of hydropathicity (GRAVY), aliphatic index,
instability index, in-vitro and in-vivo stability. Moreover, the solubility of constructed MEPV was predicted via Protein-Sol online web server available at https://protein-sol.manchester.ac.uk/ (22).

**Optimization of MEPV for overexpression in E. coli host**

The OPTIMIZER web server at http://genomes.urv.es/optimizer/ (23) was used for optimization of constructed MEPV, based on amino acid sequence, for further overexpression in E. coli host.

**Results**

**Prediction and selection of linear B cell epitopes**

The full length of PL and T4P amino acid sequences were submitted to the B cell epitope prediction tool with default threshold value 0.35 at IEDB server (BepiPred) to predict varying residual length epitopes (Table 1). Three bottle necks applied for selecting most suitable epitopes among IEDB server outputs. Firstly, regarding to the length, only B cell epitopes ≥10 mer were selected. Secondly, these selected epitopes were checked for antigenicity at VaxiJen server. Thirdly, epitopes were submitted to AlgPred server for allergenicity prediction. The epitopes did not contain any experimentally proven IgE epitopes and no hit found in blast search on Allergen Representative Peptides (ARPs). Eventually, 16 and 3 B cell epitopes were selected from PL and T4P, respectively, and used for construction of MEPV.

**Prediction and selection of T cell epitopes**

On the IEDB server, the T cell Epitope

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide</th>
<th>Length (mer)</th>
<th>Position Start</th>
<th>Position End</th>
<th>VaxiJen Score</th>
<th>Allergenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL</td>
<td>HKKDEEGKPGEDKKPEDKKPGEDKKPEDKKPGEDKKPEDKKPGKGT</td>
<td>67</td>
<td>902</td>
<td>968</td>
<td>1.3958</td>
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<tr>
<td></td>
<td>DKDSNKKKAKLPPKAGSEA</td>
<td>67</td>
<td>902</td>
<td>968</td>
<td>1.3958</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADEEEPEGTPEVQEGYATVEEAAAEAAKEALKEDKVN</td>
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<td>690</td>
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<tr>
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<td>HAGEETPELKDGYATVEEAAAEAAAEALKNDVNN</td>
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<tr>
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<td>AGKETPETPEEPKEEV</td>
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<td>388</td>
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<td></td>
</tr>
<tr>
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<tr>
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<td>T4P</td>
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<td>84</td>
<td>98</td>
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<tr>
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<td>72</td>
<td>0.9966</td>
<td></td>
</tr>
<tr>
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<td>36</td>
<td>45</td>
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</table>

The PL and T4P linear B cell epitope peptides were predicted by IEDB server (BepiPred). Predicted epitopes were selected for MEPV construction based on their length (≥10), antigenicity and allergenicity.
Antigenic properties of *Finegoldia magna* protein L and Type IV Pilin (PilA) for in-silico multi...

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Prediction Tool uses six different methods namely consensus, combinatorial library, NN-align, SMM-align, sturniolo and NetMHCIIpan methods. Totally, 6847 and 841 binding sites (15 mer) were predicted on PL and T4P, respectively. The predicted epitopes arranged by percentile ranking (cut off ≤ 0.5) where lower value shows better binding. Epitopes then checked by VaxiJen and AlgPred servers as mentioned before. Eventually, five MHC class II binding epitopes were selected for each PL and T4P (Table 2). For PL, ELKEAGITSDLYFSL, KEAGITSDLYFSLIN, TIKVNLFADGKTQT and IKVNLFADGKTQTA epitopes bind prominently to DRB3*01:01 and NPGITIDEWLLKNNAK binds to DRB1*03:01, while all 5 predicted T4P epitopes bind to DRB1*15:01. All these 10 epitopes and 3 MHC class II molecules were used for molecular docking.

**Molecular docking of selected T cell epitopes with selected HLA alleles**

The Flexible protein–peptide docking and scoring using the Internal Coordinate Mechanics (ICM) were performed using PIPER-FlexPepDock web server. For each docking a total number of 10 models were produced by PIPER-FlexPepDock web server and only the first model with the lowest binding energy was selected. The results revealed that the binding energy between ELKEAGITSDLYFSL, KEAGITSDLYFSL, TIKVNLFADGKTQT and IKVNLFADGKTQTA of PL T cell epitopes sequences and DRB3*01:01 HLA allele were -756.3, -775.4, -768.5 and -792 kcal/mol, respectively. The binding energy between NPGITIDEWLLKNNAK and DRB1*03:01 HLA allele was -375.7 kcal/mol. The binding energy between LVVLAILAILIAAV, LLELVVLAILAILIA, LLELVVLAILAILIA, ELLVVLAILAILIA and LLVVLAILAILIA of T4P T cell epitopes sequences and DRB1*15:01 HLA allele were -578.1, -613.7, -601.7, -599.1 and -601.4 kcal/mol, respectively. The results showed that all T cell epitopes acceptably bind to their corresponding HLA alleles, whereas ≤-32 kcal/mol typically is considered as good

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide (15 mer)</th>
<th>Position</th>
<th>HLA Allele</th>
<th>Consensus Methods</th>
<th>Percentile Ranking</th>
<th>Comb.lib Core Sequence</th>
<th>Smm Align Core Sequence</th>
<th>Nn Align Core Sequence</th>
<th>Sturniolo Core Sequence</th>
<th>VaxiJen Score</th>
<th>Allergenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PL</strong></td>
<td>ELKEAGITSDLYFSL</td>
<td>564-578</td>
<td>DRB3*01:01</td>
<td>comb.lib/ smm/m</td>
<td>0.03</td>
<td>ELKEAGITSDLYFSL</td>
<td>ITSDLYFSL</td>
<td>-</td>
<td>-</td>
<td>1.1186</td>
<td>-</td>
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<tr>
<td></td>
<td>KEAGITSDLYFSLIN</td>
<td>566-580</td>
<td>DRB3*01:01</td>
<td>smm/m/ sturniolo</td>
<td>0.54</td>
<td>ITSDLYFSL</td>
<td>ITSDLYFSL</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9307</td>
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<tr>
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<td>NPGITIDEWLLKNNAK</td>
<td>544-558</td>
<td>DRB3*01:01</td>
<td>smm/m/ sturniolo</td>
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<td>723-737</td>
<td>DRB1*03:01</td>
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<td>DRB3*01:01</td>
<td>smm/m/ sturniolo</td>
<td>0.54</td>
<td>ITSDLYFSL</td>
<td>ITSDLYFSL</td>
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<td>1.1014</td>
<td>-</td>
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<tr>
<td></td>
<td>LVVLAILAILIAAV</td>
<td>19-33</td>
<td>DRB1*03:01</td>
<td>smm/m/ sturniolo</td>
<td>0.23</td>
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<td>IFADGKTQTA</td>
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<td>LLELVVLAILAILIA</td>
<td>15-29</td>
<td>DRB1*03:01</td>
<td>smm/m/ sturniolo</td>
<td>0.23</td>
<td>IKVNLIFAD</td>
<td>IFADGKTQTA</td>
<td>-</td>
<td>-</td>
<td>0.9264</td>
<td>-</td>
</tr>
<tr>
<td><strong>T4P</strong></td>
<td>LVVLAILAILIAIAV</td>
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<td>DRB1*15:01</td>
<td>smm/m/ sturniolo</td>
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<td>NPGITIDEWLLKNNAK</td>
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<td>LLELVVLAILAILIA</td>
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<td>-</td>
<td>0.9678</td>
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</tr>
</tbody>
</table>

The peptides do not contain experimentally proven IgE epitopes. No hit found in Blast search on Allergen Representative Peptides (ARP) database.
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**Assessment of physicochemical parameters of MEPV**

The final MEPV construct (C\(_{3604}H_{5717}N_{961}O_{1181}S_7\)) with 797 amino acid residues is estimated to have 81.66 kDa molecular weight and theoretical isoelectric point value of 4.8, indicating negative nature of the protein with 126 negatively charged residues (E: 94 and D: 34) and 84 positively charged residues (R: 5 and K: 79). The grand average of hydropathicity (GRAVY) predicted to be -0.682 which indicates MEPV to be hydrophilic. The half-life predicted to be 30h in mammalian reticulocytes in-vitro, 20h in yeasts and 10h in *E. coli* in-vivo. The instability index of MEPV is calculated to be 21.10 which classifies it as stable. Moreover, MEPV shows aliphatic index of 66.71 as a clue for its thermos ability. The result obtained from Protein–Sol sever shows the predicted scaled solubility (0-1) of MEPV to be 0.777, where the value greater than 0.45 (the average of soluble *E. coli* proteins) is predicted to have a higher solubility than that of the average soluble *E. coli* proteins.

**Codon optimization**

The amino acid sequence of MEPV was submitted to OPTIMIZER web server for codon optimization in *E. coli* strain K-12 with One AA-one codon criteria. The result showed the CAI-value and GC content of optimized MEPV improved to 1.00 and 57%, respectively, while CAI-value and GC content of 0.88 and 59.6%, respectively, were calculated for un-optimized MEPV. The CAI-value > 0.8 is normally considered as good for high level expression in *E. coli* host cells. The suitable CG content for expression in *E. coli* host is between 30% and 70% (25).
Discussion

In recent years, in-silico progress in prediction and understanding of epitopes recognition at molecular level have enlightened the path for multi epitope peptide vaccine development.

Fig. 1. Construction of multi epitope peptide vaccine and secondary structure. a) the final construction of MEPV results from fusion of B and T cells epitopes, CTB adjuvant and 6×HisTag by GPGPG and EAAAK spacers, b) position and frequency of residues contribute to strand, helix and coil structures and c) regarding to their nature, d) the PSIPRED graphical result of MEPV secondary structure prediction with confidence for each residue in a color saturation manner.
relied on B cell and T cell epitope prediction. *F. magna* is believed to be one of the most important member of Gram Positive Anaerobic Cocci and cause of severe infections in many parts of body (1). A suitable and universal multicomponent vaccine to reinforce immune response against this bacterium is yet to be developed and is the object of the current work. Our strategy in this study was designed to select best T cell and B cell epitopes of two key virulence factors of *F. magna* by using open source servers. The predicted epitopes were checked both for antigenicity and allergenicity on VaxiJen and AlgPred web servers, respectively. VaxiJen web server developed as the first server alignment-independent prediction of protective antigens and a protein query can be submitted to this server followed by selection of three targets and antigen data bases (11). To assay allergenicity, two salient features of AlgPred server were used: firstly, the mapping of IgE approach in which the query protein sequence will be checked for any known IgE epitopes. The protein will be assign as allergen if high similarity with IgE epitopes be found. This approach has very high specificity. Secondly, a blast search on ARPs was performed. In this approach the query protein sequence is searched against almost 2890 known ARPs. If there be any hit, the query protein is assign as allergen. The accuracy of this method is very high with high sensitivity and specificity (13). Neither of the epitopes and final MEPV showed allergenicity, which strengthen the potential of MEPV as vaccine candidate. Raising suitable immune responses strongly depends on the interaction between epitopes and related receptors. All the predicted T cell epitopes were used in docking with recommended HLA alleles to determine the

**Fig. 2.** 3D structure prediction of MEPV. a) the final 3D model of MEPV, b) ramachandran plot of initial MEPV model before refinement and c) after refinement.
best and lowest binding energy by PIPER-FlexPepDock web server. This server streamlines rosetta fragment picker available at https://www.rosettacommons.org to generate precise peptide fragment ensembles. The server utilizes PIPER docking algorithm for fragment-receptor rigid-body docking and rosetta FlexPepDock for flexible full atom refinement of docked models. Moreover, rosetta cluster exerted for clustering of generated models. The best T cell epitopes with lowest binding energy were selected for further processing. The selected B cell and T cell epitopes were fused together by GPGPG spacers. This spacer in MEPV structure prohibits formation of the junctional epitopes and boosts processing and presenting of epitopes (26), while EAAAAK spacer, which used to connect CTB to the rest of MEPV, reduces the interaction between CTB and other parts of MEPV and provides effective separation. CTB is a non-toxic part of Cholera toxin which is a potent adjuvant and mucosal immunostimulant (27). A popular and highly accurate secondary structure prediction server, PSIPRED, was used to predict MEPV secondary structure which showed residues contribute to helixes, coils, strand structures, turns and bridges. The prepared MEPV then was submitted to I-TASSER server for 3D structure prediction. Top 5 models for MEPV where generated and arranged according to C-score, TM-score and RMSD. C-score or confidence score value is used for quality prediction of models by I-TASSER server and ranges between 2 and -5, where the higher indicates higher confidence. The TM-score or template modeling is another score to show the correct topology of the model where the score higher that 0.5 is preferable. RMSD along with TM-score are used to confirm reliability and accuracy of the generated 3D models. The generated 3D .pdb files of MEPV were submitted to Modrefiner server, which used for high-resolution refinement of generated 3D model. The major purpose of using Modrefiner is to refine 3D models closer to their native state and includes repositioning and adjusting of side chains, hydrogen bonds and backbone topology. Both .pdb files of MEPV, before and after refinement, were submitted to RAMPAGE server for quality and validation assessment via Ramachandran Plot Analysis which shows MEPV to be fully stable with 79.6% and 14.7% of residues (94.3%) located at favorite and allowed regions. Finally, the OPTIMIZER server used for codon usage optimization to increase protein expression level. It is possible for users to introduce specific preference optimization tables or select from more than 150 pre-computed tables for prokaryotes in OPTIMIZER server by using 3 approaches e.g., one amino acid-one codon, random or intermediate. Evaluation of effective optimization shows by The codon adaptation index (CAI) which improved from 0.88 to 1.0 for optimized MEPV. physicochemical parameters of MEPV determined by ProtParam sever revealed that this protein has acceptable stability and solubility in E. coli expression system. Moreover, hydrophobicity along with fused 6×HisTag provide clues for designing further purification methods.

Conclusion

Designing an effective multi epitope peptide vaccines needs advanced knowledge regarding to both B cell and T cell related antigen recognition which could achieve by immunoinformatics tools. To the best of our knowledge in the current study, PL and T4P are used for the first time for in-silico designing of
a multi epitope peptide vaccine with possible protective effects against *F. magna*. Further in-vitro/vivo evaluation of this in-silico designed vaccine is highly suggested to confirm its immunogenicity and protective attributes.

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**Ethical Consideration**
Authors of all ethics including non-plagiarism, Dual publishing has complied with data distortions and data making in this article.

**Conflicts of Interest**
The authors declare no conflicts of interest.

**References**
Antigenic properties of *Finegoldia magna* protein L and Type IV Pilin (PilA) for in-silico multi-


